The influence of current velocity, tidal height and the lugworm *Arenicola marina* on two species of seagrass, *Zostera marina* L. and *Z. noltii* Hornemann

DISSERTATION

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Titelbild:

Zostera marina Keimlinge (Ende März 2004) erreichen gerade die Sedimentoberfläche

Z. marina seedlings in late March 2004, just emerging at the sediment surface

List of Contents

Summary 5

1. Chapter 1: General Introduction 9

2. Chapter 2: *Arenicola marina* exclusion experiment 14

2.1 Introduction 14

2.2 Material and Methods 15

2.2.1 Study sites 15

2.2.2 Experimental set up 17

2.3 Results 20

2.3.1 Seagrass development in the donor population Puant Klent 20

2.3.2 The effects of tidal height and lugworm exclusion 24

2.3.3 The effects of current velocity and lugworm exclusion 35

2.4 Discussion 50

2.4.1 The development of the Puant Klent donor population 50

2.4.2 Seagrass seedling development in Puant Klent 51

2.4.3 The effect of tidal height and lugworm exclusion on two species of seagrass 54

2.4.4 The effect of current velocities and lugworm exclusion on two species of seagrass 60

2.5 Conclusion 66

3. Chapter 3: Carbon and nitrogen content of *Zostera noltii* 67

3.1 Introduction 67

3.2 Material and Methods 68

3.2.1 Study sites 68

3.2.2 Experimental set up 68

3.3 Results 70

3.3.1 Seagrass development in the Möwenberg meadow (donor population) 70

3.3.2 The development of adult *Zostera noltii* plants on lugworm exclusion plots 74

3.3.3 Grain size analysis 78

3.3.4 Porewater analysis 80

3.3.5 Carbon and nitrogen content of *Zostera noltii* 82

3.3.6 Carbon and nitrogen content in the sediment surrounding the seagrass transplants 86

3.4 Discussion 89

3.4.1 Seagrass biomass 89

3.4.2 Grain size 91

3.4.3 Porewater 92

3.4.4 Carbon and nitrogen content in *Zostera noltii* 94

3.4.5 Carbon and nitrogen content in the sediment 98

3.5 Conclusion 100

4. Chapter 4: Comparing the carbon and nitrogen content in the sediment and seagrass material from three different seagrass meadows 102
4.1 Introduction 102
4.2 Material and Methods 103
4.2.1 Study sites 103
4.2.2 Experimental set up 103
4.3 Results 104
4.3.1 Seagrass shoot density and biomass 104
4.3.2 Grain size analysis 107
4.3.3 Porewater analysis 108
4.3.4 Carbon and nitrogen content in seagrass samples from three different meadows 111
4.3.5 Carbon and nitrogen content in the sediment surrounding seagrasses 115
4.4 Discussion 117
4.4.1 Seagrass development in three different meadows 117
4.4.2 Grain size and porewater content 118
4.4.3 Carbon and nitrogen content of *Zostera marina* and *Z. noltii* 121
4.4.4 Carbon and nitrogen in seagrass sediment 125
4.5 Conclusion 126
5. Chapter 5: Concluding remarks 127
Acknowledgements 132
References 134
Appendices 142
Appendix 1: Statistical tables 142
T test results for experiment 1 (chapter 2) 142
Statistical tables for experiment 3 (chapter 4) 144
Appendix 2: Weather data 144
Appendix 3: Veränderungen zur Gutachterversion 149

Summary

For this PhD study three experiments with seagrasses were planned and conducted on the German North Sea island Sylt. The findings highlight yet again that hydrodynamics are the major factor influencing seagrass growth and survival. Therefore the hydrodynamic regime of a potential restoration site should be carefully investigated.

In the first experiment the influence of tidal current velocity, position on shore (tidal height) and the lugworm *Arenicola marina* on seedlings of the European seagrass species *Zostera marina* L. and *Zostera noltii* Hornemann was investigated. In the donor population, *Z. marina* seedlings appeared 3 – 4 weeks earlier at the sediment surface than *Z. noltii* seedlings. Mixed seagrass seedling cores were transplanted onto 20 m by 20 m lugworm exclusion plots to test the influence of the above mentioned factors on seagrass growth and survival. Light levels were saturating at both tidal heights throughout the experiment. After one growing season it became apparent that tidal current velocity was more influential than lugworm exclusion for seedling survival and growth. Furthermore, whilst *Z. noltii* seedlings were affected negatively by lugworm presence, *Z. marina* were not. Instead the *Z. marina* seedlings were more susceptible to strong current velocities and vanished if subjected to velocities above 8 cm s^{-1}.

For the second experiment adult *Z. noltii* plants were transplanted onto the lugworm exclusion plots to investigate the influence of lugworm exclusion, tidal height and current velocity on carbon and nitrogen content in seagrass leaves and roots/rhizomes. Lugworm presence and current velocity had a negative effect on seagrass biomass but neither had an effect on carbon or nitrogen content of the dwarf seagrass. The carbon content in the roots/rhizomes was lower in the lower intertidal plots than in the upper intertidal plots but this is not connected to the heightened nutrient availability in the lower intertidal. There was also a marked difference between the carbon content of the leaves and the roots/rhizomes.

In the third experiment seagrass, sediment and porewater samples were taken in three different seagrass beds around Sylt to investigate the carbon and nitrogen

content of seagrasses on muddy and sandy sediment, seasonal differences and the influence of porewater nutrients. Again there was a marked difference in the carbon content of the leaves and the roots/rhizomes of both seagrass species.

Neither *Z. marina* nor *Z. noltii* translocated carbon or nitrogen from their leaves to their roots and rhizomes in preparation for the cold season. Seasonal differences found in this study in the carbon and nitrogen content are due to different growth maxima, with *Z. noltii* reaching maximum biomass at a later time in the year than *Z. marina*.

Seagrass growing on muddy substrate (*Z. marina*) did not exhibit higher carbon or nitrogen content in either leaves or roots/rhizomes than those growing on sandy substrate (*Z. noltii* or mixed population). No seagrass population studied here appears to be nitrogen limited, as porewater nitrogen (ammonium) concentrations do not fall or rise with biomass development. In contrast, phosphate concentrations in the porewater are correlated with seagrass growth and may potentially limit seagrass growth.

Zusammenfassung

Für diese Dissertation wurden drei Experimente mit Seegräsern auf der deutschen Nordseeinsel Sylt geplant und durchgeführt. Die Ergebnisse zeigen den enormen Einfluss der Hydrodynamik auf Seegraswachstum und –überleben im Eulitoral auf. Daher sollten die hydrodynamischen Verhältnisse von potentiellen Ansiedelungsstellen des Seegrases genauestens auf ihre Eignung untersucht werden.

Im ersten Experiment wurde der Einfluss der Strömungsgeschwindigkeit, des Gezeitenniveaus bzw. der Position auf der Wattfläche und des Wattwurms *Arenicola marina* auf die Keimlinge zweier europäischer Seegrasarten, *Zostera marina* L. und *Z. noltii* Hornemann, untersucht. Dabei wurde beobachtet, dass in der Spenderpopulation die Keimlinge von *Z. marina* 3 – 4 Wochen vor den Keimlingen von *Z. noltii* an der Sedimentsoberfläche zu sehen waren. Seegrastransplantate beider Arten wurden aus der Spenderpopulation auf 20 m x 20 m Wattwurmausschlussflächen im Möwenbergwatt verpflanzt, um den Einfluss der oben genannten Faktoren auf das Wachstum und Überleben der Seegraskeimlinge zu untersuchen. Auf beiden untersuchten Gezeitenniveaus war ausreichend Licht für beide Seegrasarten vorhanden.
Die Strömungsgeschwindigkeit stellte sich in diesem Experiment als wichtigster Faktor für das Überleben und das Wachstum beider Seegrasarten heraus. Es zeigte sich hier ein Unterschied zwischen den beiden Arten, da *Zostera noltii* auch vom Wattwurm negativ beeinflusst wurde, die Keimlinge von *Z. marina* jedoch hauptsächlich auf die Strömungsgeschwindigkeit und nicht auf den Wattwurm reagierten. Bei Strömungsgeschwindigkeiten über 8 cm s^{-1} verschwanden die *Z. marina* Keimlinge.

Im zweiten Experiment wurde der Einfluss der oben genannten Faktoren auf den Kohlen- und Stickstoffgehalt von adulten *Z. noltii* Pflanzen untersucht, wobei auch der Nährstoffgehalt des Porenwassers untersucht wurde. Dafür wurden Seegraskerne von der angrenzenden Wiese auf die Wattwurmausschlussflächen verpflanzt.

Bei diesem Experiment zeigte sich ein negativer Einfluss des Wattwurmes und der Strömungsgeschwindigkeit auf die Biomasse des Seegrases *Z. noltii*, allerdings hatte keiner der beiden Faktoren Einfluss auf den Kohlen- oder Stickstoffgehalt des Zwergseegras. Jedoch war der Kohlenstoffgehalt in den Wurzeln/Rhizomen im unteren Eulitoral niedriger als im oberen Eulitoral, was nicht mit dem erhöhten Nährstoffangebot im Porenwasser zusammenhängt, da dieses im unteren Eulitoral erhöht war. Zusätzlich gab es einen merklichen Unterschied zwischen dem Kohlenstoffgehalt in den Blättern und den Wurzeln/Rhizomen.

Im dritten Experiment wurden Seegras-, Sediment- und Porenwasserproben aus drei unterschiedlichen Sylter Seegraswiesen untersucht, um den Kohlen- und Stickstoffgehalt von Seegräsern auf schlickigem und sandigen Untergrund, saisonale Unterschiede und den Einfluss der Nährstoffe im Porenwasser zu vergleichen. Dabei wurde festgestellt, dass es wiederum einen merklichen Unterschied zwischen dem Kohlenstoffgehalt in den Blättern und in den Wurzeln/Rhizomen beider Seegrasarten gab. Dieser Unterschied war sowohl im Sommer wie auch im Herbst vorhanden.
Es konnte keine Translokation von Kohlen- oder Stickstoff als Vorbereitung auf die Wintermonate von den Blättern zu den Wurzeln und Rhizomen bei einer der beiden Arten beobachtet werden. Saisonale Unterschiede im Kohlen- und Stickstoffgehalt der Seegraswiesen können auf die unterschiedlichen Wachstumsmaxima zurückgeführt werden, wobei *Z. noltii* die Höchstbiomasse später im Jahr (September) erreichte als *Z. marina* (Juli). Seegras auf schlickigem Untergrund (*Z. marina*) hatte einen niedrigeren Kohlen- und Stickstoffgehalt als Seegras, welches auf sandigen Untergrund wuchs (*Z. noltii* und gemischte Population). Eine Stickstofflimitierung in den Seegraswiesen kann ausgeschlossen werden, da sich der Stickstoffgehalt im Porenwasser (Ammonium) nicht mit dem Seegraswachstum oder -absterben verändert. Es könnte allerdings eine Phosphatlimitierung des Seegraswachstums geben, da der Phosphatgehalt im Porenwasser stark negativ mit dem Seegraswachstum korreliert ist.

1. Chapter 1: General Introduction

Seagrasses are the only submerged angiosperms in the seas and occur in most shallow coastal waters worldwide apart from the Antarctic (den Hartog 1970; Hemminga & Duarte 2000). They are split into two families (Potamogetonaceae and Hydrocharitaceae) and consist of about 50 species (Hemminga & Duarte 2000).

The genus *Zostera* is the most widespread seagrass genus occurring in all oceans (apart from the Antarctic). *Zostera marina L.* (eelgrass) can be found in coastal areas of the Atlantic and Pacific Ocean of the Northern Hemisphere and associated semi- enclosed areas, e. g. the Baltic Sea. *Z. noltii* Hornemann (dwarf seagrass) on the other hand can be found only in the North East Atlantic, the Baltic, Mediterranean, Black, Caspian and Aral Sea (Hemminga & Duarte 2000). Both seagrass species, *Z. marina* and *Z. noltii,* occur on the eastern site of Sylt, North Germany where they grow on sandy or muddy substrate.

Seagrasses can reproduce sexually by flowering, subsequent pollen production and dispersal, and fruit production (seeds) or vegetatively through the apical meristem (horizontal rhizome) resulting in a new ramet of one clone. They often grow on sandy or muddy sediment, but some species can grow on or overgrow rocks (e.g. *Phyllospadix terreyi*). *Z. marina* leaves can grow up to 2 m long and are between 2 – 12 mm wide. *Z. noltii* leaves grow up to 22 cm long and are between 0.75 – 1 mm wide. Nowadays both species occur intertidally on Sylt, but up until the 1930s a sublittoral meadow of *Z. marina* occurred in the Königshafen bay, Sylt-Rømø Bight. It vanished because of an outbreak of a slime mould that affected *Z. marina* meadows in most of its range (Wohlenberg 1935; Reise, Herre & Sturm 1989; Short & Wyllie-Echeverria 1996).

Seagrass blades are important habitats for small organisms such as hydroids, sponges, bryozoans, seaweeds, crustacean larvae and polychaete worm larvae (Fish & Fish 1995). Seagrass meadows are best known as breeding and nursing grounds for finfish and shellfish species (Short & Wyllie-Echeverria 1996; Polte & Asmus 2006). Because of the diverse life they support, seagrass beds are also important for commercial and recreational fisheries and globally seagrass meadows

form one of the most productive marine coastal environments, together with mangrove swamps and coral reefs (Short & Wyllie-Echeverria 1996).

Furthermore, seagrass beds also have a profound impact on their environment (and have been described as a key species and also autogenic bioengineers). They influence the material exchange (Asmus & Asmus 1998, 2000) in marine systems and enhance productivity and biodiversity (e. g. den Hartog 1970; Fonseca *et al.* 1990; Hemminga & Duarte 2000). Seagrasses also act as filters as they remove suspended matter from the water column, thus enhancing water quality, and act as a sink for nutrients (Asmus & Asmus 1998, 2000). The root and rhizome system binds and stabilises the sediment which helps counteract erosion (Hemminga & Duarte 2000), and their canopy reduces current flow and dampens wave action (Short & Wyllie-Echeverria 1996).

Seagrasses are in decline worldwide, and a number of factors have been proposed as the cause of the decline, with human-induced disturbances the most important one (Short & Wyllie-Echeverria 1996).

1. Human-induced disturbances that lead to changes in water quality and clarity. For example, increases in nutrient loads have lead to an increase in phytoplankton densities, which can, in turn, lead to a reduction of light levels for photosynthesis at the sea floor. The depth distribution of *Z. marina* is often light limited, and a further reduction in the amount of ambient light has lead to a decline in *Z. marina*. Also, increased fishing may have reduced the grazing pressures on epiphytes that live on seagrass blades. The increased densities of epiphytes could again have lead to a reduction of light available for photosynthesis (Levington 1995; Short & Wyllie-Echeverria 1996).
2. A large-scale reduction of the seagrasses *Zostera spp* and *Amphibolis antarctica* in Australia has been attributed to environmental causes such as a hot El Niño summer and is unlikely to have been caused by anthropogenic pollution (Seddon, Connolly & Edyvane 2000). Two of the possible consequences of global warming are higher rainfall and more extreme weather events. Both these factors can lead to a reduction of seagrasses, caused by either increased sediment load or physical damage from storm events (Short & Wyllie-Echeverria 1996).

3. Increased nutrient inputs from anthropogenic pollution limits *Z. marina* distribution in Europe to coastal regions with low salinity areas. When stressed because of high salinity levels, eelgrass is negatively affected by an increase of nutrients (van Katwijk *et al.* 1999).
4. A wasting disease caused by the slime mould *Labyrinthula zosterae* is affecting seagrass populations in Northern Europe at the present (Short & Wyllie-Echeverria 1996). In the 1930s, seagrass populations (*Z. marina*) suffered a serious decline worldwide, but particularly in Canada, the United States and Europe, which was caused by a wasting disease.

As seagrass decline can have profound effects on coastal ecosystems and fisheries, replanting experiments have been instigated to re-introduce *Z. marina* to coastal zones (e.g. van Katwijk *et al.* 1999) and also as mitigation actions for building work. De Jonge and Ruiter (1996) report that in the Dutch Wadden Sea in 1930, 44% of the eelgrass beds occurred sublittorally, and 56% occurred eulittorally. Because of their vegetative reproduction, they are ideally suited for transplanting. The meristem is located within the rhizome system (Ewanchuk & Williams 1996), so a transplanting unit can consist of rhizome with roots and meristem (and most often one or more shoots). Currently the replanting or establishment of seagrass meadows is undertaken by two main methods: a) transplanting sods of seagrass (or adult plants) into the new location or b) collecting seeds at a donor population and bringing them out. Whichever method one uses, the suitability of the new site has to be carefully investigated and many factors taken into account, particularly hydrodynamics.

Seagrasses are influenced by the properties of the medium surrounding them, for example salinity (Kamermans, Hemminga & de Jong 1999; van Katwijk *et al.* 1999), light availability (Philippart 1995; Peralta *et al.* 2002), nutrients (van Katwijk *et al.* 1999) or currents (Fonseca *et al.* 1982; van Katwijk & Hermus 2000; Koch 2001). As wholly submerged plants, seagrasses also depend on water movement for pollination (Ackerman 1986) and seed dispersal (Churchill, Nieves & Brenowitz 1985).

Hydrodynamics (wave action and tidal current velocity) are one of the major factors influencing seagrass survival and growth (Schanz 2003). Whilst slow to moderate water flow is beneficial to seagrasses because it reduces the thickness of the diffusive boundary layer (Fonseca & Kenworthy 1987), faster water movement is detrimental as leaves or shoots are detached or the sediment is eroded.

The aims of this PhD study were

a) to investigate the influence of bioturbation by a frequent polychaete worm, *Arenicola marina*, on mixed seagrass seedlings. Due to the set up of the experimental plots, the effect of position on shore and tidal current velocity could also be investigated;
b) the effect of the three factors mentioned above on the carbon and nitrogen content of adult *Zostera noltii* plants was studied, in particular the increased nutrient availability when the lugworm was absent (as reported by Volkenborn 2005);
c) the carbon and nitrogen content of seagrass material of three representative Sylt seagrass meadows was investigated in order to find out how sediment composition, porewater nutrients, and season influenced the carbon and nitrogen content in the leaves and root/rhizome of *Zostera marina* and *Z. noltii* and if there was any indication of either species storing carbon or nitrogen products in their roots/rhizome to prepare for winter;

In order to achieve these aims, three experiments were carried out on the island of Sylt, German North Sea. They are:

- Experiment 1: Mixed seagrass seedlings were transplanted onto the Möwenberg *Arenicola marina* (lugworm) exclusion plots looking at the influence of lugworm exclusion, tidal height and tidal current velocity on seagrass transplants. The experiment ran from March until August 2004
- Experiment 2: Adult *Zostera noltii* plants were transplanted on the Möwenberg lugworm exclusion plots investigating the effects of tidal height, current velocity and lugworm exclusion on the carbon and nitrogen content of seagrass plants (July until September 2004)
- Experiment 3: Samples were taken from three different seagrass meadows around Sylt (July and October 2004). Aim of the investigation was to find out

if carbon and/or nitrogen content in the seagrass tissue were influenced by season, sediment composition or porewater nutrients

Based on the aims and field observations it was hypothesised that

- the lugworm *A. marina* would have a negative effect on seagrass seedlings of both seagrass species based on the findings of Philippart (1994) and that the seedlings would benefit from the increased nutrient content at the experimental plots where the lugworm was absent
- strong hydrodynamics were assumed to have a negative influence on seedling survival and growth, based on the work done by Schanz & Asmus (2003)
- as *Zostera noltii* often grows as a perennial plant, it was expected to find some evidence of organic carbon and/or nitrogen products being translocated from the leaves to the rhizomes with the onset of autumn; also as different reproductive strategies are often adopted by the two seagrass species, a possible difference between the annually occurring *Z. marina* population and the perennial *Z. noltii* meadow was expected to be seen
- Short (1987) reports a difference in nitrogen content in the leaves and rhizomes of *Zostera marina* depending on sediment composition (mud or sand) and it was expected to find a similar difference here when the seagrasses were also growing on different sediments

2. Chapter 2: Arenicola marina *exclusion experiment*

2.1 Introduction

Both seagrasses and *Arenicola marina* occur in the intertidal zone of the Wadden Sea, and both are considered bioengineers. Bioengineers are species that either modify, maintain or create habitats for other species (Jones, Lawton & Shachak 1994). They are separated into two groups, allogenic and autogenic bioengineers. *Arenicola marina* is an allogenic bioengineer, i.e. a species that changes a resource from one state to another (e.g. organic rich sand into organic poor). Seagrasses on the other hand are autogenic bioengineers, as they change a habitat by their own physical structure, e.g. slow down water flow and filter suspended matter out of the water column with their canopy (Asmus & Asmus 1998, 2000).

The lugworm *A. marina* is a very abundant bioturbating polychaete worm in the Wadden Sea with up to 20 – 40 individuals per m^2 (Beukema 1976). It lives in a U-shaped burrow within the sediment and feeds on the organic matter of intertidal sands. The cleaned sand is defecated back up to the surface again, forming the characteristic coiled fecal casts (Riisgård & Banta 1998). The upper centimetre of the sediment is cleansed several times per year (Cadée 1976). Individuals grow up to 15 – 25 cm in length (Riisgård & Banta 1998).

It has been speculated by Philippart (1994) that *Arenicola marina* bioturbation is detrimental to the seagrass *Zostera noltii* because the seagrass shoots could be covered up by sediment. Furthermore, lugworm activities could indirectly affect seagrass meadows, e.g. vegetative propagules, seeds or seedlings could be covered up. Adult lugworms are expected to negatively influence seagrass meadows. On the other hand, dense seagrass mats can potentially be a hindrance to burrowing activities of lugworms. The root/rhizome mats may hinder the establishment of young lugworms. Thus either species may negatively influence the other.

It became apparent during the conduct of this experiment that not only the absence or presence of the lugworm *Arenicola marina* and the position of the experimental plots on the shore were affecting the seagrass transplants, but also current

velocities. Thus the results were also analysed for the effect of current velocity on the experimental parameters.

To investigate the influence of the lugworm *Arenicola marina* on the seedling development of two species of seagrass, *Zostera marina* and *Zostera noltii*, mixed seagrass transplants were brought out onto lugworm exclusion and control plots. Seagrasses can reproduce sexually or vegetatively, but for this study seedlings from both seagrass species were taken from a mixed donor population. The influence of current velocity and tidal height was also tested as well as any interaction between these three factors.

2.2 Material and Methods

Mixed seagrass seedlings were transplanted onto the Möwenberg *Arenicola marina* (lugworm) exclusion plots. The experiment ran from March until August 2004 and investigated the effect of lugworm exclusion, tidal height and current velocity on mixed seagrass patches

2.2.1 Study sites

Sylt-Rømø Bight

The Sylt-Rømø Bight (54°52’ to 55°10’ North, 8°20’ to 8°40’ East) is situated in the northern part of the German Wadden Sea (North Sea). It is a shallow tidal lagoon which has an area of 404 km^2 and is formed by the barrier islands Rømø and Sylt and causeways to the mainland (Gätje & Reise 1998). Water exchange with the North Sea is through one tidal inlet. Tides are semidiurnal in the Sylt-Rømø Bight and have a mean tidal range of 1.8 m. Salinity ranges between 28 to 32 psu (Backhaus, Hartke & Hübner *et al.* in Gätje & Reise 1998). The water temperature ranges between -1°C in winter and 20°C in summer (Baird, Asmus & Asmus 2004). Around one third of the bight (135 km^2) is not covered by water during medium low tide (eulittoral areas). Almost the entire sublittoral and 72% of the eulittoral are dominated by sand flats, mud flats comprise 3% of the eulittoral and 25% of the eulittoral is mixed sediments (Reise & Riethmüller in Gätje & Reise 1998). Seagrass beds cover approximately 15.2 km^2 of the entire bight (Asmus & Asmus 2000).

Möwenberg tidal flat

The Möwenberg tidal flat is a sandy tidal flat characterised by great ripples and is situated within the Königshafen, an inner tidal bight lying within the Sylt-Rømø Bight. The aerobic zone of the sediment is around 1 cm deep. The intertidal seagrass meadow consists mainly of *Zostera noltii* plants which reproduce via their rhizome systems (asexually) and are often perennial. Salinity in the porewater ranged from 32.3 psu in July to 30.0 psu in October 2004. Grazing by waterfowl occurs.

Hörnum Deep

The Hörnum Deep (54°53' to 54°42' North, 8°17' to 8°40' East) is also situated in the northern part of the German Wadden Sea (North Sea). It lies south of the Hindenburgdamm (the causeway which connects Sylt to the mainland) and is bordered by the southern part of the barrier island Sylt and the northern part of the barrier island Föhr. It covers an area of 250 km^2 and has been partly designated as a 'zero-use-zone' since 1999. All resource use including fishery is prohibited in the 'zero-use-zone', but shipping along navigational routes is allowed (Kellermann & Eskildsen 2005). Tides are semidiurnal. Around 50% of the bight (estimated) is not covered by water during medium low tide (eulittoral areas). The substrate consists of silt, mixed sediments and sands.

Puant Klent donor population

The Puant Klent tidal flat also has sandy sediment with a shallow aerobic zone (3 - 4 mm deep). The intertidal seagrass meadow consists of both *Z. marina* and *Z. noltii*. *Z. noltii* reproduces both asexually and to a lesser extent sexually here and is often perennial. *Z. marina* reproduces mostly via seeds in this meadow, but a few adult plants also survive the grazing by waterfowl and harsh winter conditions or grow back from rhizome fragments left in the soil. Salinity in the porewater ranged between 34.0 psu in July and 31.2 psu in October 2004.

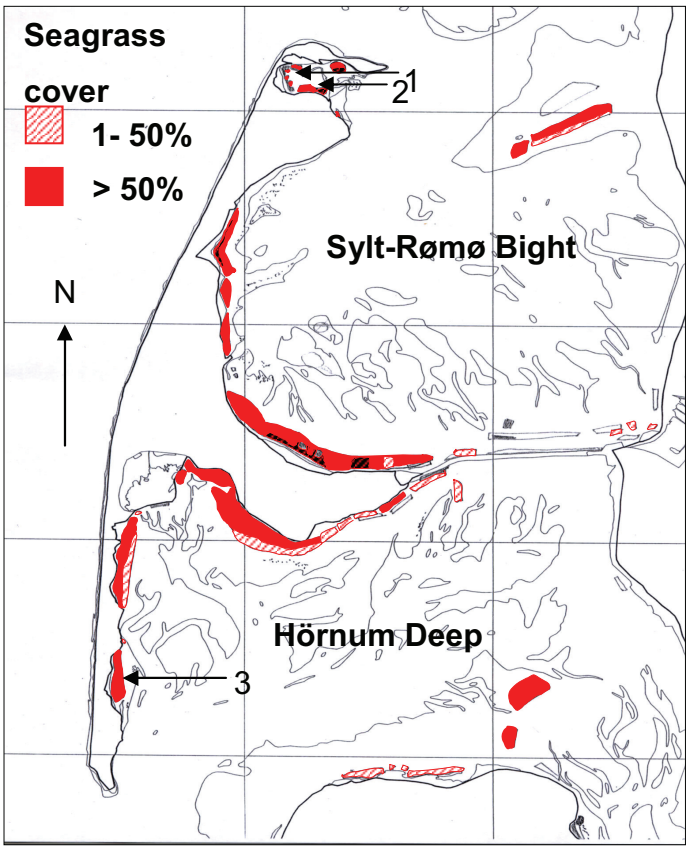

Figure 1: Map of the island Sylt showing the Sylt-Rømø Bight and the Hörnum Deep. The red areas show the seagrass meadows in both bights. The striped areas have a seagrass cover of 1 - 50%, the full areas have a seagrass cover of more than 50 % (Map modified from the Seagrass monitoring report 2003). 1 = Gröning tidal flat; 2 = Möwenberg tidal flat; 3 = Puant Klent tidal flat; 1 cm on the map = 3.8 km

2.2.2 Experimental set up

The 18 experimental plots were set up by Nils Volkenborn (Volkenborn 2005) in 2002. They consist of 20 m by 20 m plots arranged in blocks of three treatments each at two different tidal heights (see fig. 2). One block consists of one A*renicola marina* (lugworm) exclusion plot, one sediment disturbance plot (achieved by mixing the sediment) and one control (untreated) plot. The distance between the two rows of blocks was approximately 200 m, between the blocks lay roughly 100 m. The lugworm exclusion plots were created by embedding plastic gauze (mesh size 1 mm) in the sediment which prevents the lugworm from burrowing. As the control and the sediment disturbance plots showed no statistically significant difference after two years of the experiment, only the control and the lugworm exclusion plots were used in the seagrass experiments.

The water depth was 130 cm on the lower eulittoral plots and 80 cm at the higher eulittoral plots measured during medium high tide. The irradiance at the surface of the lower eulittoral plots was 932 µmol m^{-2} s^{-1} and 559 µmol m^{-2} s^{-1} at the sea floor on the 9th September 2004. On the same day but on the upper eulittoral plots, the irradiance at the surface was 904 µmol m^{-2} s^{-1} and 715 µmol m^{-2} s^{-1} at the sea floor.

54 sediment cores with seeds and seedlings from both seagrass species were cut from the Puant Klent meadow (see above for description of the meadow) on 30th / 31st March 2004. Care was taken not to disturb the sediment and also to exclude adult *Zostera noltii* or *Z. marina* plants which had survived from the previous year. The seagrass cores were cut out using a plastic tube (diameter 11.8 cm, height approx. 20-25 cm) and stored overnight in a temperature control room at 6 ° C.

Three mixed seagrass cores per experimental plot were transplanted. Due to adverse weather conditions, the transplantation was repeated on 5th / 6th April 2004 with the same number of cores and with the same experimental set up resulting in two data sets.

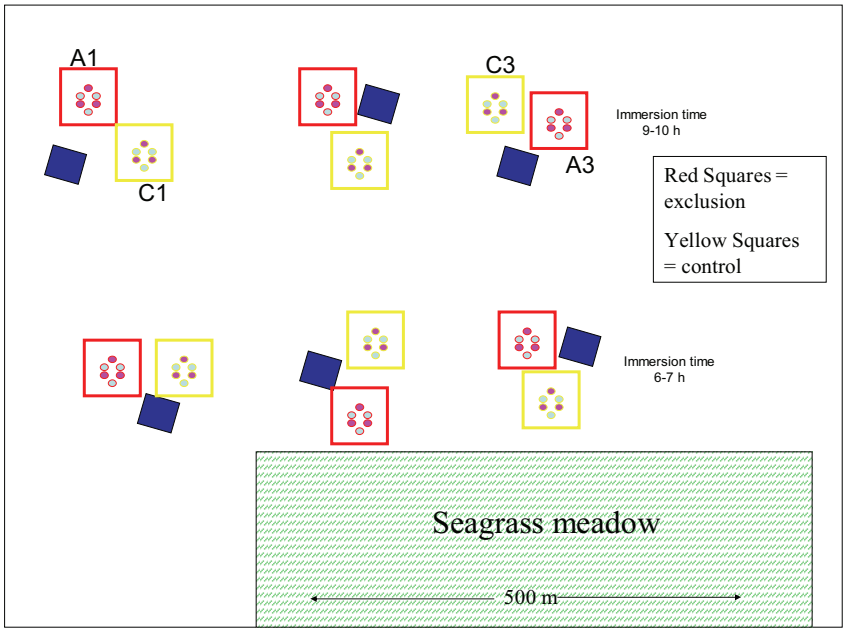

Figure 2: Experimental set up of the lugworm exclusion plots on the Möwenberg tidal flat. Red squares = lugworm exclusion plots; yellow squares = control plots; blue squares = disturbed sediment plots, not used. The circles indicate transplanted seagrass cores.

Because of the high current velocity, and the resulting difference in the replicates of the experimental plots in the Möwenberg tidal flat, the data from the experiment were analysed also for the factor current velocity. Instead of combining the replicates (i.e. the experimental plots) the plots A1, C1, A3 and C3 (see fig. 2) were analysed individually for the effect of current velocity and lugworm exclusion on seagrass growth and development.

Current velocity

Current velocity was measured on the western and eastern side of the lugworm exclusion plots in the upper and lower eulittoral (experiments 1 and 2).

The current velocity was measured on the 6th and 7th July 2005 on the left and right side of the experimental plots in the lower eulittoral zone with a Flow-Mate 2000 (Marsh McBirney) and Portable Water Current Meter 201D (Marsh McBirney). Measurements were taken at 5 – 15 cm above the sediment during high tide (incoming water) for 30 min (1 measurement per minute). On the 5th July 2005, the current velocity was recorded in the upper eulittoral zone using the same method and flow metres.

Seagrass parameters

Measurements were either taken in the field or in the laboratory.

- biomass: samples were either taken with a box corer (side length 10 cm by 10 cm) which was pushed approximately 20 cm into the sediment or the whole experimental unit (transplant) was taken. Care was taken to include all shoots during sampling. The samples were then washed and sieved in freshwater, all macrofauna and sediment was removed and the seagrass separated into above ground (shoots and leaves) and below ground (roots and rhizome) biomass. The separated samples were dried for a minimum of three days at 70 – 80°C until weight consistency was achieved. Dry weight (DW) was recorded and then the samples were combusted for 12 hours at 500°C. Ash free dry weight (AFDW) was calculated as the difference between dry weight (DW) and ash weight.
- shoot density: number of shoots in a sampling quadrate (12.5 cm by 12.5 cm). The density was calculated by multiplying the number of shoots with the factor 64 to get the seagrass density per square metre.

- number of shoots: number of shoots per experimental unit were counted in the field or in the laboratory if sample was removed from field.
- shoot length: Shoot length was measured from the shoot stem to the tip of the longest seagrass blade of six seagrass shoots per experimental unit.
- shoot elongation rate (SER): Shoot elongation rate is based on the method used by Peralta, Brun & Hernandez (2005). It measures the shoot growth rate from one sampling date to the next using the following formula:
  $(SL_{fi} - SL_{st})/(T_{fi} - T_{st})$
  where SL = shoot length; T = number of days; Fi = finish; St = start.
- number of leaves per shoot: the number of leaves of six different seagrass shoots were either counted in the field or in the laboratory.
- Leaf appearance rate (LAR): leaf appearance rate is based on the method used by Peralta, Brun and Hernandez (2005). It measures the leaf appearance rate from one sampling date to the next using the following formula:
  $(NL_{fi} - NL_{st})/(T_{fi} - T_{st})$

where NL = number of leaves; T = number of days; Fi = finish; St = start.

All data were analysed using the JMP software package.

2.3 Results

Seagrass cores were taken from the donor population in Puant Klent and were transplanted onto the *Arenicola marina* exclusion plots in the Möwenberg tidal flat. The development of the donor population was monitored and biomass, shoot density, shoot length and shoot elongation rate were recorded and analysed.

2.3.1 Seagrass development in the donor population Puant Klent

The Puant Klent seagrass meadow is situated in the Hörnum Deep (see material and methods for further description). It is a mixed seagrass bed consisting of both *Zostera marina* and *Z. noltii*. Seagrass transplants or samples from this meadow were used in experiments 1 and 3. The development of the seagrass bed was monitored from April to October 2004.

Biomass development

In May, June and July 2004 the above and below ground biomass (see fig. 3) are of a similar height. In August and October the above ground biomass is higher than the below ground. In April and May on the other hand the below ground biomass is higher than the above ground biomass. Furthermore, the seedling biomass could only be given in April and May when it was still possible to distinguish between plants from seeds, and plants that had survived or grown from rhizome fragments.

In general though there was an increase in biomass over time until a maximum biomass was achieved with 139.02 g m^{-2} in August. The data had to be log transformed to achieve homogenous variances. The results for the above and below ground biomass and seedling biomass increased significantly in time (Oneway ANOVA, above ground: $F_{5, 35} = 61.3393$, $p = <0.0001$, below ground: $F_{5, 35} = 13.8489$, $p = <0.0001$; seedlings: $F_{1, 11} = 27.0605$, $p = 0.0004$).

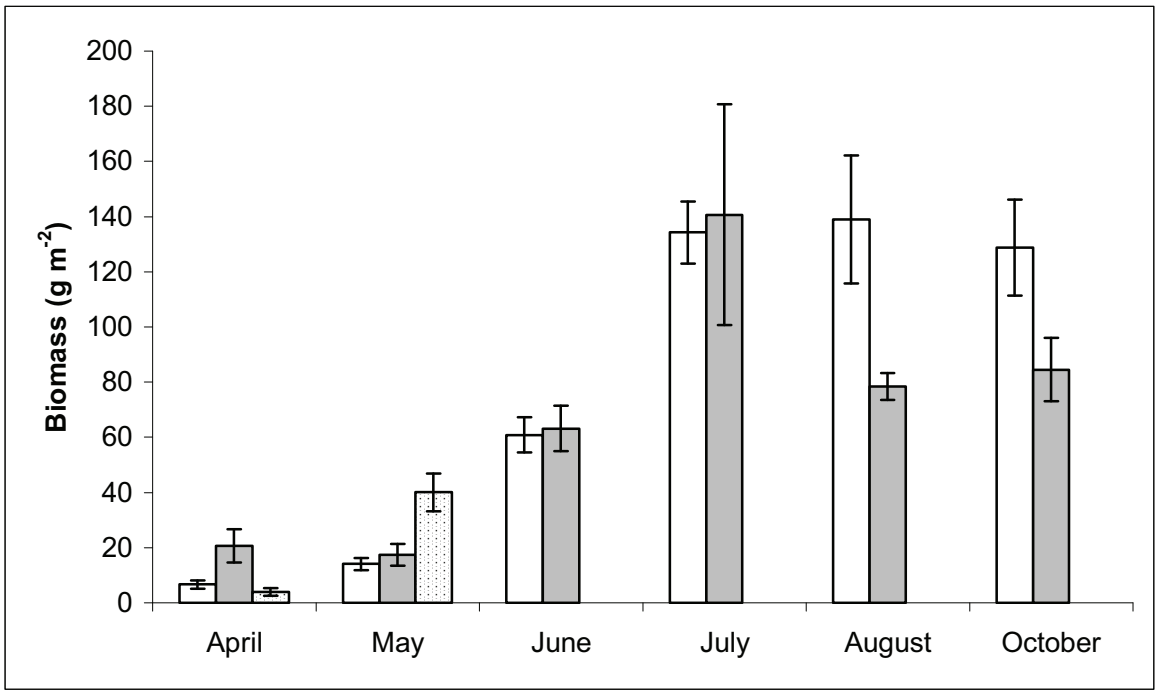

Figure 3: Above and below ground biomass results from Puant Klent. The results differ significantly in time. White columns = above ground biomass, grey columns = below ground biomass, dotted columns = seedling biomass.

Shoot density

As expected the shoot density (see fig. 4) increased over time in the Puant Klent meadow. There was a marked decrease in October; the maximum shoot density was found in late August with 10,880 shoots m^{-2}. The maximum seedling density was reached in mid April with 568 seedlings m^{-2}. There was no significant

difference in the seedling density from 24th March till 15th April 2004. The seagrass density from mid April to late June did increase significantly over time (Oneway ANOVA, $F_{3, 92} = 117.6658$, $p = <0.0001$). As it was impossible to count as many squares as before in the sampling quadrate, the number of squares was reduced, so the data from July till October is based on 6 replicates instead of 24. From July till October the shoot density still differed but less significantly so (One way ANOVA $F_{2, 15} = 4.4471$, $p = 0.0304$).

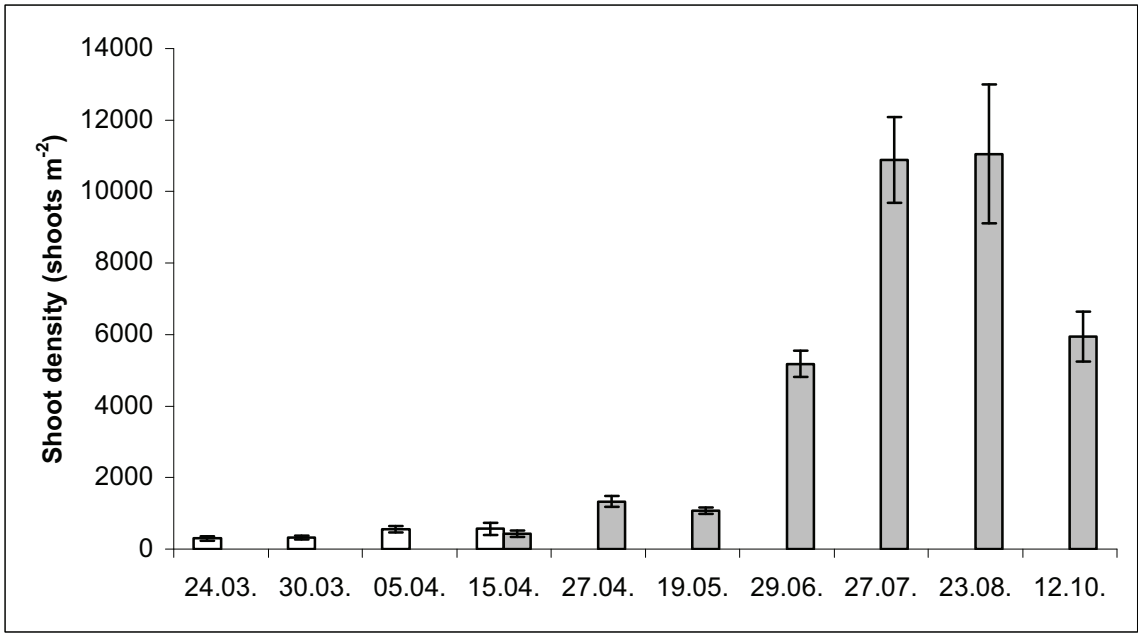

Figure 4: Shoot density in the Puant Klent meadow. White columns = seedlings, grey columns = seagrass density.

Shoot length

The shoot length of both *Zostera marina* and *Z. noltii* increased over time (see fig. 5). In the seedlings, there was a marked difference between the two seagrass species (Scheirer Ray Hare (nonparametric equivalent of a 2 factorial ANOVA with interaction, Dytham 1999), species: $p = <0.0001$). Both increased in length over time (Scheirer Ray Hare, time: $p = <0.0001$) but the results differed between the species. *Z. marina* seedlings occurred earlier (early April) and were longer (11.72 cm on the 26th May), the *Z. noltii* seedlings were longest in late June with 9.07 cm and only started to occur in late April.

From July onwards, it was no longer possible to differentiate between plants from seeds or rhizome or last-year's plants. The shoot length increased in both species, reaching a peak for *Z. marina* with 22.75 cm in October and for *Z. noltii* also in

October with 15.69 cm, it also differed significantly between the two species (Two way ANOVA with interaction, species: $F_{2, 166} = 38.0402$, $p = <0.0001$). The shoots also lengthened significantly in time (Two way ANOVA with interaction, time: $F_{1, 166} = 48.6438$, $p = <0.0001$) and there was also interaction between time and species (Two way ANOVA with interaction, time x species: $F_{2, 166} = 11.6326$, $p = <0.0001$).

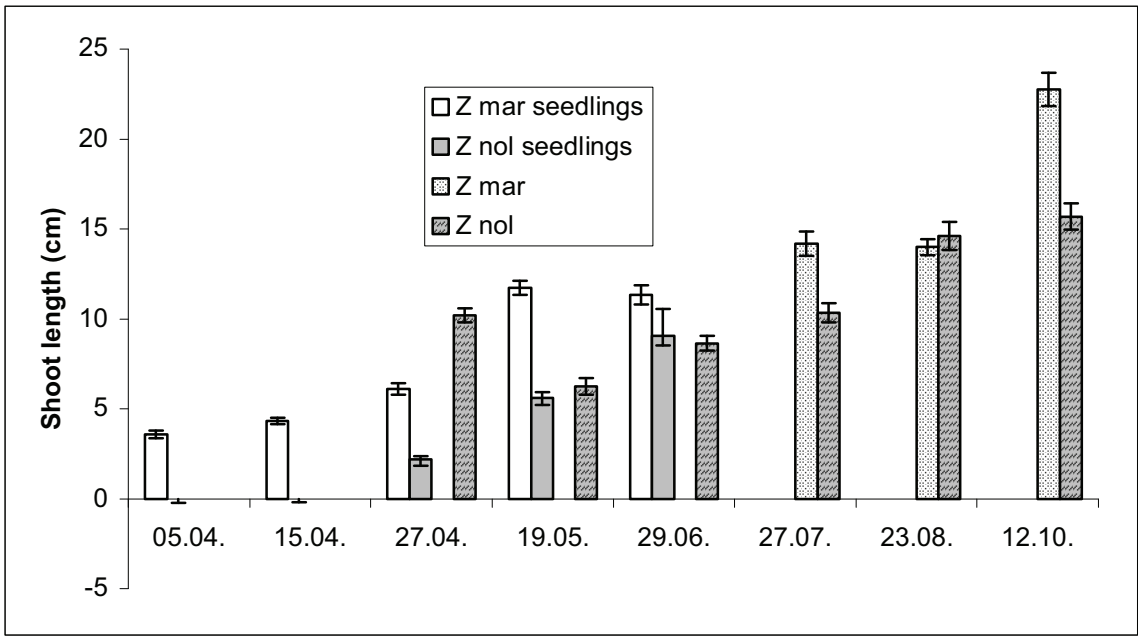

Figure 5: Shoot length in the Puant Klent meadow. White columns = *Z. marina*, grey columns = *Z. noltii*, striped or dotted= adult plants.

Shoot elongation rate

The shoot elongation rate (SER) in the Puant Klent meadow was calculated for *Zostera marina* and *Z. noltii* (see fig. 6). The SER of *Z. noltii* could only be calculated by combining the measurements of the rhizome plants and the seedlings. From mid April to late June, the *Z. marina* data was recorded from seedlings only. From July onwards it could no longer be differentiated between rhizome and seedling plants for both *Z. marina* and *Z. noltii*.

The SER of *Z. marina* increased till May (0.19 cm plant^{-1} day^{-1}), but then decreased rapidly again and reached a plateau at 0.09 cm plant^{-1} day^{-1}. The SER of *Z. marina* was significantly higher than the SER of *Z. noltii* (Scheirer Ray Hare test, species: $p = <0.0001$).

The SER of *Z. noltii* was highest in mid May also (0.08 cm plant^{-1} day^{-1}) but decreased afterwards. It was generally lower than the SER of *Z. marina* apart from August were the growth rates overlap.

Both growth rates differ significantly in time (Scheirer Ray Hare test, time: p = 0.002) and there is also interaction between time and species (Scheirer Ray Hare test, time x species: p = 0.003).

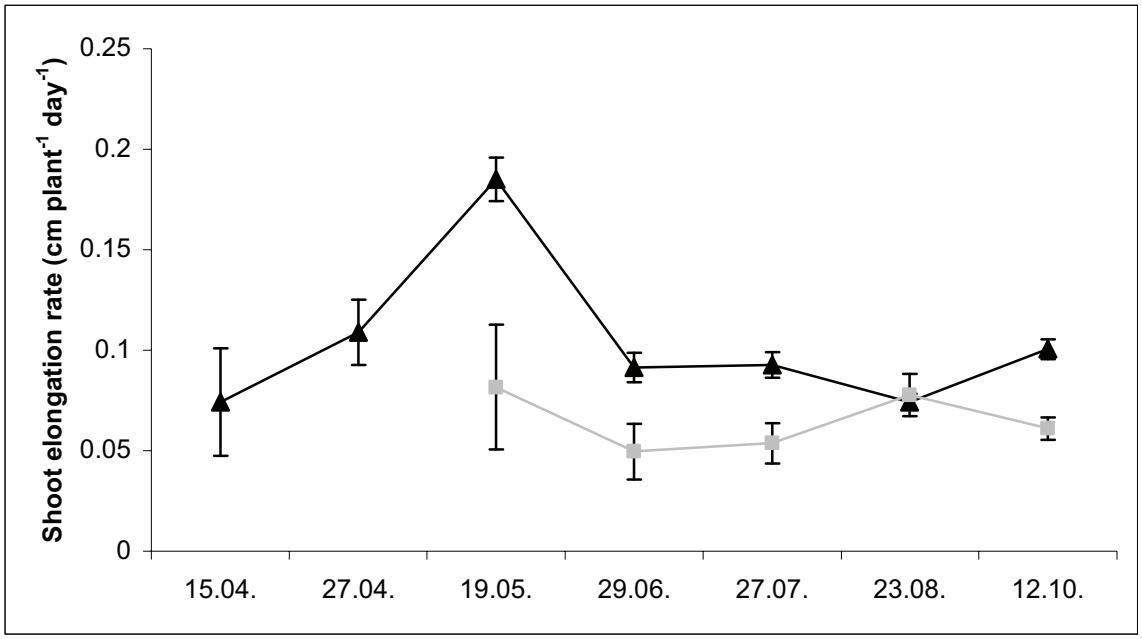

Figure 6: Shoot elongation rate in the Puant Klent meadow. The data was taken from seedlings and rhizome plants, from July onwards it was no longer differentiated between seedling or rhizome plant. Black triangles = *Z. marina*, grey squares = *Z. noltii*.

2.3.2 The effects of tidal height and lugworm exclusion

Several seagrass parameters were measured in the experiment (see materials and methods for further description of each parameter). The data were analysed for a) the effects of lugworm exclusion and tidal height and b) for the effects of current velocities and lugworm exclusion. As the transplantation of the seagrass cores occurred twice (due to unfavourable weather), the seagrass data consists of two separate sets (first and second transplantation). T tests were carried out for each seagrass parameter to see if the results could be combined (see app.1). The transplants were taken from the seagrass meadow in Puant Klent.

At the start of the experiment when the cores were taken from the donor population it was not possible to differentiate between seedlings from *Zostera marina* or *Z. noltii*. Therefore the number of shoots was recorded in the beginning as the overall

number of shoots, and could only later be seperated into the individual seagrass species. Shoot length and shoot elongation rate were only obtained for all seagrass shoots, and not each species. In general there were less *Zostera noltii* shoots present than *Z. marina* shoots, so these parameters tend to be dominated by *Z. marina*.

Number of shoots per experimental unit (seagrass core) of both seagrass species combined

The t tests showed no significant differences so the data sets were combined, resulting in 6 cores per experimental plot. The Scheirer Ray Hare test was used to test for interaction of effects.

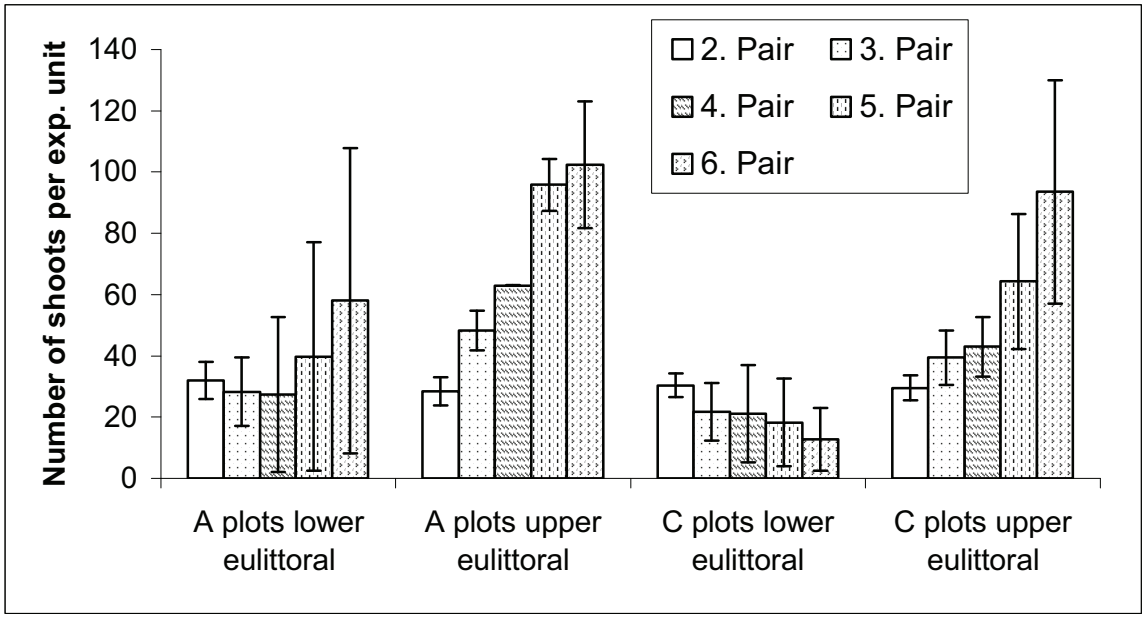

Figure 7: Results for number of shoots per experimental unit in the lugworm exclusion experiment, Pairs 2-6 (April - August 2004). A = lugworm exclusion plots, c = control plots.

The number of shoots (see fig. 7) did not differ significantly between plots from 10th April (Pair 2) until 22nd June (Pair 4) and on 10th August 2004 (Pair 6). However, in July 2004 (Pair 5) the number of shoots per experimental unit were significantly lower (the data from Set 4 and 5 had to be log transformed to achieve homogenous variances, Scheirer Ray Hare test, tidal height: $p < 0.001$) in the lower eulittoral plots (on average 39.78 shoots on the lugworm exclusion plots and 18.22 shoots on the control plots) than in the upper eulittoral plots (on average 95.89 shoots on the lugworm exclusion plots and 64.28 on the control plots).

Number of shoots for each species separately

From June 2004 onwards, the seagrass plants could be separated into the two distinct seagrass species and the data were analysed accordingly. The t tests showed no significant difference, so the data pairs were combined (see table 12 in app.1).

Significantly more *Zostera marina* shoots grew (see fig. 8, 10, 12) in the upper eulittoral (81.78 shoots) than in the lower eulittoral (11.33 shoots) in all three data pairs (June/Pair 4: 2 factorial ANOVA with interaction, tidal height: $F_{1, 68} = 5.89210$, $p = 0.0179$; July/Pair 5 (data was log transformed): 2 factorial ANOVA with interaction, tidal height: $F_{1, 68} = 26.4060$, $p < 0.0001$; August/Pair 6: 2 factorial ANOVA with interaction, tidal height: $F_{1, 68} = 9.5182$, $p = 0.0029$).

The *Zostera noltii* shoots (see fig. 9, 11, 13) were also more numerous in the upper eulittoral (42.94 shoots) than in the lower eulittoral (1.22 shoots) in all 3 data pairs, in August (Pair 6) the interaction between tidal height and lugworm exclusion was also significant (June/Pair 4: 2 factorial ANOVA with interaction, tidal height: $F_{1, 68} = 8.7873$, $p = 0.0042$; July/Pair 5: 2 factorial ANOVA with interaction, tidal height: $F_{1, 68} = 16.4581$, $p = 0.0001$; August/Pair 6 (data were log transformed): 2 factorial ANOVA with interaction, tidal height: $F_{1, 68} = 20.8418$, $p < 0.0001$, Interaction: $F_{1, 68} = 4.9359$, $p = 0.0296$).

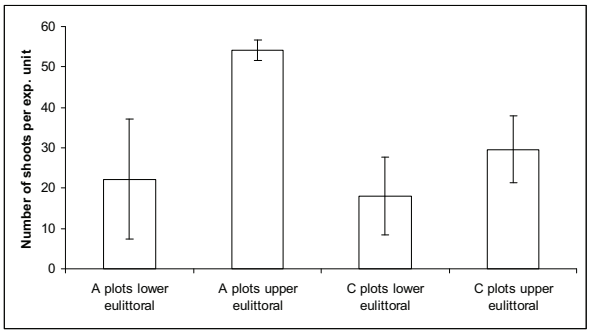

Figure 8: Results for number of shoots from *Z. marina* Pair 4 (16 June/22 June 2004). A = lugworm exclusion plots, c = control plots. The factor tidal height was statistically significant.

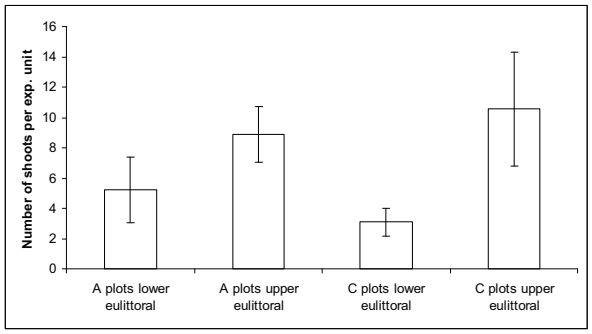

Figure 9: Results for number of shoots from *Z. noltii* Pair 4 (16 June/22 June 2004). The factor tidal height was statistically significant.

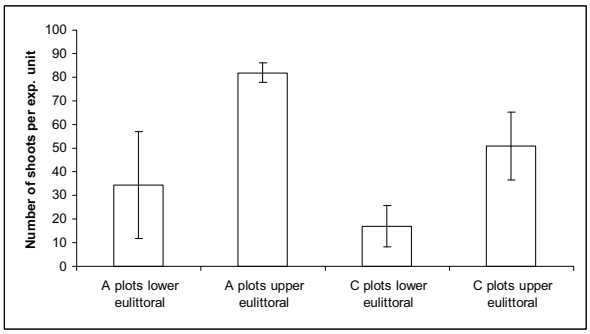

Figure 10: Results for number of shoots from *Z. marina* Pair 5 (16 July/19 July 2004). The factor tidal height was statistically significant.

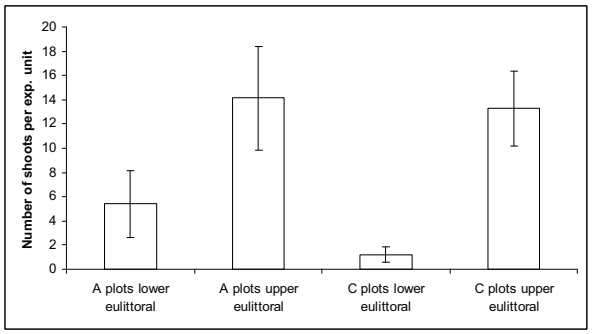

Figure 11: Results for number of shoots from *Z. noltii* Pair 5 (16 July/19 July 2004). The factor tidal height was statistically significant.

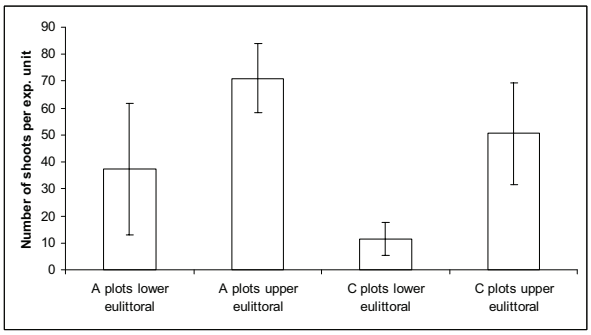

Figure 12: Results for number of shoots from *Z. marina* Pair 6 (10 August/10 August 2004). The factor tidal height was statistically significant.

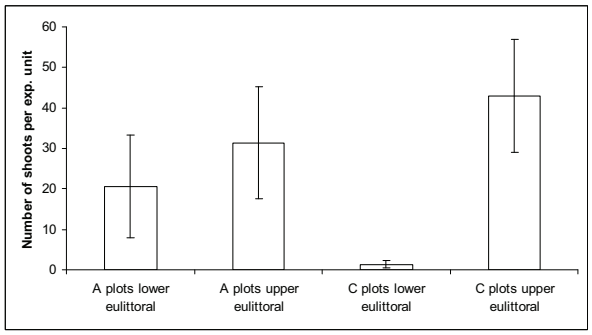

Figure 13: Results for number of shoots from *Z. noltii* Pair 6 (10 August/10 August 2004). The factors tidal height and the interaction between lugworm exclusion and tidal height were statistically significant.

Shoot length

The t test showed a significant difference in the data from 10th/ 14th April 2004 (Pair 2) so these data were analysed separately from the remaining data but all other data were combined. The results from the first transplantation (see fig. 14) were not statistically significant. The shoot length ranged from 3.94 cm on the lugworm exclusion plots in the lower eulittoral zone to 3.3 cm on the control plots in the upper eulittoral. From May onwards, data were only analysed for the effect of current velocity and lugworm exclusion.

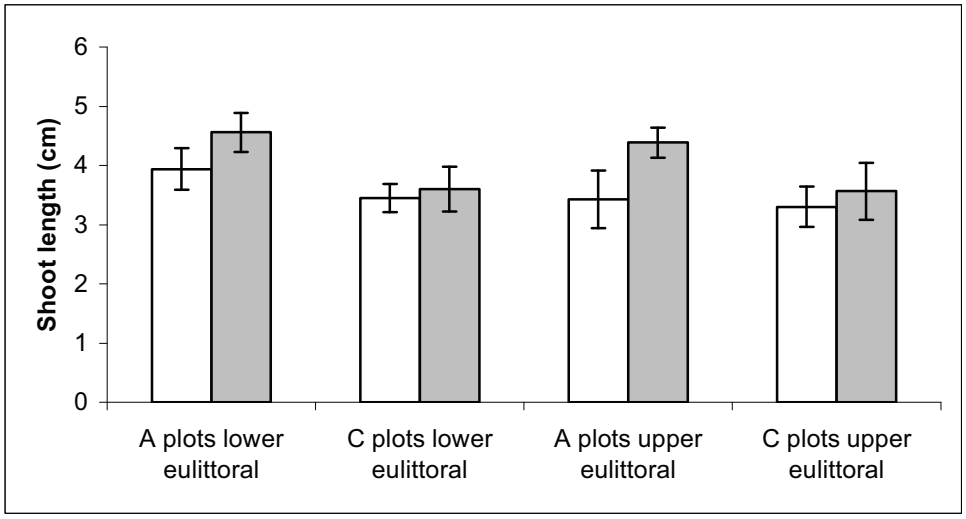

Figure 14: Shoot length results for Pair 2 (10 / 14 April 2004) A = lugworm exclusion plots, C = control plots. White columns = first transplantation, grey columns = second transplantation. Lugworm exclusion was significant in the second transplantation.

Lugworm exclusion did result in longer shoots in the transplants from the second transplantation (see fig. 14) on the lugworm exclusion plots compared with the control plots (2 factorial ANOVA with nested block design and interaction, lugworm exclusion: $F_{2, 33} = 9.7561$, $p = 0.0037$).

In the combined data, the variances were homogenous in the data on 3rd/8th April (Pair 1), 20th/23rd April (Pair 3), 28th April/5th May (Pair 4) but only in early April were the data statistically significant (see fig. 15). Again lugworm exclusion resulted in longer shoots on the lugworm exclusion plots (2 factorial ANOVA with interaction, lugworm exclusion: $F_{1, 68} = 10.4515$, $p = 0.0019$). Only the data from Pair 1 is shown.

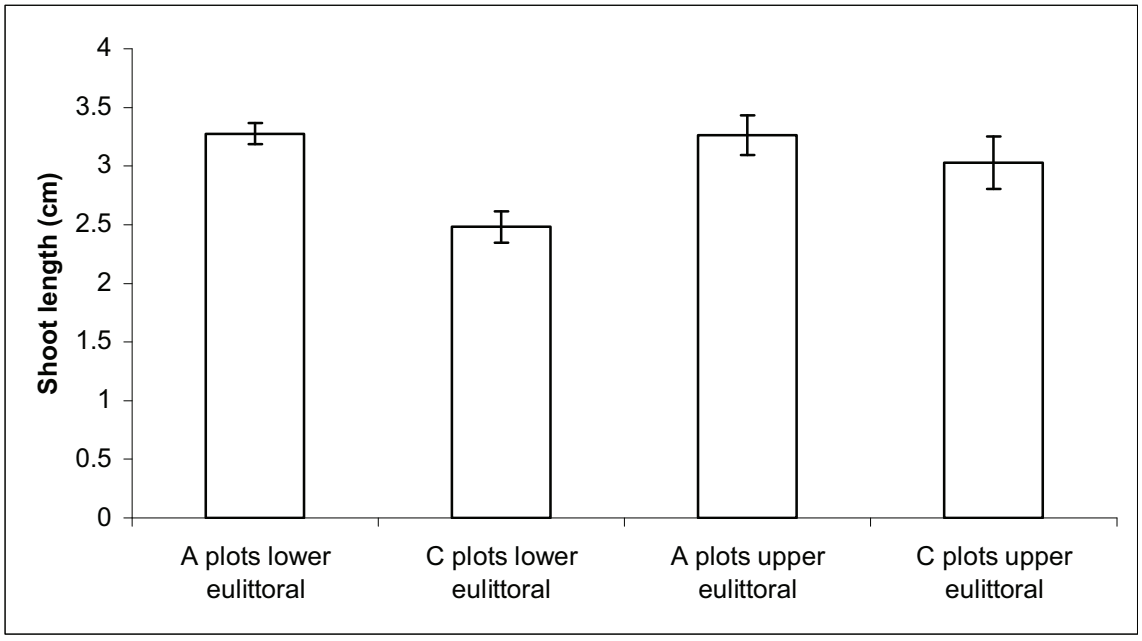

Figure 15: Shoot length results for Pair 1 both transplantations (3 / 8 April 2004), showing the effect of lugworm exclusion on seagrass seedlings. A = lugworm exclusion plots, C = control plots. Lugworm exclusion was statistically significant.

Shoot elongation rate (SER)

The t tests results show that all data pairs apart from the data from 10th/14th April 2004 (Pair 1) can be combined. Pair 1 was therefore analysed split into two separate data sets (see fig. 16). From April onwards the data was only analysed for the effect of current velocity and lugworm exclusion.

Lugworm exclusion has a negative effect (2 factorial ANOVA with nested block design, $F_{1, 32} = 8.5088$, $p = 0.0064$) on the SER in the results from the first transplantation as the rate is higher on the control plots (0.16 cm plant $^{-1}$ day^{-1}) than the lugworm exclusion plots (0.024 cm plant $^{-1}$ day^{-1}). The results for the second transplantation were not statistically significantly different.

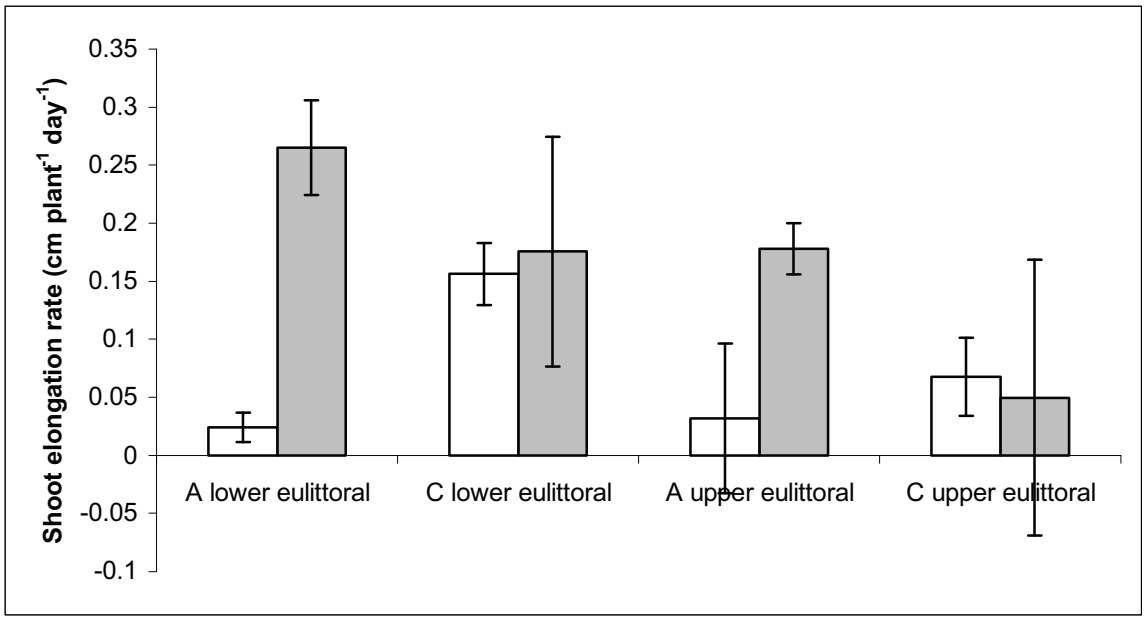

Figure 16: Results for SER Pair 1 first (10th April) and second (14th April) transplantation, showing the effects of lugworm exclusion on seagrass growth rate. The results are significant in the first transplantation where lugworm exclusion has a negative effect. White columns = First transplantation, grey columns = Second transplantation. A = lugworm exclusion, C = control.

Combined above and below ground biomass of each seagrass species

Tidal height (Scheirer Ray Hare test, tidal height: p = 0.028) significantly influenced the *Zostera marina* biomass (see fig. 17), as the ash free dry weight was higher in the upper eulittoral (59.65 ash free dry weight (AFDW) g m^{-2}) than it was in the lower eulittoral (15.85 AFDW g m^{-2}). The data were square root transformed to achieve homogenous variances.

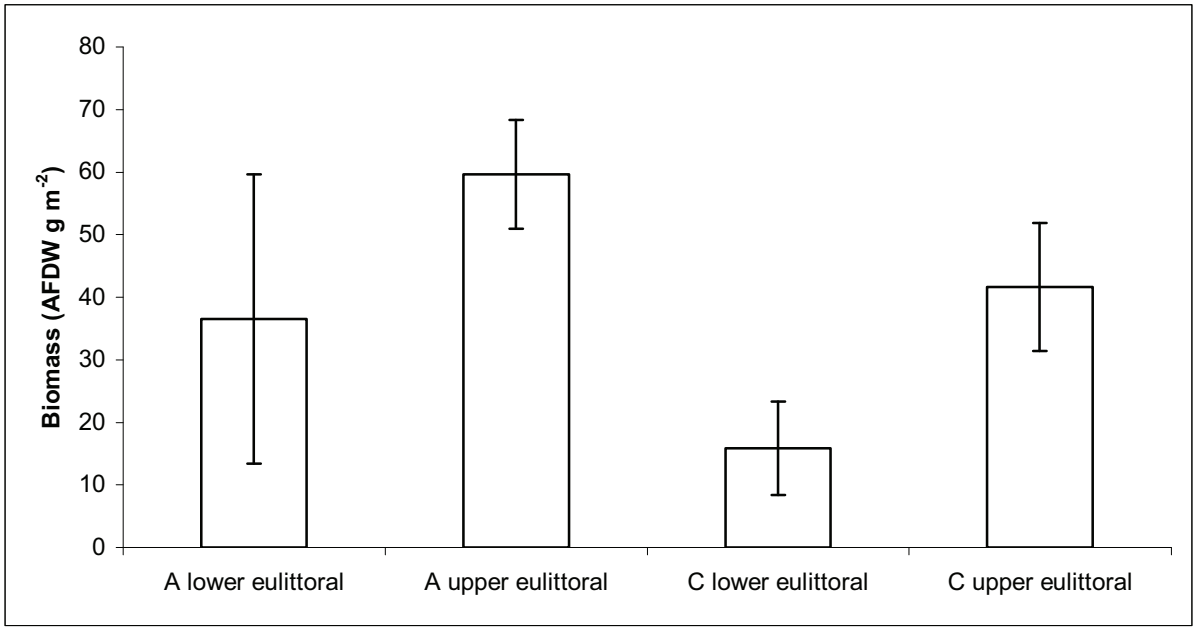

Figure 17: Results for the combined (above ground and below ground) biomass of *Zostera marina* after 4 months. The results were significant for the factor tidal height. A = lugworm exclusion, C = control.

The biomass of *Zostera noltii* (see fig. 18) on the other hand was influenced significantly by the interaction between tidal height and lugworm exclusion (Scheirer Ray Hare test, interaction: p = 0.038). This resulted in the highest biomass on the lugworm exclusion plot in the lower eulittoral (5.35 AFDW g m^{-2}). The lowest biomass was found on the control plot in the lower eulittoral with 0.25 AFDW g m^{-2}. The data were square root transformed to achieve homogenous variances.

Figure 18: Results for the combined (above and below ground) biomass of *Zostera noltii* after 4 months. The results were significant for the interaction between tidal height and lugworm exclusion. A = lugworm exclusion, C = control.

Separated above and below ground biomass of each seagrass species

If the above and below ground biomass are not combined, then the data are not statistically significant for *Z. marina* (data not shown) or for the below ground biomass of *Z. noltii* (see fig. 19), but are for the above ground biomass of *Z. noltii* (Scheirer Ray Hare test, tidal height: $p = 0.037$). The biomass in the upper eulittoral plots is higher (2.58 AFDW g m^{-2}) than the biomass in the lower eulittoral plots (0.30 AFDW g m^{-2}).

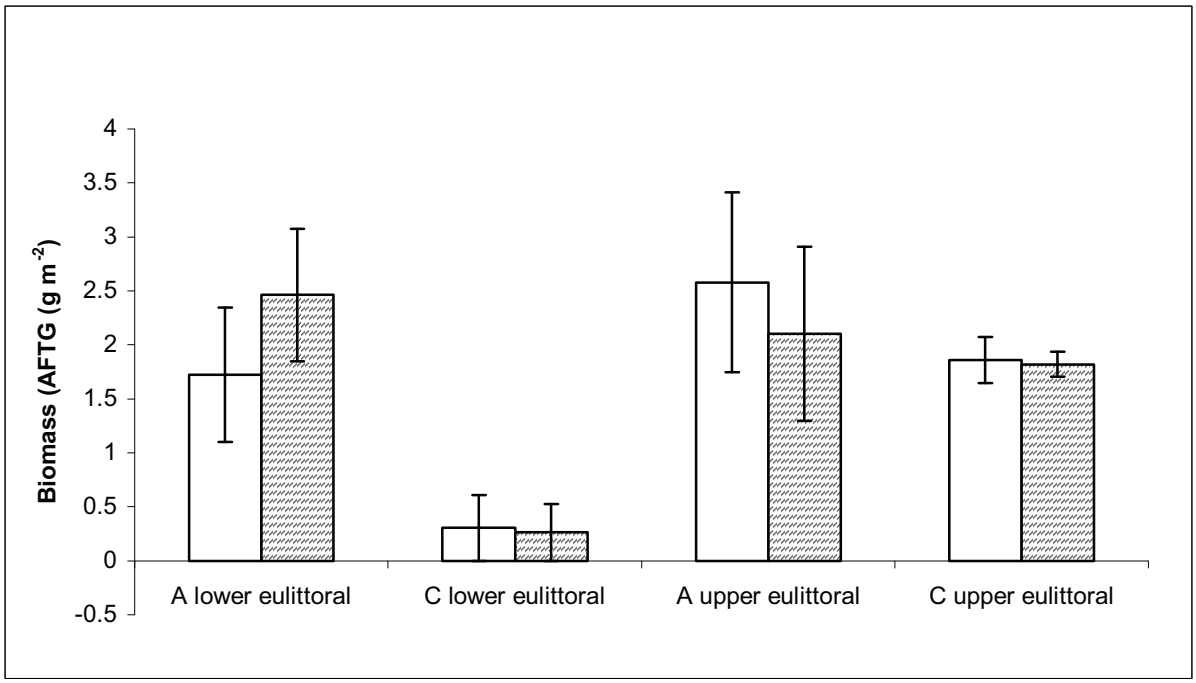

Figure 19: Results for the above ground (AG) and below ground (BG) biomass of *Zostera noltii* after 4 months. The results were significant for the factor tidal height in the above ground biomass. White columns = AG biomass of *Z. noltii*, striped columns = BG biomass of *Z. noltii*. A = lugworm exclusion, C = control.

Summary table

The following table summarises all results of this section of experiment 1. Not shown are the results for the number of leaves per seagrass shoot and the leaf appearance rate (LAR), as the results were not statistically significant. These parameters have significant results when analysed for the effect of current velocity (see below) and are included in that section.

Table 1: Results from the Möwenberg lugworm exclusion experiment (first part). Sg = Seagrass, + = positive, - = negative, Z. mar = *Zostera marina*, Z. nol = *Zostera noltii*

Parameters	**Tidal height**		***Arenicola marina***	
	Lower eulittoral	**Upper eulittoral**	**Excluded**	**Control**
Combined no. of shoots	Sg -	Sg +	No effect	No effect
No. of shoots sep. spec	Z. mar - Z. nol -	Z. mar + Z. nol +	No effect No effect	No effect No effect
Shoot length	No effect	No effect	Sg +	Sg -
Shoot elongation rate (SER)	No effect	No effect	Sg -	Sg +
Combined AG and BG biomass	Z. mar - No effect	Z. mar + No effect	No effect Z. nol +	No effect Z. nol -
Sep. AG and BG biomass	Z. mar not significant Z. nol -	Z. mar not significant Z. nol +	Z. mar not significant No effect	Z. mar not significant No effect

2.3.3 The effects of current velocity and lugworm exclusion

Due to the strong influence of current velocity from April 2004 onwards, the data from the transplanted seagrass cores were also analysed for the effects of current velocities and lugworm exclusion on seagrass. Only the data from the lower eulittoral plots and then only those on the most western and eastern side (i.e. plots A1, C1 and A3, C3, see fig. 2 in material and methods) were tested because the current velocity measurements had been taken here and the differences were the most extreme.

At the start of the experiment, when the seagrass cores were taken from the donor population, it was not possible to differentiate between seedlings from *Zostera marina* or *Z. noltii* without digging up the transplants. Therefore the number of shoots was recorded in the beginning as the overall number of shoots per seagrass core, and could only later be seperated into the individual seagrass species. Shoot length, shoot elongation rate, number of leaves per shoot and leaf appearance rate were only obtained for all seagrass shoots present per transplant, and not separated into individual species. In general there were less *Zostera noltii* shoots present than *Z. marina* shoots, so these parameters tend to be dominated by *Z. marina*.

Current velocity measurements

Current velocities were measured in July 2005 (see table 2). The results showed that the Möwenberg tidal flat is an area of high current velocities, and high local variability. Thus it is difficult to compare the large experimental plots (= replicates) with each other, as they are governed by different current velocities.

The data of the experimental plots in the lower eulittoral zone showed a highly significant (t test $_{29,\ 0.05}$, < 0.001) increase in current velocity from western to eastern plots. The same results were obtained twice, on July 6th and 7th 2005. On July 5th, the current velocity in the upper eulittoral experimental plots was measured but there was no significant difference amongst the plots.

Table 2: Current velocities in the Möwenberg tidal flat during July 2005. The measurements were taken on the left and right hand side of the experimental plots.

	Left upper eulittoral (cm s^{-1})	Right upper eulittoral (cm s^{-1})	Left lower eulittoral (cm s^{-1})	Right lower eulittoral (cm s^{-1})	Left lower eulittoral (cm s^{-1})	Right lower eulittoral (cm s^{-1})
	5th July 2004		6th July 2004		7th July 2004	
Mean	3.3	3.6	8.7	15.2	8.1	15.1
SE	0.22	0.16	0.19	0.57	0.29	0.4

Combined number of shoots

The number of shoots were influenced greatly by current velocity, as can be seen in fig. 20. They also increased when growing on plots exposed to weak current velocity, or decreased when growing on plots affected by strong current velocities. By August 2004, most seagrass shoots remained on the lugworm exclusion plots exposed to weak current velocities compared with the other 3 groups. Only in August did lugworm exclusion have a significant effect.

Data pairs 3 - 5 of the combined seagrass data did not have homogenous variances. The data of Pair 3 were root transformed. The data of Pair 4 and 5 were analysed using a nonparametric test.

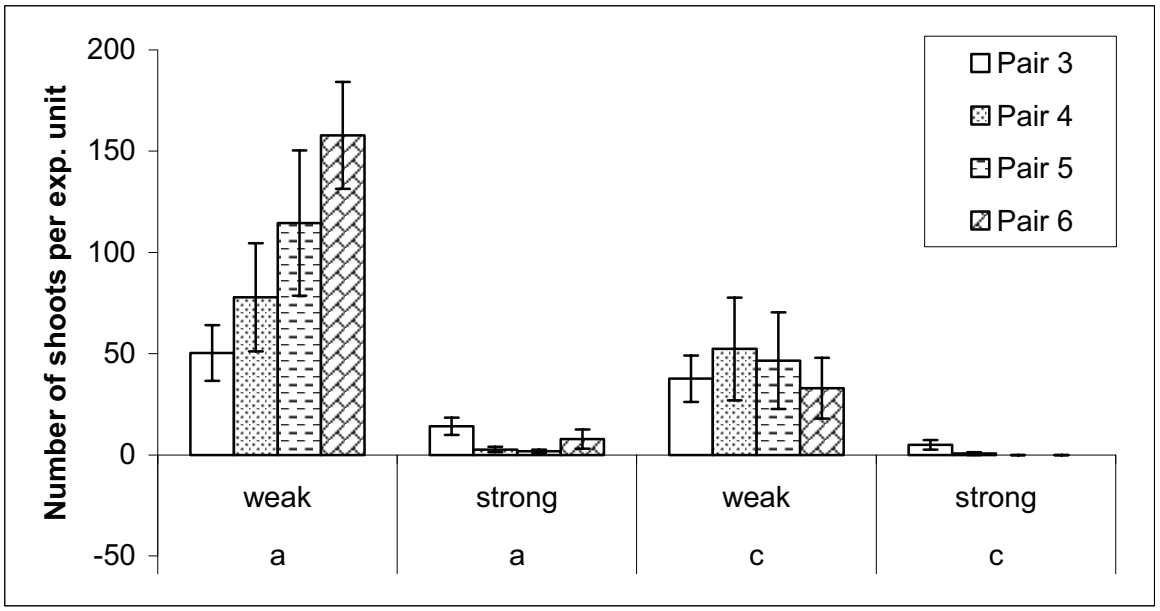

Figure 20: Results for number of shoots per experimental unit in the lugworm exclusion experiment, Pair 3 (May / early June 2004), Pair 4 (June 2004) and Pair 5 (July 2004) showing the effect of current velocity on seagrass. Pair 6 (August 2004) shows the effect of current velocity, lugworm exclusion and their interaction on seagrass. A = lugworm exclusion plots, c = control plots.

Strong current velocities led to decreased number of shoots in late May/early June 2004 (Pair 3; 2 factorial ANOVA with interaction, current velocity $F_{1, 20} = 20.4465$, $p = 0.0002$). The most shoots (50.33 shoots) were found on the lugworm exclusion plots affected by weak current velocities, on the ones affected by strong current velocities only 14.2 shoots remained.

In mid June (Pair 4), the number of shoots was also higher on the plots affected by a low current velocity (Scheirer Ray Hare Test, current velocity, $p = < 0.001$, other factors not significant). The highest value was again found on the lugworm exclusion plot exposed to low current velocities (77.83 shoots) compared to the lowest value of 0.67 shoots on the control plot exposed to strong current velocities.

By July (Pair 5), the number of shoots was still significantly higher on the lugworm exclusion plots exposed to weak current velocities with 114.5 shoots (Scheirer Ray Hare Test, current velocity, $p = 0.001$, other factors not significant). On the control plots exposed to strong current velocities no seagrass shoots remained at all.

The results in August (Pair 6) differ from the previous three pairs. Here both factors and their interaction are statistically significant (2 factorial ANOVA with interaction, current velocities: $F_{1, 20} = 35.5640$, $p = <0.0001$; lugworm exclusion: $F_{1, 20} = 18.6910$, $p = 0.0003$; interaction: $F_{1, 20} = 14.5372$, $p = 0.0011$). However, most seagrass shoots remained on the plots exposed to weak current velocity (see fig. 20 above) compared with those growing on plots exposed to stronger current velocities. Lugworm exclusion has also become important with more shoots growing on the lugworm exclusion plots than on the control plots. Again there are no shoots left on the control plots exposed to strong current velocities, but there has been a slight recovery on the lugworm exclusion plots affected by strong current velocities with 7.8 shoots. Tukey's HSD (test result not shown) showed that the results from the lugworm exclusion plots affected by weak current velocities is significantly different from the three other plots.

Number of shoots for each species separately

The number of shoots were also analysed for the two species separately. The results for *Z. marina* (see fig. 21, 23, 25) showed a marked effect of current velocity, in that more shoots were growing on the plots exposed to weak current velocities.

Lugworm exclusion had no effect and the number of shoots also increased over time.

In June 2004 (Pair 4, see fig. 21), neither lugworm exclusion nor current velocity had a statistically significant effect on the number of shoots. The most shoots were found on the lugworm exclusion plots affected by weak current velocities compared with the control plots. No shoots were left on the plots exposed to strong current velocities.

The results from July 2004 (Pair 5, see fig. 23) showed a higher number of shoots on the experimental plots exposed to weak current velocities (Scheirer Ray Hare test, $p < 0.001$) compared to the plots affected by strong current velocities (as in June, no shoots left).

In August (Pair 6, see fig. 25), the overall number of shoots increased compared to July (146.67 compared to 141 shoots). Again there was a statistically significant effect of current velocity with more shoots growing on plots exposed to weak current velocities (Scheirer Ray Hare Test, $p < 0.001$).

The results for *Z. noltii* (see fig. 22, 24, 26) differ slightly from those of *Z. marina* (see above). The overall number of shoots is lower than in *Z. marina* but increased in time and both current velocity and lugworm removal had an effect.

In June 2004 (Pair 4, see fig. 22), both current velocity and lugworm removal had a marked effect on *Z. noltii* (data was square root transformed, 2 factorial ANOVA with interaction, lugworm exclusion ($F_{1, 20} = 4.6969$, $p = 0.0425$), current velocity ($F_{1, 20} = 11.3361$, $p = 0.0031$). The seagrass grew best on the lugworm exclusion plot exposed to weak current velocity (11.83 shoots) but only 0.67 shoots remained on the control plots exposed to strong current velocities.

In July 2004 (Pair 5, see fig. 24) only current velocity influenced the number of shoots in that more shoots grew on the experimental plots exposed to weak current velocity (data was loq transformed, 2 factorial ANOVA with interaction, current velocity ($F_{1, 20} = 14.2247$, $p = 0.0012$). Lugworm exclusion no longer had any effect

on the number of shoots left. The overall number of shoots did not significantly change compared with the June data.

In August (Pair 6, see fig. 26) however the results are no longer statistically significantly different from each other. The number of shoots growing on the lugworm exclusion plot affected by weak current velocities increased to 45.67 *Zostera noltii* shoots.

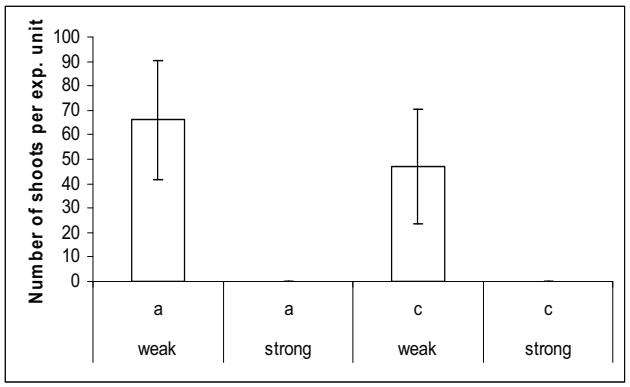

Figure 21: Results for number of shoots from *Z. marina* Set 4 (16/ 22 June 2004). A = exclusion plots, c = control plots. The results were not statistically significant.

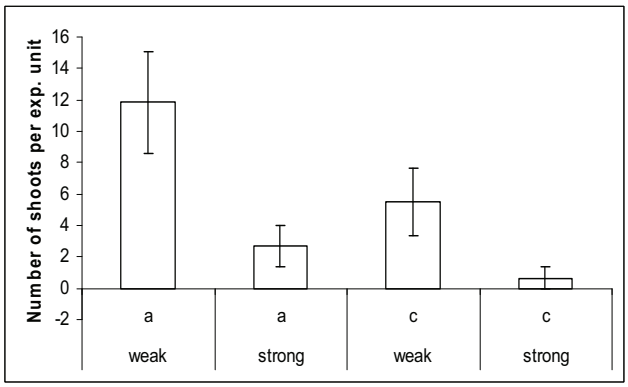

Figure 22: Results for number of shoots from *Z. noltii* Set 4 (16/22 June 2004). The factors current velocity and treatment were statistically significant.

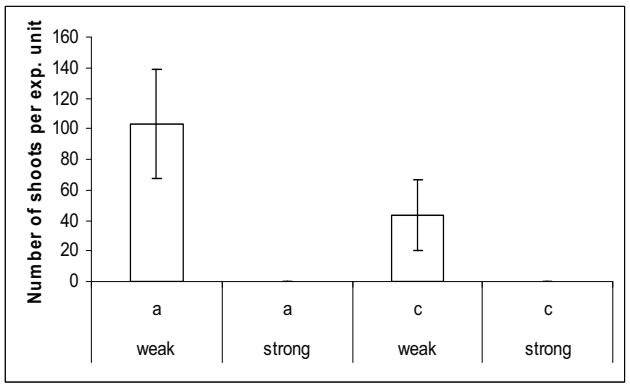

Figure 23: Results for number of shoots from *Z. marina* Set 5 (16/19 July 2004). The factor current velocity was statistically significant.

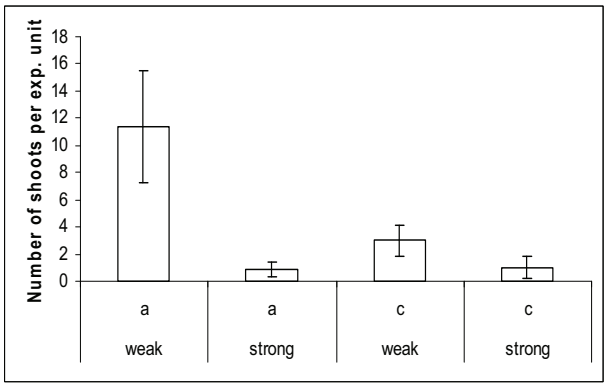

Figure 24: Results for number of shoots from *Z. noltii* Set 5 (16/19 July 2004). The factor current velocity was statistically significant.

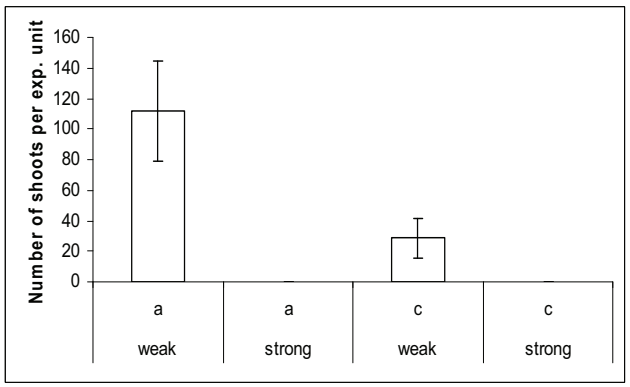

Figure 25: Results for number of shoots from *Z. marina* Set 6 (10/10 August 2004). The factor current velocity was statistically significant.

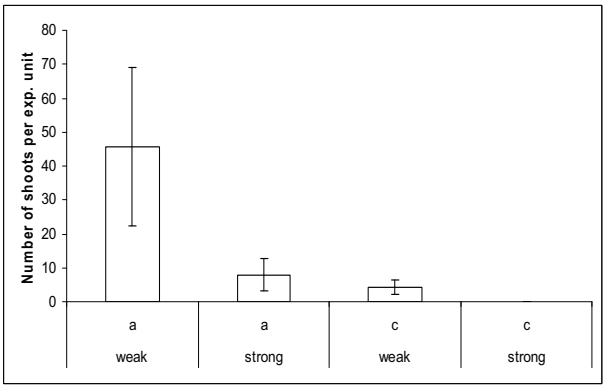

Figure 26: Results for number of shoots from *Z. noltii* Set 6 (10/10 August 2004). The results were not statistically significant.

Shoot length

The shoot length results were recorded from May 2004 onwards. The shoot length increased over time on the experimental plots affected by weak current velocities but stagnated or decreased on the others. Lugworm exclusion had no effect on shoot length.

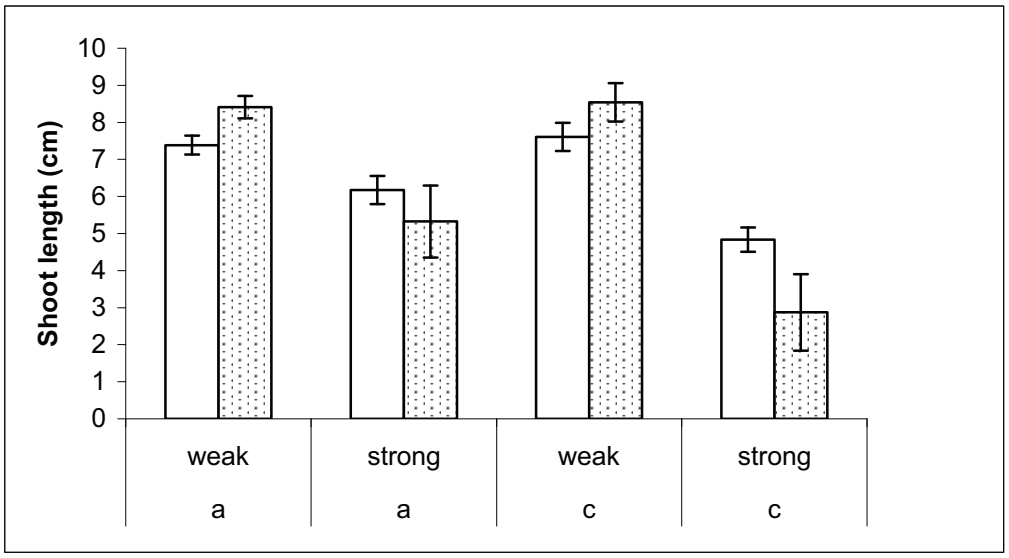

Figure 27: Shoot length results on 14th/18th May 2004 (Pair 5) and 25th May/03rd June 2004 (Pair 6). The results are significant in Pair 5 for current velocity and interaction between lugworm exclusion and current velocities, in Pair 6 only for current velocity. A = lugworm exclusion plots, c = control plots, white columns = 14th /18th May 2004, dotted columns = 25th May/03rd June 2004.

On 14th/18th May 2004 (Pair 5, see fig. 27), the seagrass shoots were longest on the control plots exposed to weak current velocities (7.61 cm). Both current velocity ad the interaction between lugworm removal and current velocity were found to be statistically significant (2 factorial ANOVA with interaction, current velocity: $F_{1, 20}$= 34.1354, p = <0.0001, interaction: $F_{1, 20}$= 5.2203, p = 0.0334).

By 25th May/03rd June (Pair 6, see fig. 27), only current velocity had an effect on the seagrass shoot length. Again shoots were longer when exposed to weak current velocities compared with those shoots growing on plots affected by stronger current velocities (2 factorial ANOVA with interaction, current velocity: $F_{1, 20}$= 32.2975, p = <0.0001).

By mid June (Pair 7, see fig. 28), the shoot length had not increased much from late May/early June. On the plots exposed to high current velocity the shoot length had decreased from 2.88 cm to 0.27 cm but the highest shoot length was still found on the experimental plots affected by low current velocities (Scheirer Ray Hare Test, p = 0.0005).

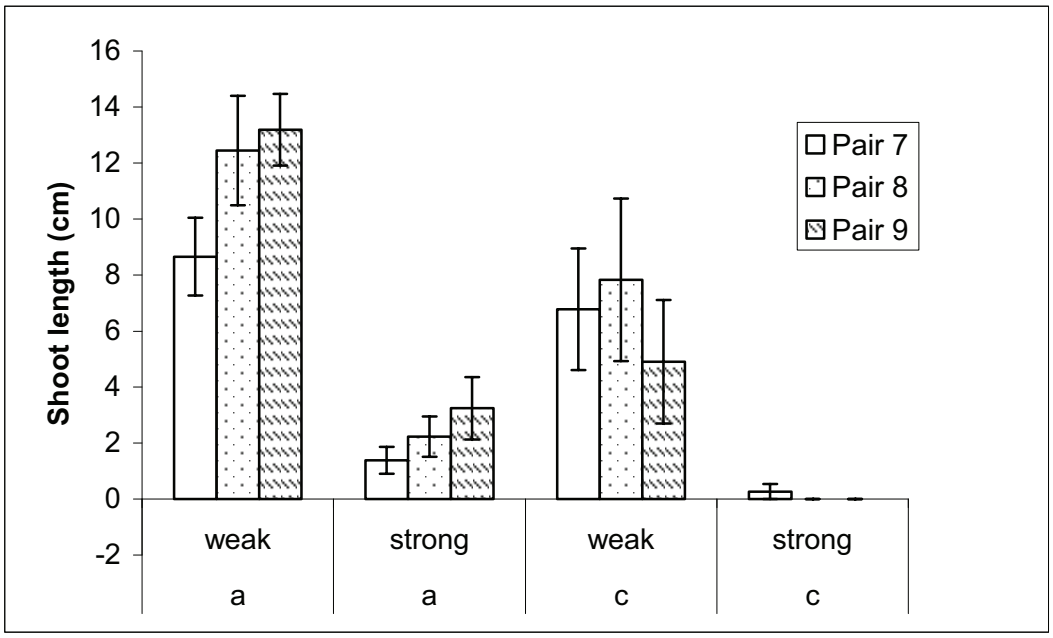

Figure 28: Shoot length results for Pair 7 (June 2004), 8 (July 2004) and 9 (August 2004). The results are significant in all three pairs for the factor current velocity. A = lugworm exclusion plots, c = control plots.

The shoot length had increased by July (Pair 8, see fig. 28) to 12.45 cm on the experimental plots exposed to weak current velocities (Scheirer Ray Hare Test, p = 0.001). No shoots were left on the control plots affected by high current velocities.

By August 2004 (Pair 9, see fig. 28) the maximum shoot length on the experimental plots exposed to low current velocities had reached 13.19 cm. Again current velocity had a statistically significant effect on shoot length with the longest shoots growing on the plots exposed to weak current velocities (Scheirer Ray Hare Test, p = 0.0001).

Shoot elongation rate (SER)

The shoot elongation rate (SER) was influenced by current velocity and lugworm exclusion. Over time the SER remained constant or decreased. The t tests of the data pairs 2 – 7 were not significant so the data were combined. Data Pair 1 could not be combined so was analysed separately.

The results from the first and the second transplantation showed a statistically significant result but for different factors (see fig. 29). Lugworm exclusion had a negative effect on the SER of the seagrass cores from the first transplantation (2 factorial ANOVA with interaction, lugworm exclusion: $F_{1, 8} = 15.2121$, $p = 0.0045$). The highest SER was found on the control plots exposed to weak current velocities.

The results for the second transplantation differ in that lugworm exclusion had no effect but strong current velocities resulted in lessened SER (2 factorial ANOVA with interaction, current velocities: $F_{1, 8} = 8.612$, $p = 0.0189$). The highest SER was found on the lugworm exclusion plots exposed to weak current velocities with 0.35 cm plant $^{-1}$ day^{-1}, which was also higher than the SER from the first transplantation with 0.19 cm plant $^{-1}$ day^{-1}.

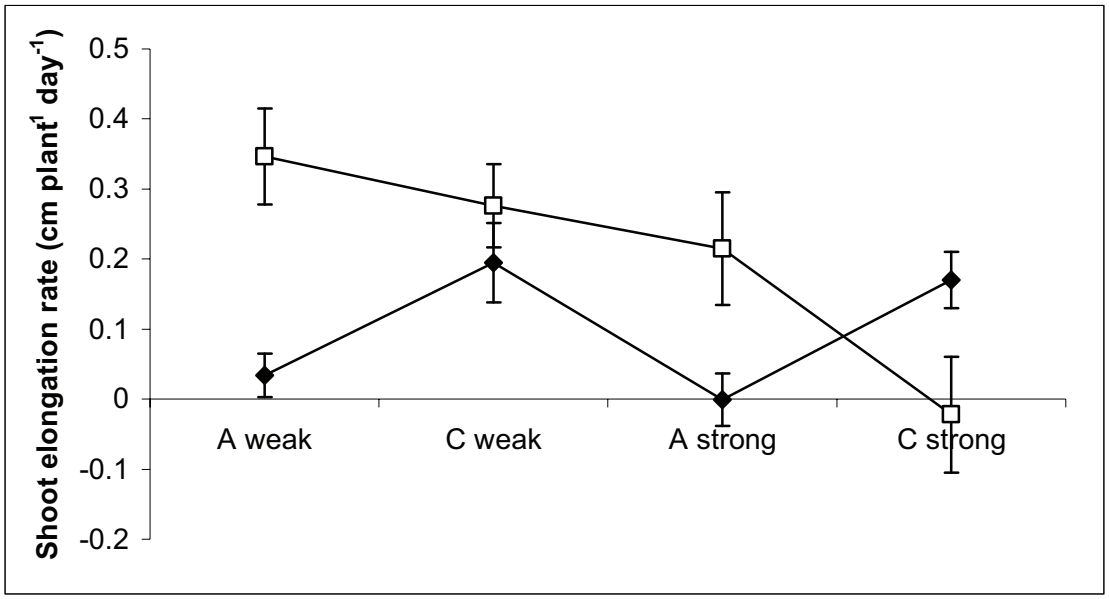

Figure 29: Results for SER on 10th/14th April 2004 (Pair 1), showing the effects of current velocities and lugworm exclusion on seagrass growth rate. Squares = first transplantation, diamonds = second transplantation. A = lugworm exclusion plots, c = control plots.

The results from 20th/ 23rd April (Pair 2) and on 16th/19th July 2004 (Pair 7) were not statistically significant (see fig. 30).

By 28th April/05th May (Pair 3, see fig. 30), lugworm exclusion had no effect on the data. Strong current velocity on the other hand resulted in lessened SER (2 factorial ANOVA with interaction, current velocity: $F_{1, 20} = 8.8802$, $p = 0.0074$). The highest SER with 0.13 cm plant $^{-1}$ day^{-1} was still lower than in April.

The results on 14th/18th May (Pair 4, see fig. 30) are similar to those during late April/ early May. Again strong current velocities resulted in reduced SER (2 factorial ANOVA with interaction, current velocity: $F_{1, 20} = 21.0578$, $p = 0.0002$).

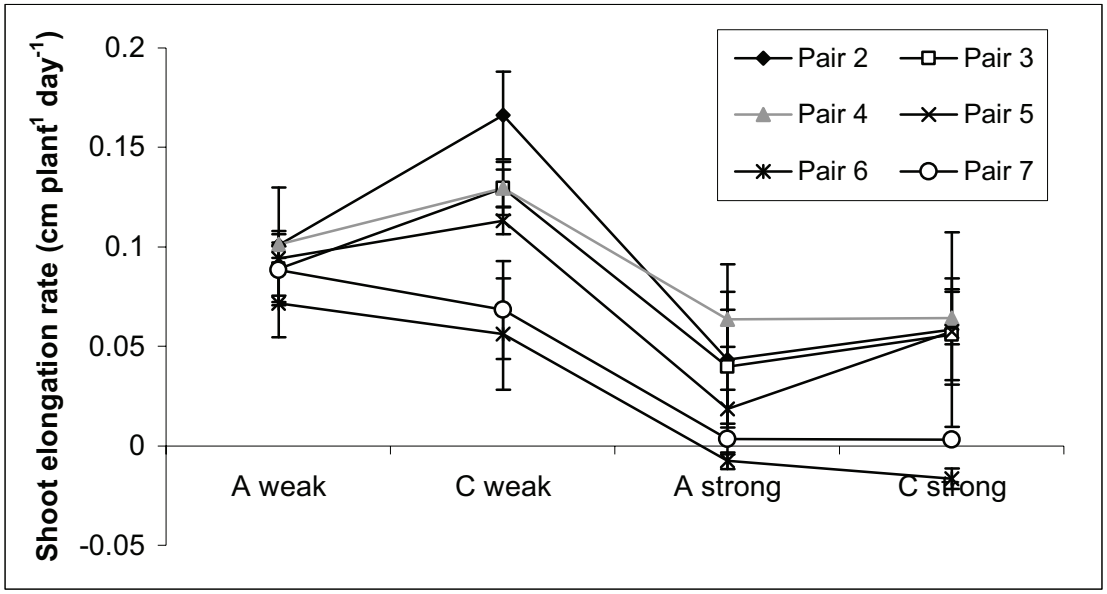

Figure 30: SER results from April – July 2004. The results for 20th/23rd April (Pair 2) and 16th/19th July (Pair 7) are not significant. The results were significant for the factor current velocity on 28th April/ 05th May (Pair 3), 14th/18th May (Pair 4), and 16th/22nd June (Pair 6), and on 25th May/03rd June 2004 (Pair 5) for current velocity and treatment. A = lugworm exclusion plots, c = control plots.

The maximum rate (from the same experimental plot) was the same in early and mid May but the lowest SER was slightly higher in mid May.

By late May/early June (Pair 5, see fig. 30), the SER had decreased overall and both current velocity and lugworm removal had an effect. Lugworm exclusion had a negative effect on the SER and strong current velocities resulted again in decreased SER (2 factorial ANOVA with interaction, current velocity: $F_{1, 20} = 29.0917$, $p = <0.0001$; lugworm exclusion: $F_{1, 20} = 5.8338$, $p = 0.0266$).

In mid June (Pair 6, see fig. 30), the SER was still further reduced compared with the earlier results. The values from the plots exposed to weak current velocities are still higher than those from strong current velocities, which even became negative (Scheirer Ray Hare Test, $p = <0.0001$). The maximum rate (with 0.07 cm plant $^{-1}$ day^{-1}) is much lower than the previous ones.

Above and below ground biomass of Zostera marina *and Z.* noltii

The above and below ground biomass of *Z. marina* was influenced by current velocity (and also the interaction between current velocity and lugworm removal), but the biomass of *Z. noltii* was influenced by lugworm exclusion only. As expected, *Z. marina* had more above and below ground biomass than *Z. noltii* if it was still able to remain at the site.

As can be seen clearly in fig. 31 below, current velocity had a strong influence on seagrass biomass as only on the plots affected by low current velocities any *Z. marina* was present and none was left on the plots exposed to strong current velocities (Scheirer Ray Hare test, above ground biomass p = 0.038, interaction above biomass x current velocity p = 0.007; below ground biomass p = 0.038, interaction below biomass x current velocity p = 0.015). The above and below ground biomass of *Z. marina* was of a similar height apart from the lugworm exclusion plot exposed to weak current velocities where the above ground biomass was much higher. The highest below ground biomass was also found there.

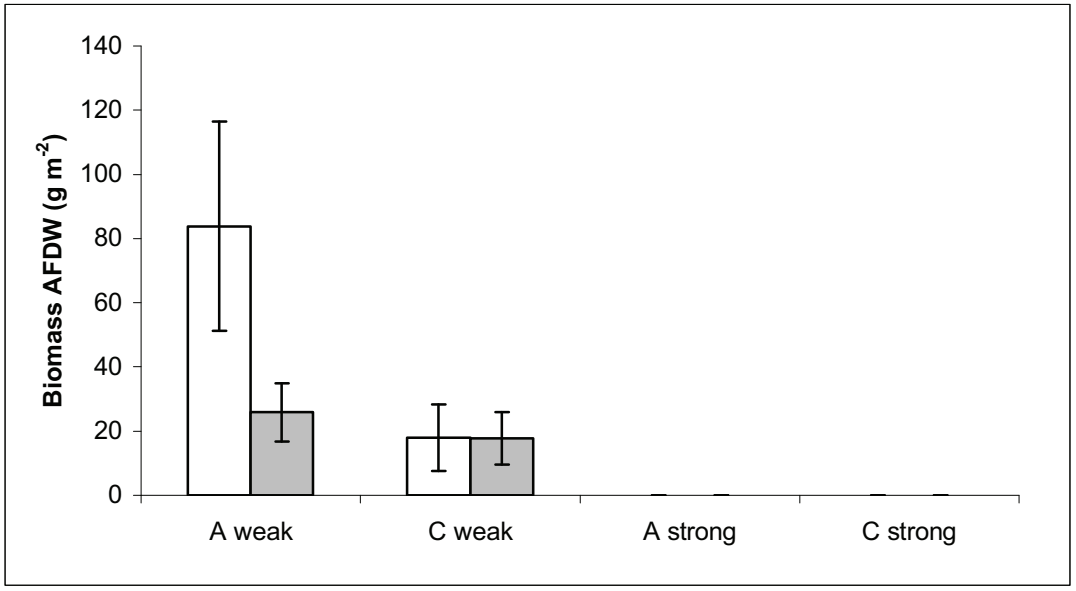

Figure 31: Results for above ground and below ground biomass of *Zostera marina* after 4 months. The results were significant for the factors current velocity and the interaction between treatment and current velocity. White columns = AG biomass, grey columns = BG biomass of *Z. marina*. A = lugworm exclusion plots, c = control plots.

The above and below ground biomass of *Zostera noltii* (see fig. 32) was influenced by lugworm exclusion and not by current velocity. Even though the biomass was much lower than the biomass of *Z. marina*, some *Z. noltii* remained on the lugworm

exclusion plots affected by strong current velocity where no *Z. marina* remained. The most above and below ground biomass was found on the lugworm exclusion plots compared with the control plots (Scheirer Ray Hare test, lugworm exclusion above ground p = 0.013, lugworm exclusion below ground p = 0.0056). The above and below ground biomass was again of a similar height (slightly higher in the above ground biomass) apart from lugworm exclusion plot exposed to high current velocity where the below ground biomass was much higher than the above ground.

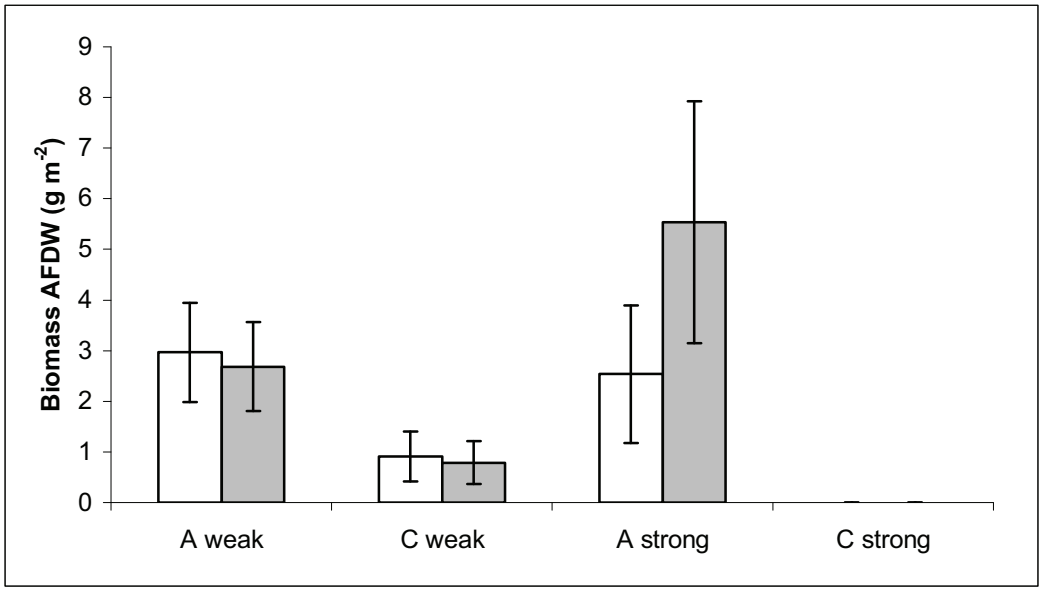

Figure 32: Results for the above ground (AG) and below ground (BG) biomass of *Zostera noltii* after 4 months. The results were significant for the factor treatment. White columns = AG biomass, grey columns = BG biomass of *Z. noltii*. A = lugworm exclusion, C = control plot.

Number of leaves per shoot

The t test results for this parameter were not significant so all data pairs were combined. The number of leaves per seagrass shoot (not split up into seagrass species) increased in time on the lugworm exclusion plot exposed to weak current velocities but decreased or plateaued on the other plots. Current velocity, lugworm removal and the interaction between the two all influenced the number of leaves per seagrass shoot (see fig. 33).

In late May/early June, low current velocity resulted in a higher number of leaves shoot^{-1} (4.08 on the lugworm exclusion plot) compared with the other plots (Scheirer Ray Hare test, current velocity p = 0.009).

By mid June, the number of leaves shoot^{-1} was still greatly affected by current velocity, but also by the interaction between current velocity and lugworm exclusion

(2 factorial ANOVA with interaction, current velocity: $F_{1, 20} = 27.9283$, $p = <0.0001$; interaction: $F_{1, 20} = 4.4443$, $p = 0.0478$). Overall the number of leaves has slightly increased to 4.22 leaves shoot^{-1}, but has also drastically decreased from 1.75 to now 0.20 leaves shoot^{-}1 on the lugworm exclusion plots exposed to strong current velocity.

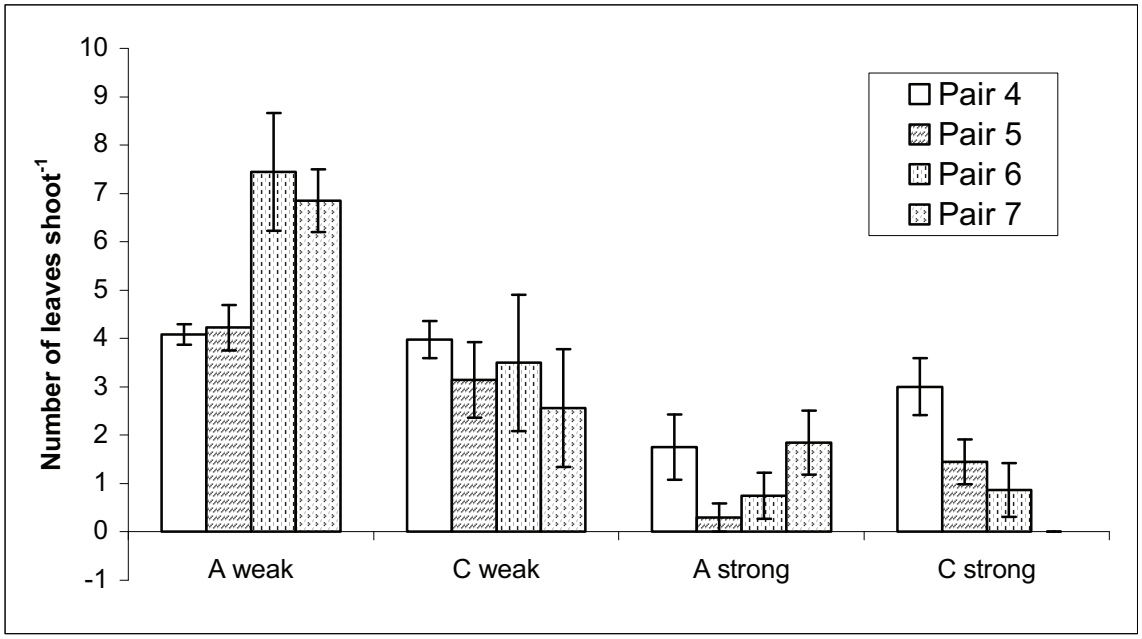

Figure 33: Results for the number of leaves per shoot. The results were statistically significant for the factor current velocity on 25th May/03rd June 2004 (Pair 4) and 16th/19th July 2004 (Pair 6), on 16th/22nd June 2004 (Pair 5) for current velocity and interaction between treatment and current velocity, and on 10th/10th August 2004 (Pair 7) for current velocity and treatment. A = lugworm exclusion, C = control plot.

In mid July (Pair 6) the most leaves per seagrass shoot were found again on the lugworm exclusion plot exposed to weak current velocities (7.44 leaves shoot^{-1}). Only 0.75 leaves remained on the lugworm exclusion plot affected by high current velocities. Strong current velocities resulted in a decrease of number of leaves, whereas the number of leaves increased on the plots exposed to low current velocity (2 factorial ANOVA with interaction, Current velocity: $F_{1, 20} = 21.7477$, $P = 0.0001$) compared with the previous month.

By August 2004 (Pair 7), the number of leaves shoot^{-}1 decreased slightly on the plots exposed to weak current velocity but increased on the lugworm removal plot exposed to strong current velocity. Both current velocity and lugworm exclusion had a significant effect this month, i.e. strong current velocity resulted in a lower number of leaves and lugworm exclusion resulted in higher number of leaves (Scheirer Ray Hare test, current velocity $p = 0.01$; lugworm exclusion $p = 0.02$).

Leaf appearance rate (LAR)

The t test results for the leaf appearance rate were not significant on 28th April/ 5th May (Pair 1), 14th/18th May (Pair 2) and on 16th/17th July (Pair 5) so the data were combined. The results of Pair 3 and 4 had to be analysed in two separate sets as the data sets from the first and second transplantation could not be combined.

The results of the leaf appearance rate on 28th April/ 5th May (Pair 1), 14th/18th May (Pair 2) and on 16th/17th July (Pair 5) were not statistically different from each other. The rates ranged from a maximum of 0.07 to a minimum of 0.01 new leaves day^{-1}. The results from the 16th June (Pair 4 first transplantation) and the 22nd June 2004 (Pair 4 second transplantation) range from 0.05 new leaves day^{-1} and no plant left and again do not differ significantly from each other (data not shown).

The results from the 25th May (Pair 3 first transplantation, see fig. 34) were also not different from each other. However, the highest leaf appearance rate (0.08 new leaves day^{-1}) from all the data was measured than on the control plot exposed to strong current velocities.

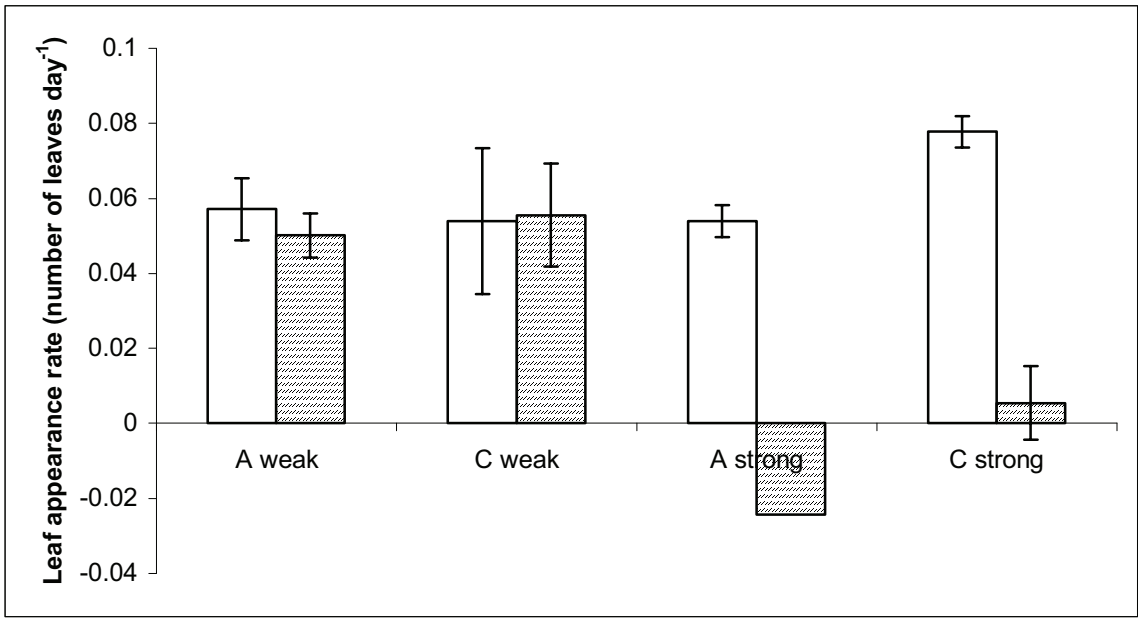

Figure 34: Leaf appearance rate results on 25th May and 3rd June 2004 (Pair 3 first and second transplantation). The results were statistically significant only in Pair 3 second transplantation. 25th May 2004 (Pair 3 first transplantation) = white columns, 3rd June 2004 (Pair 3 second transplantation) = striped columns. A = lugworm exclusion, C = control plot.

Only on 3rd June 2004 (see fig. 34) do the results differ significantly from each other. Strong current velocity lead to decreased or negative leaf growth, but on the

plots affected by weak current velocity the leaf appearance rate was not affected by lugworm exclusion at all (2 factorial ANOVA with interaction, current velocity: $F_{1, 6}$ = 24.3218, p = 0.0026).

Summary table

The following table summarizes all results from this part of the experiment. Not shown in this summary table are the results for the combined above and below ground biomass because the results were not statistically significant.

Table 3: All results from the Möwenberg lugworm exclusion experiment (part 2). Sg = Seagrass, + = positive, - = negative, Z. mar = *Zostera marina*, Z. nol = *Zostera noltii*

Parameters	**Current velocities**		***Arenicola marina***	
	Weak	***Strong***	***Excluded***	***Control***
Combined no. of shoots	Sg +	Sg -	No effect	No effect
No. of shoots sep. spec	Z. mar + Z. nol +	Z. mar – Z. nol -	No effect Z. nol +	No effect Z. nol -
Shoot length	Sg +	Sg-	No effect	No effect
Shoot elongation rate (SER)	Sg +	Sg -	No effect	No effect
Sep. AG and BG biomass	Z. mar + No effect	Z. mar – No effect	No effect Z. nol +	No effect Z. nol -
No. of leaves per shoot	Sg +	Sg -	No effect	No effect
Leaf appearance rate (LAR)	Sg +	Sg -	No effect	No effect

2.4 Discussion

2.4.1 The development of the Puant Klent donor population

The biomass development of the donor population in Puant Klent increased from April to October as expected. It was not differentiated between the two seagrass species present. The below ground biomass was higher than the above ground biomass early in the year (April/May) when the meadow was still recovering from e.g. grazing by water fowl and harsh winter conditions. By August, the above ground biomass was higher than the below ground biomass. There is a slight decrease in biomass from August to October in above and below ground biomass, but not markedly so.

The maximum above ground biomass is higher in this mixed meadow than it is in a sheltered monospecific meadow, e.g. *Zostera noltii* meadow with 117.42 g ash free dry weight m^{-2} (Schanz, Polte & Asmus 2002). The monospecific *Zostera marina* meadow in the Gröning tidal flat (as described in material and methods and also in chapter 4) however had a higher maximum biomass than the mixed Puant Klent meadow, but both a lesser shoot density and below ground biomass. It can be assumed that the above ground biomass is mostly made up of *Z. marina* shoots in Puant Klent, but shoot density and below ground biomass mostly consist of *Z. noltii* shoots. In this study (experiments 1 and 3) it was generally found that *Zostera marina* had a higher above ground biomass than *Z. noltii*, even though it had a lower shoot density. As the *Z. marina* shoots grew longer and wider, it is not surprising that *Z. marina* has more biomass.

As with biomass, the shoot density also increased over time. From 24th March till 5th April only seedlings were found, after 15th April it was no longer distinguished between annual or perennial plants. Shoot density reached a peak at late August with 10,880 shoots m^{-2}. Afterwards the shoot density decreased again. The maximum shoot density is well above the mean plant density for e.g. a sheltered *Zostera noltii* bed with 8,817 individuals m^{-2}, as reported by Schanz, Polte & Asmus (2002). The shoot density in a monospecific *Z. marina* meadow (Gröning tidal flat, < than 1,000 shoots m^{-2} at maximum density) is much lower.

Overall the Puant Klent meadow was densely grown with a medium high biomass compared to monospecific meadows. The shoot length increased in time but the *Z. marina* shoots did not grow as long as the shoots from the monospecific Gröning meadow (maximum length in July 25.37 cm, n = 30) but were more numerous. The combined shoot elongation rate of annual and perennial plants showed a marked increase in *Z. marina* growth, followed by a decrease and then a stable growth rate of 0.1 cm plant^{-1} day^{-1}. The growth rate of *Z. noltii* in the same meadow was lower and ranged between 0.04 and 0.08 cm plant^{-1} day^{-1}.

2.4.2 Seagrass seedling development in Puant Klent

Seedling biomass greatly increased from April to May, actually being higher than the above or below ground biomass of the perennial plants.

In early April, when the transplants were cut out, only the hypocotyls and in some cases the cotyledons of *Z. marina* were visible. The seedlings of *Z. noltii* emerged later at the surface and the first hypocotyls and cotyledons of *Z. noltii* seedlings were visible in late April. Seedlings from *Z. marina* were longer at first, but by June the shoot length of the two species was nearly the same (9 – 10 cm). The shoot elongation rate of the *Z. marina* seedlings showed a marked increase until May 2004, and then a rapid decrease. Whilst the length of the *Z. noltii* seedling also rapidly increased and nearly matched the overall length of the *Z. marina* seedlings by late June 2004 (see fig. 5), the seedling elongation rate was higher than that of the *Z. marina* seedlings (data not shown, maximum elongation rate 0.18 cm plant^{-1} day^{-1} on 27th April 2004).

Several different triggers of germination in *Zostera* seeds have been described by different authors so far, such as water temperature, oxygen availability and salinity (Hootsmans, Vermaat & van Vierssen 1987; Loques, Caye & Meinesz 1990; Moore, Orth & Nowak 1993, Probert & Brenchley 1999). According to Hootsmans, Vermaat & van Vierssen (1987), both *Zostera* species showed an optimum seed germination at 30°C and 1 psu salinity *ex situ*. In general they reported an increased germination at low salinities for both species, but also a strong interaction between salinity and temperature. Interestingly they also report that the requirements for seedling survival are different to those of germination, and also differ between the two *Zostera* species. According to them, *Z. marina* seedlings survived best at 10°C

and 10 – 20 psu salinity. *Z. noltii* seedlings survived best also at 10°C but at 1 psu salinity.

In comparison, Loques, Caye & Meinesz (1990) found that the germination success of *Z. noltii* was much higher at low salinities (1 – 10 psu) than at high (20 – 38 psu) where no germination occurred. They conducted their experiments at 13, 18 and 25 °C and found that temperature had no effect on *Z. noltii* germination. It should be noted that these temperatures are below the optimum germination temperature reported by Hootsmans, Vermaat & van Vierssen (1987). They also found that incising the seed coat and then lowering the salinity resulted in the highest germination rates and that the germlings survived better if the salinity was lowered (10 psu). However, the ambient salinity of the Mediterranean Sea is 38 psu and the experiments were conducted in fluid media without sediment, thus not reflecting natural conditions.

Neither the optimum seed germination requirements nor seedling survival requirements as described by Hootsmans, Vermaat & van Vierssen (1987) or Loques, Caye & Meinesz (1990) are normally experienced at the time of year on Sylt when germination and seedling sprouting (i.e. the first sign of seedlings at the sediment surface) happened (mid March/early April for *Z. marina* and late April (3-4 weeks later) in *Z. noltii*). Seed germination in both *Zostera* species did occur, and seedlings also survived at ambient conditions. Probert & Brenchley (1999), working in a *Zostera marina* meadow in The Fleet, UK, report that most *Z. marina* seeds germinated between October and March, but do not report any sightings of seedlings or their fate. In most northern European *Z. marina* populations, seed germination occurs between October and March (Harrison 1993; Probert & Brenchley 1999; this study) with the emergence of seedlings at the sediment surface beginning in March.

Harrison (1993) also mentioned that *Z. marina* seedlings were most abundant in March, but could not find any germinated *Z. noltii* seeds or seedlings at any time in Zandkreek, The Netherlands. The only other *Z. noltii* seedlings reported in scientific literature found by this author seems to be Goubin & Loquès (1991) on Corsica, France. From the two *Zostera noltii* stands studied in this PhD study, only in one

(Puant Klent, Hörnum Deep) were any *Z. noltii* seedlings found abundantly. In the monospecific *Z. noltii* meadow in the Sylt-Rømø Bay only very few seedlings were found at all, even though flowering and seed production occurred. It is not clear which process governs the occurrence of *Z. noltii* seedlings, as the two meadows mentioned above are situated in similar locations and would experience similar water temperatures and rain fall (which could reduce the salinity).

Moore, Orth & Nowak (1993) reported that oxygen availability can also be a factor in *Zostera marina* seed germination in that low oxygen concentrations in combination with low temperature initiate germination. As salinity remained relatively high in their experiments (at ambient levels, ranging between 17 to 24 psu) and was much higher than those reported by others to stimulate seed germination, they conclude that salinity has a minor effect on the germination of American subtidal *Z. marina* populations. These findings were further supported by Probert & Brenchley (1999) working with intertidal *Z. marina* in the United Kingdom.

Alexandre *et al.* (2006) suggest in their study that the faster reproductive process of *Z. marina* (as observed in other studies, e.g. De Cock 1980; Hootsmans, Vermaat & van Vierssen 1987) may be due to the faster flowering and fruiting processes and may also be related to the annual life cycle of *Z. marina*. However, the results reported in this study clearly show that the coexisting *Z. noltii* population produces viable seeds which germinate and grow into seedlings and have a faster growth rate than the *Z. marina* seedlings. As they lag behind the *Z. marina* seedlings 3 – 4 weeks, this could be an essential disadvantage in their survival struggle as essential resources (e.g. nutrients, light, space) will already be exploited by perennial *Z. noltii* plants, *Z. noltii* ramets (from rhizome particles), and *Z. marina* seedlings.

The germination of *Z. marina* has been linked to triggers such as water temperature and oxygen availability in the sediment. Conversely, the germination of *Z. noltii* seeds has been shown to be related only to reduced salinity (Loques, Caye & Meinesz 1990). Probert and Brenchley (1999) conclude in their paper that the main factor influencing germination of *Zostera marina* seeds *in situ* is water temperature. They show that *Zostera marina* seeds are well adapted to germinate under

anaerobic conditions, and do so at lower water temperatures (2° - 6°C) compared to germinating under aerobic conditions.

These different triggers of germination could be responsible for the delayed emergence of *Z. noltii* seedlings in comparison with *Z. marina* seedlings found in this study, and could also be the reason why *Z. noltii* populations are mostly linked to vegetative reproduction – when occurring in mixed seagrass beds, the seedlings are simply outcompeted. As both species are capable of reproducing either sexually or vegetatively, which form of reproduction is adapted or dominant in a population depends on specific circumstances (see also Jacobs 1982). However, as Orth *et al.* (2000) mention in their review, there is a need for 'ecologically meaningful' experiments and it is simply not known if e.g. anaerobic conditions will also promote germination of *Z. noltii* seeds at lower temperature. It is however plausible that different environmental germination triggers or thresholds exist for the two seagrass species, as well as differing between seeds of the same species.

2.4.3 The effect of tidal height and lugworm exclusion on two species of seagrass

Both seagrass species, *Zostera marina* and *Zostera noltii*, occur around the North Sea island Sylt. They either occur in single species meadows with only occasional patches of the other seagrass species, or in mixed communities. One of the factors thought to govern the occurrence and survival of seagrasses is bioturbation, i.e. the reworking or displacement of the surrounding sediment by e.g. polychaete worms (Philippart 1994; Short & Wyllie - Echeverria 1996; Hughes *et al.* 2000). Therefore the removal of one of the key species of the German Wadden Sea, the lugworm *Arenicola marina*, should have a positive effect on seagrass transplants.

The results highlight that only some seagrass parameters (shoot length, shoot elongation rate, combined biomass of *Z. noltii*) are affected by the lugworm, but nearly all are affected either by tidal height or current velocities. The findings from Philippart (1994) could not be transferred onto eelgrass, as position on shore and current velocity were the two major factors influencing *Zostera marina*. It should be noted though that the lugworm densities on the control plots (and the adjacent tidal flat) were decreasing in the 3 year course of the experiment and by the third year had reached 5 – 15 individuals m^{-2} (Volkenborn 2005). This decrease in lugworm

population (normal densities would be roughly 30 individuals m^{-2}, see Beukema 1976) could be a possible reason why the effect of lugworm bioturbation was not more apparent in the results from this study. Nonetheless the effects of complete lugworm exclusion were still apparent, e.g. changed sediment characteristics (see Volkenborn 2005).

The combined number of shoots (which were not counted for each individual species until the plants were large enough to differentiate between them) for both species was not affected by the lugworm. However, they were affected by tidal height. There was a higher combined number of shoots in the upper eulittoral than in the lower eulittoral plots. The results were only significant for one of the data pairs (Pair 5, mid July) and not statistically significant before or after July.

When looking at the results for each individual species, again neither is affected by *Arenicola marina* but by position on shore. *Zostera noltii* is affected by tidal height in that more shoots grew in the plots situated in the upper eulittoral than in the lower eulittoral. These plots were close to a naturally occurring *Z. noltii* meadow so one can infer that the environmental conditions were favourable for *Z. noltii* plants. However, the *Z. marina* plants also grew more numerous in the upper eulittoral than in the lower eulittoral so even though only very few individual *Z. marina* plants occurred naturally in the area, the environmental conditions were also favourable for them. As the upper eulittoral clearly provides a suitable habitat for both species, other factors than position on the shore and lugworm activities limit the natural occurrence here.

The naturally occurring *Z. noltii* meadow reproduces mostly vegetatively, and not via seeds. Whilst the plants had flowers and produced seeds (personal observation), hardly any seedlings could be found in the meadow at any time. It is not known if the produced seeds were not viable, but it seems more likely that either germination did not occur or the seedling mortality was so high that none were found at the sediment surface. This would leave only shoots and rhizome fragments as a method of dispersal, and it has be shown in another seagrass species to be highly unlikely (Ewanchuk & Williams 1996). The Möwenberg monospecific *Z. noltii* meadow can expand via vegetative growth and even though

the conditions in the upper intertidal plots and the surrounding tidal flat would be suitable for seagrass habitation, it could only disperse there if the seagrass meadow expands this far.

Shoot length (again it was not differentiated between species) on the other hand was not affected by tidal height, but instead was influenced by presence or absence of *Arenicola marina*. When the lugworm was present, the shoot length was reduced. On average, the shoots were between 6 to 8 mm longer when the plants were growing on the lugworm exclusion plot. This effect was only noticeable in the early stages of the experiment (early to mid April); later on there was no difference in shoot length.

Hüttel (1990) found that whilst the presence of *Arenicola marina* increased the nitrate concentrations at the same time the ammonia and silicate concentrations were reduced. Volkenborn (2005) showed in his study that nutrient levels (ammonium, silicate and phosphate) increased on lugworm exclusion plots and that organic matter also increased on these plots. Volkenborn took his samples in April 2004, so at the same time as the shoot length of the seagrass transplants was increased on the lugworm exclusion plots. The findings of Hüttel (1990) and Volkenborn (2005) indicate that on the lugworm exclusion plots there should be a decreased nitrate concentration compared with the control plots, and an increased concentration of silicate, phosphate and ammonium. This increased amount of nutrients is still noticeable in July 2004 (Volkenborn 2005) and could be the reason for the increased shoot length early in the year.

Most seagrass nutrient studies focus on nitrogen and phosphorus (Hemminga & Duarte 2000) and it has also been shown that sediment fertilization resulted in increased seagrass growth. As seagrasses have been shown to utilise both ammonium and nitrate as a source of nitrogen (Hemminga & Duarte 2000), the decreased nitrate concentrations in the porewater of the lugworm exclusion plots should not have been detrimental to the seagrass seedlings, as nitrate is mostly taken up via the leaves (Touchette & Burkholder 2000). Ammonium uptake rates are also higher than nitrate uptake rates (Hemminga *et al.* 1994), indicating that ammonium is the most important source of inorganic nitrogen.

The observed beneficial effect of heightened nutrient availability in the sediment did not result in a permanently higher biomass of *Zostera marina* on the lugworm exclusion plots. This could be due to the fact that at later development stages the seagrass seedlings began to absorb nutrients through their leaves as well as their root/rhizome system and thus the heightened nutrient levels in the sediment were no longer the main source of nutrients for the seedlings. Hemminga *et al.* (1994) showed that in an annual population of *Z. marina* the leaves accounted for 70% of plant nitrogen uptake throughout the growing season. It seems likely that the seagrass seedlings in this study also mainly used their leaves for nitrogen uptake, and that the seedlings growing on the lugworm exclusion plots with their heightened nutrient availability could not benefit later than around mid April 2004 from them. This would also explain why in the beginning of the experiment shoots were longer on the lugworm exclusion plots, but no long term effect was found in any of the seagrass parameters and at the end of the experiment the *Zostera marina* biomass showed no differences.

Not surprisingly the shoot elongation rate (SER) was also only affected by the absence or presence of *A. marina*, as it is calculated from the shoot length. However, the SER was influenced exactly the other way round. The SER was highest on the control plots (where *A. marina* was present) and lesser by 1.7 mm $plant^{-1}$ day^{-1} on the lugworm exclusion plots. It is not known why the SER would be higher when *Arenicola marina* was present. It should be noted though that only the data from the first transplantation was affected by lugworm exclusion, the second batch of transplants which had not been exposed to the easterly storm were not influenced by either lugworm exclusion or tidal height. Furthermore, this positive effect of the lugworm presence was only transient (see results) and did not last longer than 20th April 2004.

The above and below ground biomass was combined for each species, and the eelgrass *Zostera marina* was influenced by tidal height and the dwarf seagrass *Z. noltii* by lugworm exclusion.

Z. marina grew better in the upper eulittoral (the eelgrass grew roughly four times as much as in the lower eulittoral at the maximum difference). The sediment in the lower eulittoral plots consisted of smaller particles and was muddier, and more nutrients were present in the porewater of the lower intertidal plots. Even thought at first glance the environmental conditions should have been more suitable for *Zostera marina* in the lower intertidal plots, the transplants did better in the upper intertidal. The lower intertidal plots were exposed to higher current velocity than the upper intertidal plots where current velocities only reached 3.3 – 3.6 cm s^{-1} compared with 8.1 – 15.2 cm s^{-1} in the lower intertidal. Furthermore, due to the reduced water depth at high tide in the upper intertidal (roughly 48 cm difference), more light was also available for the eelgrass in the upper intertidal plots. Koch & Beer (1996) state that the light requirement for growth of *Zostera marina* (Long Island Sound population) is 300 µmol quanta m^{-2} s^{-1} which includes the growth of the below ground tissue (which is supported by leave photosynthesis). The irradiance at both tidal heights should have been more than enough to support growth (see Methods and Materials section), but as the measurements were taken on a sunny day, light might have been limiting during overcast days.

The dwarf seagrass grew better on the lugworm exclusion plots (roughly 21 times more seagrass biomass at the maximum difference was found on the exclusion plots) than on the control plots. This effect is particularly obvious in the lower eulittoral but can still be observed (to a lesser extent) in the upper intertidal. It was also growing better on the control plots in the upper eulittoral than in the lower eulittoral.

The results from the lower intertidal therefore supports the findings from Philippart (1994) that lugworms had a negative effect on *Zostera noltii*. The effect of the lugworm seems to be amplified by the harsher tidal regime in the lower intertidal and the increased water depth (and thus less available light) as it is far less pronounced in the upper intertidal.

Hughes *et al.* (2000) found in south-east England that the presence of another polychaete worm, *Nereis diversicolor,* had a negative effect on *Zostera noltii*. They concluded that *N. diversicolor* can affect seagrass by damaging and eating the

leaves, and also by damaging the roots/rhizomes with their burrowing activities. Furthermore, they report that *N. diversicolor* has also been observed eating *Z. noltii* seeds and has been reported to consume *Salicornia* spp. seeds and seedlings (Paramor and Hughes 2004). It is unlikely that seed burial by bioturbation would have a negative effect on seeds of either *Zostera* species as they seem to be well acclimatised to anoxic sediment conditions. However, the only trigger of germination reported in scientific literature for *Z. noltii* is reduced salinity, and it is possible that deeply buried *Z. noltii* seeds have reduced germination success because the salinity of the porewater is higher than that at the surface. Thus the bioturbation of *Arenicola marina* may have an affect on germination success of *Z. noltii* seeds, as well as affecting seedling survival rate by burying them.

If the above and below ground biomass are looked at individually, then neither the above nor the below ground biomass of *Z. marina* is affected by tidal height or lugworm exclusion. The reason for this is the immense standard variation in the data. It was caused by the current velocity which affected the replicates in the lower eulittoral. Where the current velocity was weak, the eelgrass biomass was high, but on the experimental plots exposed to strong current velocities the eelgrass disappeared completely. The combination of these replicates in turn led to the high standard deviation. Despite this, the trend in the data (data not shown) suggests a negative effect of *A. marina* on the above ground biomass of *Z. marina*, both in the lower and upper intertidal plots. The below ground biomass is much lower than the above ground biomass, and not affected by the lugworm presence.

Zostera noltii, on the other hand, was also affected by the strong current velocities. However, here the shoot density was generally much lower than in *Z. marina*, but opposite to the eelgrass the dwarf seagrass did not completely disappear from the plots that were exposed to strong current velocities. The below ground biomass was not statistically significantly affected by either tidal height or lugworm exclusion, but the above ground biomass was affected by tidal height. The biomass was higher in the upper eulittoral than in the lower eulittoral, which is due to more favourable conditions in the upper eulittoral (e.g. decreased current velocities in the upper eulittoral).

Despite the other seagrass parameters showing clear effects of either tidal height or lugworm exclusion, neither the number of leaves nor the calculated leaf appearance rate (LAR) showed any effect. This is surprising because with increased shoot length and biomass one would have expected that the number of leaves per shoot would also increase and thus serve as an indicator of the well being of the plant. However, till mid May (data pair 4), the number of leaves was not affected by either tidal height or lugworm exclusion. From May onwards, the data was only analysed for the effect of lugworm exclusion and current velocities because of the high standard deviation in the data.

In summary it can be said that position on the shore had a greater influence on either seagrass species than presence or absence of lugworm. Only shoot length, shoot elongation rate and combined above and below ground biomass showed any effect of lugworm exclusion. On the whole *Zostera marina* seems to be less affected by lugworm presence and more by tidal height, whereas *Zostera noltii* seems to be affected both by lugworm exclusion and position on the shore. The seagrass seedlings growing in the nutrient-richer sediment were unable to maintain their advantage, as the effect was not permanent.

2.4.4 The effect of current velocities and lugworm exclusion on two species of seagrass

Strong water dynamics can have a negative effect on seagrass, e. g. by uprooting shallow seagrass rhizomes and roots (Kirkman & Kuo 1990) or reducing leaf number per shoot, shoot and leaf length (Schanz 2003). It also decreases the shoot density (van Katwijk & Hermus 2000; Schanz & Asmus 2003). Moderate water flow can have a positive effect by reducing the thickness of the diffusive boundary layer and thus allowing increased nutrient uptake and a heightened rate of photosynthesis (Fonseca & Kenworthy 1987).

The combined number of shoots (both seagrass species) was not affected by the lugworm *Arenicola marina* but instead by current velocities. From May until July, there were more seagrass shoots growing on the plots subjected to weak current velocities than on the ones exposed to stronger current velocities. Consistently the most shoots were growing on the lugworm exclusion plots compared to the control

plots, but the difference was only significant in August 2004. It is possible that the burrowing activities of the lugworm had a negative influence on the seagrass transplants, but it seems more likely the cause of an indirect effect of the lugworm (i.e. more nutrients than on the control plots). The growth maxima of both seagrass species were reached around this time (August to September), and more nutrients would be needed to support this growth. As the lugworm exclusion plots accumulated more organic material and nutrients, the plants were able to utilise these extra resources in low current regimes.

In August, there were less shoots (71.1 shoots) in the upper eulittoral plots than in the lower eulittoral plots (112.7 shoots). Thus it appears that the environmental conditions in the lower eulittoral were more advantageous for *Z. marina* as long as the current velocities were low. This could be because of the higher nutrient concentration in the porewater of the lower intertidal plots. Furthermore, the tidal emersion times were also shorter in the lower intertidal plots and thus any environmental stress (e.g. dehydration, sun burn etc) would be higher in the upper eulittoral plots. Another explanation could be the reduced wave action at larger water depth as mentioned by van Katwijk & Hermus (2000) as a cause for seagrass loss. The difference in water depth between lower and upper intertidal is roughly 50 cm, but the plots at both tidal heights fall dry during low tide. As such the plots at both tidal heights are exposed to the incoming tide and the water dynamics connected to it, and it seems unlikely that the water dynamics would be higher in the upper eulittoral plots when the current velocities are actually reduced in the upper intertidal (see results). Wind-induced water movement could indeed be larger in the upper eulittoral but has unfortunately not been measured in this study.

The effect of current velocities and lugworm exclusion were looked at for each species separately. *Zostera marina* was only affected by current velocities, and not by lugworm exclusion. *Z. noltii* on the other hand was affected both by current velocity and *Arenicola marina*.

From June till August, more *Z. marina* shoots were found on the plots exposed to weak current velocities, with the maximum in August with 112.7 shoots. These results were expected for *Z. marina*, which has been shown in previous studies to

be negatively influenced by strong water dynamics (e.g. Fonseca & Bell 1998). Van Katwijk & Hermus (2000) report that *Z. marina* transplants were unable to establish at depth below -0.20 m MSL because of severe water dynamics at greater depths. Reduced water movement by enclosures also prevented the loss of transplants. Fonseca & Kenworthy (1987) report more seedlings at locations with low current velocities compared with locations subjected to high current velocities.

Zostera noltii on the other hand was affected by both lugworm exclusion and current velocities. Most *Z. noltii* shoots were found on the lugworm exclusion plots exposed to weak current velocities, and the maximum number was reached in August with 45.7 shoots. Overall, *Z. noltii* had more shoots on the lugworm exclusion plots than on the control plots, so benefited from lugworm removal. Contrasting these results with the ones from the upper eulittoral, one sees that there is hardly a difference in the maximum number of shoots (42.9 shoots in August on the control plot). It would appear that *Z. noltii* was not affected by lugworm presence in the upper eulittoral where the current velocities were relatively weak (see results). However, when exposed to stronger current velocities, it was advantageous for the plants to grow on the lugworm exclusion plots. This could be due to the added bioturbation activities of the lugworm or by the indirect effect of accumulated nutrients on the lugworm exclusion plots.

Comparing the results of the two seagrass species, it is clear that *Z. marina* had the higher number of shoots overall and was not affected by lugworm removal. *Z. marina* did benefit from low current velocities and grew better in the lower intertidal plots as long as current velocity was low. On the other hand, *Z. noltii* shoots were still present on plots exposed to strong current velocities on which *Z. marina* shoots had disappeared, even though only in very low numbers. Subtidal *Z.marina* beds have been estimated to tolerate a maximum of 120 – 150 cm s^{-1} (Fonseca *et al.* 1983). As has been shown in this study, much lower current velocities (15 cm s^{-1}) already lead to reduced biomass and number of shoots in intertidal *Z. marina* transplants. Schanz & Asmus (2003) reported that *Z. noltii* had a reduced shoot density and changed shoot morphology (e. g. shortened leaves) at current velocities as low as 8 cm s^{-1}. However, Schanz & Asmus also report that *Z. noltii* can tolerate current velocities up to 33 cm s^{-1}. It should be noted, however, that

these current velocities relate to established seagrass meadows whereas the findings from this study concern seagrass transplants that would, due to small patch size, offer less protection against strong water dynamics.

In general, however, *Zostera noltii* had less shoots than *Z. marina*. This could also be connected to the later appearance of the *Z. noltii* seedlings (roughly 4 weeks later than *Z. marina*) as an earlier appearance would also lead to a competitive advantage (see above).

In conclusion, it seems that *Zostera noltii* has a higher tolerance for increased current velocities and can grow both in the upper and lower eulittoral, albeit in lower numbers. Furthermore, only *Z. noltii* is affected by the presence of absence of the lugworm. *Z. marina* on the other hand is both affected by tidal height and current velocities. Whilst the conditions in the lower eulittoral led to increased shoot numbers of *Z. marina* compared with the upper eulittoral, this was only true if *Z. marina* was not also affected by strong current velocities at the same time.

The shoot length of the two seagrasses in this setup was not affected by *Arenicola marina*, but instead by current velocities. The shoots grew consistently longer on the experimental plots exposed to weak current velocities (shoot length maximum was reached in July with 12.45 cm). The shoot elongation rate was also higher on the plots subjected to low current velocities compared with the plots exposed to high current velocities. These results confirm the findings from other studies which show that increased current velocities will lead to reduced shoot length, for example Schanz & Asmus (2003).

Interestingly the SER data from mid April could not be combined, which could be due to the adverse weather conditions that made the second transplantation of seagrass cores necessary. The seagrass cores from the first transplantation had been affected by a strong easterly wind and an extreme low tide that lasted for several days. The SER from these seagrass seedlings is affected by lugworm removal in that the SER was higher on the control plots (0.19 cm plant $^{-1}$ day^{-1}) but not by current velocity. This would indicate a beneficial effect of lugworm presence, which has not been found in any of the other seagrass parameters measured here.

It is not clear why the presence of the lugworm would lead to an increased SER, but it could be speculated that these plots did not dry out as much as the others did.

The SER of the second transplants however is affected by current velocity and not by lugworm exclusion. The seedlings had a higher SER when growing on plots exposed to low current velocities (0.35 cm plant $^{-1}$ day^{-1}). As the SER is higher in the seedlings from the second transplantation which were not affected by the above mentioned extreme low tide, it can be assumed that the different results between the first and second sets of transplants is due to the adverse weather conditions. However, this effect only lasted for a short time and after ten days no difference could be found in the seedlings from the first and second transplantation.

When looking at the results, it becomes apparent that the SER was again higher on the control plots than the lugworm exclusion plots (on 10th April 2004). It is not clear why the presence of the lugworm should lead to an increased growth rate, as more nutrients can be found on the lugworm exclusion plots. Perhaps some feature of the lugworm burrow helped to retain water and thus prevented dehydration and abrasion of the seagrass seedlings. The only other time that the SER was positively affected by the presence of the lugworm is in late May/early June (see results). Again it is not clear what the cause is, but by mid June the SER is higher on the lugworm exclusion plots than on the control plots. It is not clear what has led to the reversal and fluctuations of the growth rates of the seagrass transplants, which is otherwise mostly and consistently affected by current velocity.

The above and below ground biomass of *Zostera marina* was affected by current velocity and not by lugworm exclusion. On the plots exposed to weak current velocities, the eelgrass grew better than on the plots subjected to strong current velocities. These results are consistent with the results for the number of shoots as discussed above. The above ground biomass is also roughly three times higher at the maximum values than the below ground biomass. Kautsky (1987), Coops, Boeter & Smith (1991) and Fonseca & Bell (1998) report an increased below ground biomass for marine plants in exposed versus sheltered sites. These findings are not supported by the results from this study, where increased current velocity has a negative effect on below and above ground biomass of *Zostera marina*.

The above and below ground biomass of *Zostera noltii* was affected by lugworm removal but not by current velocity, in direct contrast to the results for *Z. marina*. The dwarf seagrass grew better on the lugworm removal plots than on the control plots. Interestingly, at the maximum values the below ground biomass is nearly twice as high as the above ground biomass, which is also the opposite of the case with *Zostera marina*. Unexpectedly the highest below ground biomass was found on the lugworm exclusion plot exposed to high current velocity. What stimulated the growth of the below ground biomass on these plots is unknown, but it could be a response to the strong current velocities. Schanz & Asmus (2003), also working in the Königshafen, report a decreased above and below ground biomass of *Z. noltii* when comparing exposed and sheltered sites. However, an increased below ground biomass has been found in *Z. marina* by Fonseca & Bell (1998). In contrast, Kirchner (2005) reports a higher below ground biomass in two of her three studied seagrass meadows (also in the Sylt-Rømø Bight). Both of those were sheltered sites, and the below ground biomass in the exposed site was lower than the above ground biomass. The difference may lay in the fact that Fonseca & Bell (1998) studied a sublittoral meadow, whereas Schanz & Asmus (2003) and Kirchner (2005) studied intertidal seagrass meadows. The most likely explanation for the findings from this study (i.e. increased below ground biomass at plots subjected to high tidal current velocity) is that whilst the seagrass shoots had been separated from the rhizomes and washed away, the rhizomes and roots remained in the deeper layers of the sediment and thus lead to the increased biomass.

The number of leaves per seagrass shoot were affected by lugworm exclusion and also by current velocity. Current velocity had a more consistent and predictable influence through time, as shoots exposed to high current velocities had fewer leaves than shoots growing on plots subjected to low current velocities. The effect of the lugworm was less pronounced and also changed over time, but in general the shoots on the control plots had fewer leaves than those on the lugworm exclusion plots. Lugworm exclusion had a beneficial effect later in the year (August) whereas it did not play a role earlier in the growing season (May). The best growth conditions were again found on the plots subjected to low current velocities and without lugworms.

The leaf appearance rate (LAR) is calculated from the number of leaves. The results are mostly not significantly different from each other, apart from the data recorded in early June. The results from the first and second transplantation could not be combined here, and only the results from the second transplantation are statistically significant. More leaves per seagrass shoot are produced on the plots exposed to weak current velocities compared with the ones influenced by strong current velocities. This is the expected result as the increased water movements would stress the plants and thus result in fewer leaves produced.

2.5 Conclusion

Summarising the findings of the Möwenberg lugworm exclusion experiment, it becomes clear that current velocity and position on shore (i.e. tidal height) play a major role in growth and survival of seagrass transplants. Unfortunately, the effect of tidal height cannot be separated from the influence of tidal current velocity as the current velocity was reduced higher up the shore. When current velocity was low, then the conditions in the lower intertidal were more suitable for both seagrass species.

The lugworm *Arenicola marina* did influence the seagrass growth, and in the most part negatively, but had less influence than current velocity. It also became clear that the lugworm had a negative influence on *Zostera noltii*, in particular when *Z. noltii* was also subjected to high hydrodynamics, but did not influence *Zostera marina* to the same extent or at all.

In contrast to expectations Z. *noltii* turned out to be the more robust of the two seagrass species, as it managed to withstand the negative effects of high current velocity for longer. *Zostera marina* was suited to grow both in the upper and lower intertidal, and also on fine and medium sand, as long as current velocities were low. Even though reports *of Z. noltii* seedlings are sparse in the literature, the *Z. noltii* seeds from the Puant Klent meadow did germinate but emerged 3 – 4 weeks later than the *Z. marina* seedlings at the sediment surface. At least in this populations, both species reproduce sexually as well as vegetatively.

3. Chapter 3: Carbon and nitrogen content of Zostera noltii

3.1 Introduction

Zostera noltii occurs from the upper intertidal zone down to the low water line around the German North Sea island Sylt and is often found growing on sandy sediment. Not much data has been published about the carbon and nitrogen content of *Z. noltii* populations in the North Sea but some data is available on Mediterranean or Portuguese populations (e.g. Pirc & Wollenweber 1988; Cabaço & Santos 2007), and it is not known if disturbance by bioturbation would have an effect on the carbon or nitrogen balance. Cabaço & Santos (2007) show that burial or erosion can have a profound effect on *Z. noltii* and can lead to significant changes in carbon and nitrogen content of leaves and rhizomes. As lugworm activity can potentially cover the dwarf seagrass plants, the lugworm could have a direct influence on *Z. noltii* and also the carbon and nitrogen content in leaves and roots/rhizomes. Furthermore the dwarf seagrass could benefit from the absence of the lugworm and the accumulation of nutrients in the sediment and porewater which could in turn be expressed in the carbon and nitrogen content.

Schanz & Asmus (2003) have shown what a profound effect hydrodynamics can have on the morphology and development of *Z. noltii*. They did not, however, look at the effect it might have on the carbon and nitrogen balance of the plants. Water movement decreases the diffusive boundary layer (Fonseca & Kenworthy 1987) which allows enhanced photosynthesis, but stronger water movement stresses the plants and destabilises the sediment. It is therefore possible that plants that are subjected to strong water movements have a different carbon and nitrogen balance than those growing in reduced hydrodynamics.

The aims of this experiment were to find out which influence bioturbation, tidal height and tidal current velocity have on the carbon and nitrogen content of the dwarf seagrass *Zostera noltii*.

3.2 Material and Methods

Adult *Zostera noltii* plants were transplanted on the Möwenberg lugworm exclusion plots investigating the effects of tidal height and lugworm exclusion on the carbon and nitrogen content of seagrass plants and sediment (July until September 2004).

3.2.1 Study sites

The experiments were conducted in the Königshafen bay which lies inside the Sylt-Rømø Bight. For a detailed description of the bight, the Möwenberg *Arenicola marina* exclusion plots and the Möwenberg donor population please see chapter 2.

3.2.2 Experimental set up

On the 26th July 2004, 36 *Zostera noltii* seagrass cores were transplanted from the middle of the Möwenberg seagrass meadow onto the adjacent lugworm exclusion plots (see fig 2 and also description of lugworm exclusion plots in chapter 2). The cores were cut out using a plastic tube (diameter 11.8 cm, height approx. 20-25 cm) and care was taken to include all seagrass material and sediment. Three seagrass cores were transplanted onto each experimental plot. The transplants were harvested 8 weeks later (in September 2004) and the samples analysed. Porewater (n = 3) and grain size (n = 6) samples were taken in late July and early September. For a detailed description of seagrass parameters and current velocity measurements please see chapter 2. A description of how the seagrass and sediment samples for the carbon and nitrogen analysis were treated and the settings of the C/N analyser can be found in chapter 4.

Porewater

72 samples were taken with corers (diameter 3.6 cm) on the experimental plots (3 samples per plot) on 23 and 24 July 2004 and on 3 and 4 September 2004. Only the upper 8 cm of the sample were analysed. On the Möwenberg exclusion plots, the gauze hindered the sampling so cores of varying depths had to be taken. If more than 8 cm were in the corer, than only the upper 8 cm were used for analysis.

Porewater was extracted from the cores on the same day as they had been collected. The cores were stored in a temperature control room at 6°C before extraction. Analysed nutrients were ammonium (NH_4^+), phosphate (PO_4) and silicate (Si_xO_y). Porewater was blown out of the sediment with nitrogen gas at 2 bar

pressure and filtered (pore size 0.45 µm), then frozen before analysis (ammonium and phosphate) or stored in a refrigerator (salinity and silicon sub sample). The phosphate and ammonium sample was analysed with an auto analyser (BRAN+LUEBBE AA3) in deionised water (Hedtkamp 2005). Porewater salinity was measured using a portable refractometer (Reichert Scientific Instruments). The concentration of the dissolved silicon was measured photometrically after dilution of the porewater (Grasshoff *et al.* 1983).

Grain size

Sediment samples were taken using a small corer (diameter 2.5 cm) in Puant Klent on 28th July 2004, in the Möwenberg tidal flat (centre and edge) on 26th July 2004, in Gröning on 26th July 2004; experimental plots were sampled on 16th July 2004. Sub samples were taken from each sample because only a small sample size is needed for laser diffraction.

The sub samples were treated with 10% H_2O_2 for 24 hours to remove organic material, then rinsed twice with deionised water. To remove shell particles, samples were treated with 10% acetic acid for 24 hours, and then rinsed twice with deionised water.

Samples from Gröning and Möwenberg tidal flat were not treated as above, but instead were freeze dried and sieved (mesh size 2 mm). Shell particles such as mud snail shells were removed by hand. Samples were re suspended in water and analysed.

All samples were analysed with the QUANTACHROME CILAS 1180 and the software package ‘The Particle Expert’. The grain size distributions were calculated using the software programme GRADISTAT. The parameters median, phi (Φ), sorting coefficient QD, percentage (%) sand and mud (fine fraction, particle size smaller than 63 µm) were used in the analysis.

Table 4: Wentworth classification of particle grades and phi scale

	Sand				Silt		
	Coarse	Medium	Fine	Very fine	Very coarse	Coarse	Medium
Particle size range (µm)	1000 - 500	500 - 250	250 - 125	125 - 63	63 - 31	31 - 16	16 - 8
Phi units	0-1	1-2	2-3	3-4	4-5	5-6	6-7
	Silt (cont.)				Clay		
					Fine	Very fine	
Particle size range (µm)					8 - 4	4 - 2	<2
Phi units					7-8	8-9	9

All data were analysed using the JMP software package.

3.3 Results

Adult *Zostera noltii* cores were transplanted from the Möwenberg *Z. noltii* meadow onto the adjacent lugworm exclusion plots. The data was analysed for the effect of lugworm exclusion, tidal height and current velocity on several seagrass and sediment parameters.

3.3.1 Seagrass development in the Möwenberg meadow (donor population)

The data from the Möwenberg meadow were recorded from May until July at the centre and at the edge of the meadow, and in August and October only in the middle of the meadow. Seagrass transplants from this meadow were used in experiments 2 and 3. It is dominated by *Z. noltii* with only single *Z. marina* plants (see further description in chapter 2).

Biomass

The biomass increases over time both above and below ground (see fig. 35), but the above ground biomass is generally lower than the below ground until July, after which these positions are reversed. The highest biomass was found in the centre of the seagrass meadow (below ground in July with 101.53 g m^{-2}, above ground biomass in October with 93.42 g m^{-2}). The data were log transformed and results differ significantly between months both for the above and below ground biomass (Oneway ANOVA, above ground: $F_{4, 29} = 90.4441$, $p = <0.0001$, below ground: $F_{4, 29} = 94.2821$, $p = <0.0001$).

The above ground biomass in the centre and at the edge of the seagrass meadow increased in time but there was no significant difference between them (Repeated measurements ANOVA, Time $F_{2, 9} = 222.0265$, $p = <0.0001$). However, the below ground biomass differed between centre and edge and over time (Repeated measurements ANOVA, time $F_{2, 9} = 94.9808$, $p = <0.0001$; location $F_{1, 10} = 9.9770$, $p = 0.0102$).

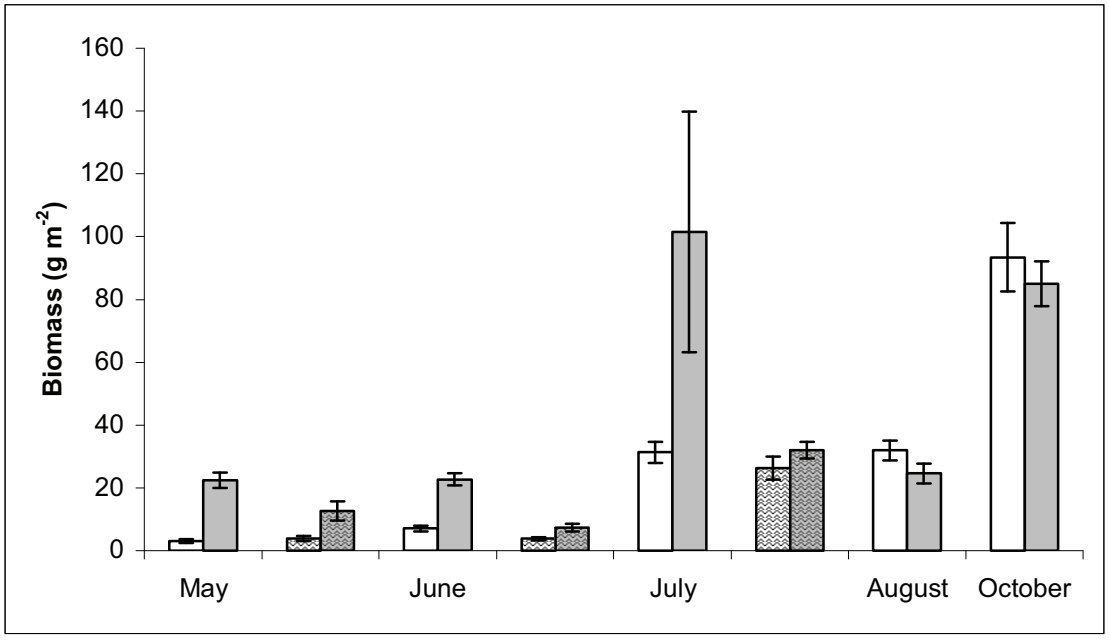

Figure 35: Above and below ground biomass results from Möwenberg. The results differ significantly in time. White columns = above ground biomass, grey columns = below ground biomass, wavy columns = edge biomass.

Shoot density

The shoot density in the Möwenberg meadow (see fig. 36) increased as well as the biomass over time (Oneway ANOVA, $F_{5, 102} = 11.1158$, $p = <0.0001$). There was a marked difference between the edge and centre of the meadow in April and June, but not in May or July (Oneway ANOVA, April: $F_{1, 46} = 5.2089$, $p = 0.0271$; June: $F_{1, 46} = 19.9755$, $p = <0.0001$). Overall, the highest shoot density was found in October with 7,690.67 shoots m^{-2} at the centre of the meadow. Tukey's HSD test showed that the shoot density was similar from July to October was similar , and dissimilar from April to June.

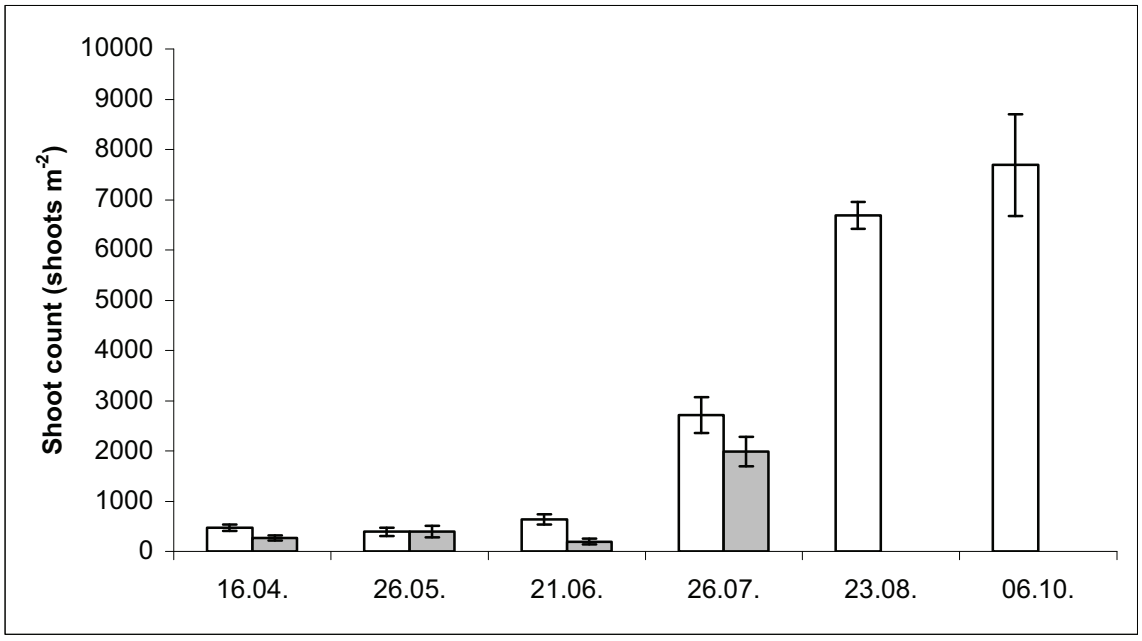

Figure 36: Shoot density in the Möwenberg meadow. White columns = meadow centre, grey columns = meadow edge.

Shoot length

As with biomass and shoot density, the shoot length also increased with time significantly (see fig. 37, Oneway ANOVA, $F_{5, 174} = 39.8103$, $p = <0.0001$). Tukey's HSD showed that there were four significantly different groups: April ≠ May, July, August ≠ June, July, August ≠ October.

Comparing the edge and the centre of the meadow over time showed that the shoots in the centre were longer but also increased over time. The shoots at the edge also increased over time but were shorter with 8.6 cm at their longest (Repeated measurements ANOVA, location: $F_{1, 58} = 6.4419$, $p = 0.0139$; time: $F_{3, 56} = 30.5269$, $p = <0.0001$). The shoots in the centre were longest in October with 14.94 cm.

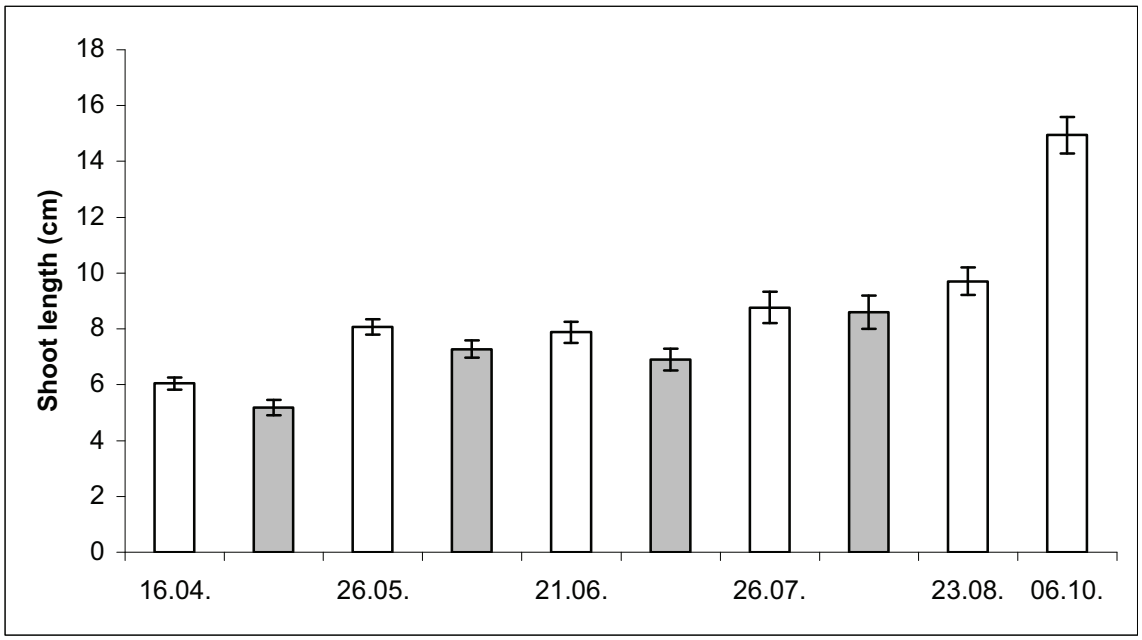

Figure 37: Shoot length in the Möwenberg meadow. White columns = meadow centre, grey columns = meadow edge

Shoot elongation rate

The shoot elongation rate (SER) decreases over time from May to July (see fig. 38), but then increases again in the Möwenberg meadow. The SER differs significantly in time (Wilcoxon test, p = 0.0019). The highest growth rate at the centre was measured in October with 0.051 cm plant^{-1} day^{-1}. There is no difference between the SER of the centre and the edge from May until July. The highest growth rate at the centre was measured in May with 0.05 cm plant^{-1} day^{-1}, at the edge also in May with 0.052 cm plant^{-1}day^{-1}.

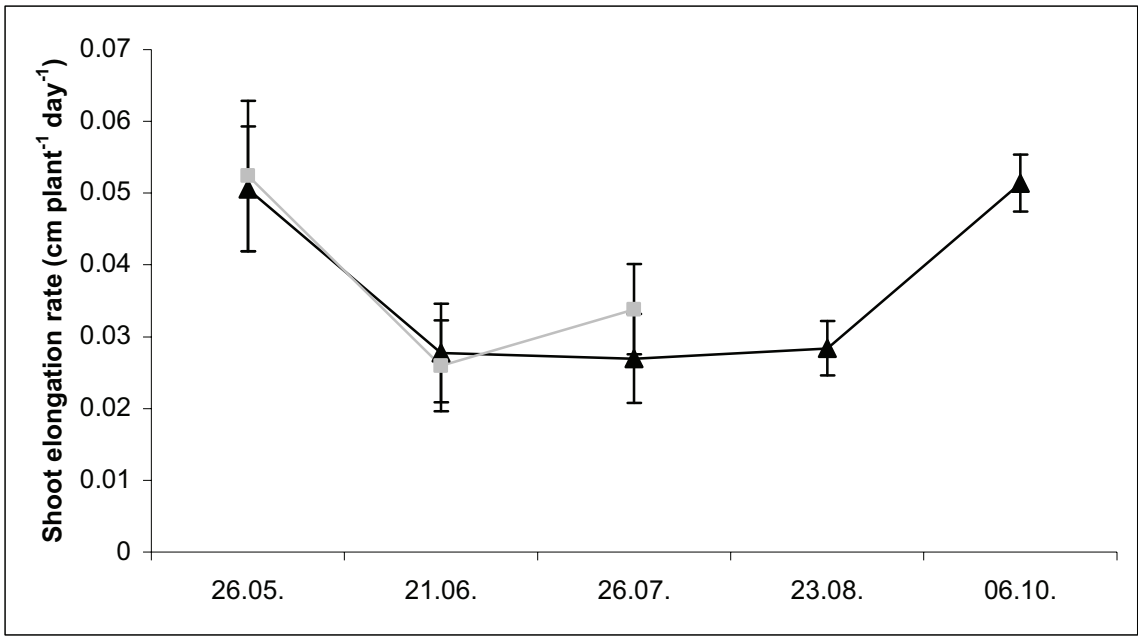

Figure 38: Shoot elongation rate in the Möwenberg meadow. Black triangles = meadow centre, grey squares = meadow edge

3.3.2 The development of adult *Zostera noltii* plants on lugworm exclusion plots

Seagrass cores were taken from the donor population and transplanted onto the *Arenicola marina* exclusion plots. A number of seagrass parameters were recorded as well as the nitrogen and carbon content of the seagrass plants after 8 weeks.

Number of shoots per experimental unit and ash-free dry weight (AFDW) affected by tidal height

The numbers of shoots per experimental unit were recorded as well as the ash-free dry weight (AFDW). It was expected to see a high AFDW if the number of shoots was also high, which was the case here. [1]

The number of shoots per experimental unit (= seagrass transplant) was influenced by tidal height (see fig. 39). More seagrass shoots were counted on the plots growing in the upper eulittoral than in the lower eulittoral (2 factorial ANOVA with nested block and interaction, tidal height $F_{1, 11} = 24.1640$; $p = 0.0012$, other factors not significant).

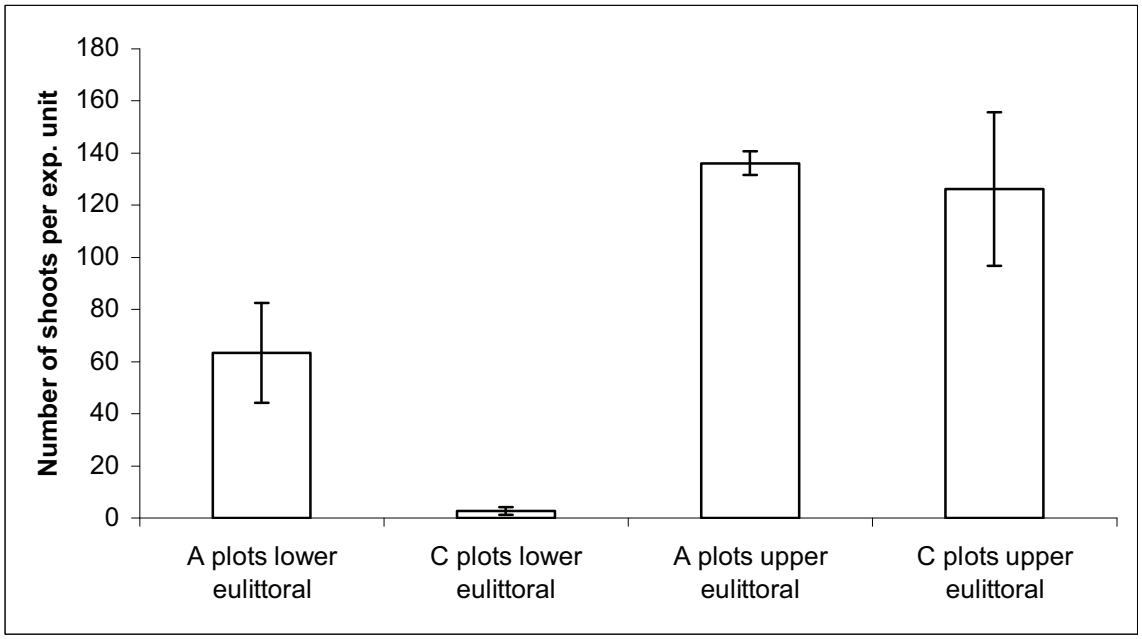

Figure 39: Results for number of shoots per experimental unit in the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effect of tidal height and lugworm exclusion. The results are significant for the factor tidal height. A = lugworm exclusion plots, c = control plots.

The ash free dry weight (see fig. 40 below) on the other hand was influenced both by tidal height and the interaction between tidal height and lugworm exclusion (2 factorial ANOVA with nested block design, tidal height: $F_{1, 8} = 41.6949$; $p = 0.0002$; lugworm exclusion x tidal height: $F_{1, 8} = 5.6233$; $p = 0.0452$). The biomass was

[1] Note: I used this parameter because the data is not extrapolated for area as one would do to calculate biomass per area. Some of my biomass samples were very small, but if you use the small area (several millimetres) in the calculation and than extrapolate you get a large biomass even if the sample was actually small.

higher from the seagrass transplants growing in the upper intertidal. However, in the lower intertidal the lugworm exclusion plots yielded the higher biomass but in the upper intertidal the biomass was slightly higher on the control plot.

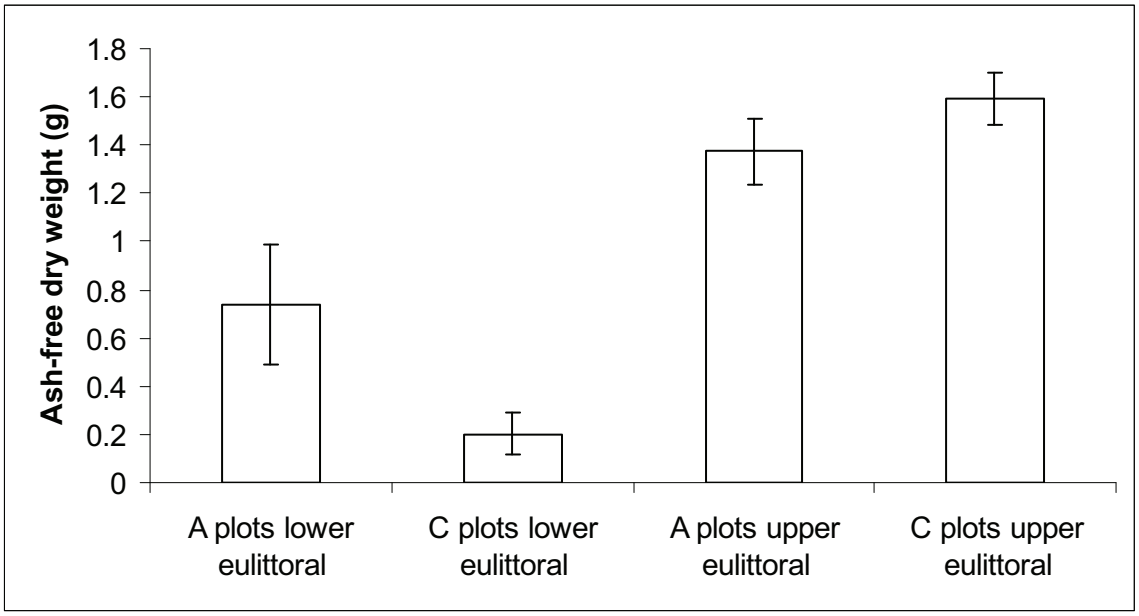

Figure 40: Ash-free dry weight (AFDW) results of the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effect of tidal height and lugworm exclusion. The results are significant for the factors tidal height and interaction. A = lugworm exclusion plots, c = control plots.

Number of shoots per experimental unit and ash-free dry weight (AFDW) affected by current velocity and lugworm exclusion

Both current velocity and lugworm exclusion had an effect on the number of shoots per seagrass transplant (see fig. 41). More shoots were growing on experimental plots affected by weak current velocities than on those affected by stronger current velocities. Lugworm exclusion also had a beneficial effect in that more shoots were growing on plots where the lugworm had been excluded compared with the control plots (2 factorial ANOVA with interaction, current velocities: $F_{1, 8} = 6.6965$; $p = 0.032$; lugworm exclusion: $F_{1, 8} = 17.1848$; $p = 0.0032$).

Less shoots remain on the lugworm exclusion plot affected by strong current velocity compared with that affected by weaker current velocities. However, even when the number of shoots are reduced by current velocity, more shoots remain on plots where the lugworm was excluded than on those where the lugworm was still present (control plots).

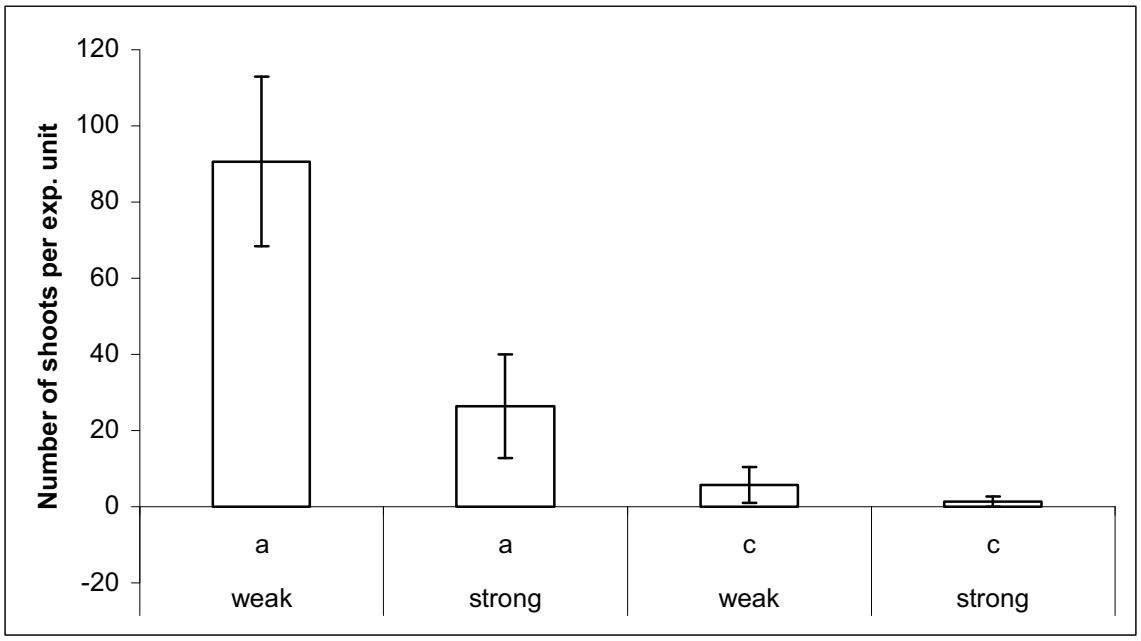

Figure 41: Results for number of shoots per experimental unit in the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effect of current velocities and lugworm exclusion. The results are significant for the factors current velocities and lugworm exclusion. A = lugworm exclusion plots, c = control plots.

The biomass was also affected by lugworm exclusion and current velocity (see fig. 42), but also by the interaction between these two factors. More biomass had developed on the lugworm exclusion plots compared with the control plots. Current velocity was also influential and the most biomass had developed on the plot where there was low current velocity and lugworm exclusion (2 factorial ANOVA with interaction, lugworm exclusion: $F_{1, 8} = 43.2181$; $p = 0.0002$; current velocities: $F_{1, 8} = 20.7984$; $p = 0.0018$; current velocity x treatment: $F_{1, 8} = 25.8855$; $p = 0.0009$). However, when the current velocity was strong the biomass was slightly higher on the control plot compared with the lugworm exclusion plot.

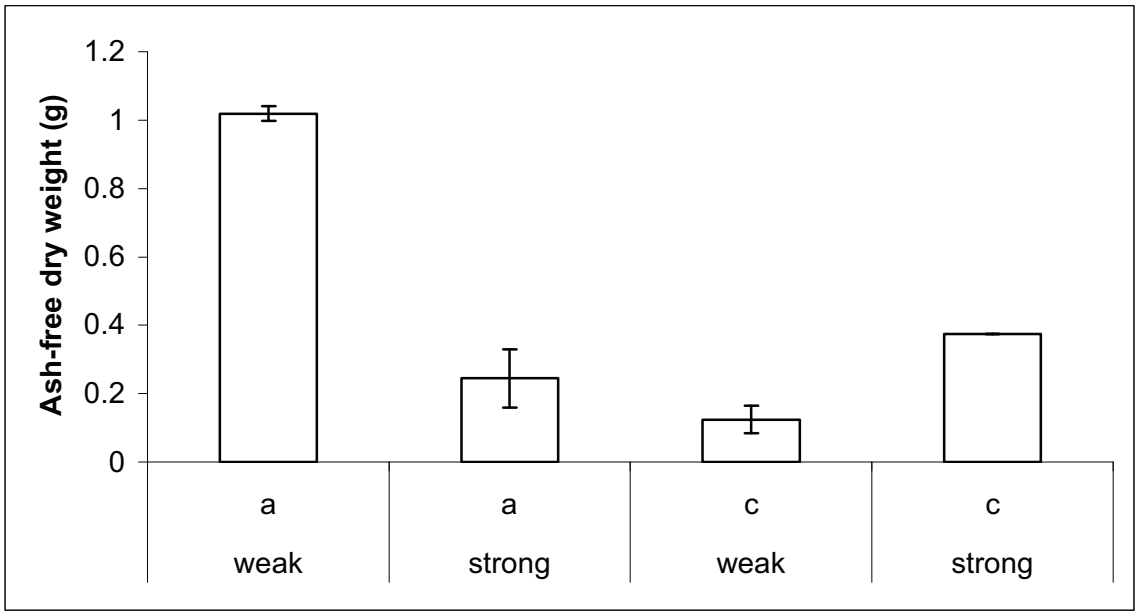

Figure 42: Ash-free dry weight (AFDW) results of the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effect of current velocity and lugworm exclusion. The results are significant for the factors current velocities, lugworm exclusion and interaction. A = lugworm exclusion plots, c = control plots.

3.3.3 Grain size analysis

Five different parameters were analysed in the grain size analysis: median, *phi*, sorting coefficient QD, percent sand (< 1,000 µm >63 µm) and percent mud (<63 µm). Only tidal height had an effect on grain size distribution (see table 5), the absence or presence of *Arenicola marina* had no effect. Percentage sand and mud were also analysed for an effect of current velocity but the results were not significant and are not shown. All samples were taken in July 2004.

Table 5:Grain size results of the C/N experiment on the Möwenberg lugworm exclusion plots. A = lugworm exclusion plots, c = control plots. The results are significant for the factor tidal height (not for sorting coefficient).

Experimental plots	Median (µm)	Phi	Sorting coefficient QD	% Sand	% Mud
A plots lower eulittoral	180.18	2.49	1.59	81.83	18.17
C plots lower eulittoral	182.36	2.46	1.43	81.41	18.59
A plots upper eulittoral	274.53	1.87	0.84	92.56	7.44
C plots upper eulittoral	253.08	1.98	0.92	89.87	10.13
2-factorial ANOVA with interaction, $F_{1,8}$	99.8454	105.498	2.4260	13.2288	13.2288
p value	<0.0001	<0.0001	Not significant	0.0066	0.0066

The sediment from the lugworm exclusion plots in the lower eulittoral has the second lowest sand and the second highest mud fraction compared with the other experimental plots. The sediment particles have the lowest median and the biggest *phi* value and it would be classed as fine sand (see material and methods section for classification). It was the most poorly sorted (high sorting coefficient) sediment from the four stations.

The samples from the control plots in the lower eulittoral have the lowest sand and highest mud fraction of the four stations. The particle median was slighter higher than that in the samples from the lugworm exclusion plots, but the *phi* value was slightly lower. It would also be classed as fine sand. The sediment was again poorly sorted if slightly better than the lugworm exclusion plots in the lower eulittoral.

The lugworm exclusion plots from the upper intertidal have the highest sand section and the lowest mud section from all four stations. The particle median was the highest from all the stations and the *phi* value the lowest. The sediment would be classed as medium sand and was better sorted than the sediment from the lower intertidal (lowest sorting coefficient).

The sediment from the control plots in the upper intertidal have the second highest sand and the second lowest mud section of all four stations. The particle median was the second highest and the *phi* value the second lowest. The sediment would be classed as medium sand and had the second lowest sorting coefficient.

In summary, the particle size of the sediment on the experimental plots was only affected by tidal height (see table 5 above), not lugworm exclusion or current velocity.

3.3.4 Porewater analysis

The porewater samples were analysed for salinity, silicate, phosphate and ammonium concentration. The samples were taken in July and September 2004. The data was also tested for the effect of current velocity, but there was no statistically significant result for any of the porewater parameters (data not shown).

Salinity

The salinity in the porewater (see fig. 43) was higher in July than in September, but neither tidal height nor *Arenicola marina* exclusion had any effect. The salinity ranged in July 2004 from 32.3 to 33.1 psu, and in September from 31.9 to 32.1 psu (MANOVA with interaction, time: $F_{3,8} = 9.9863$; $p = 0.0134$).

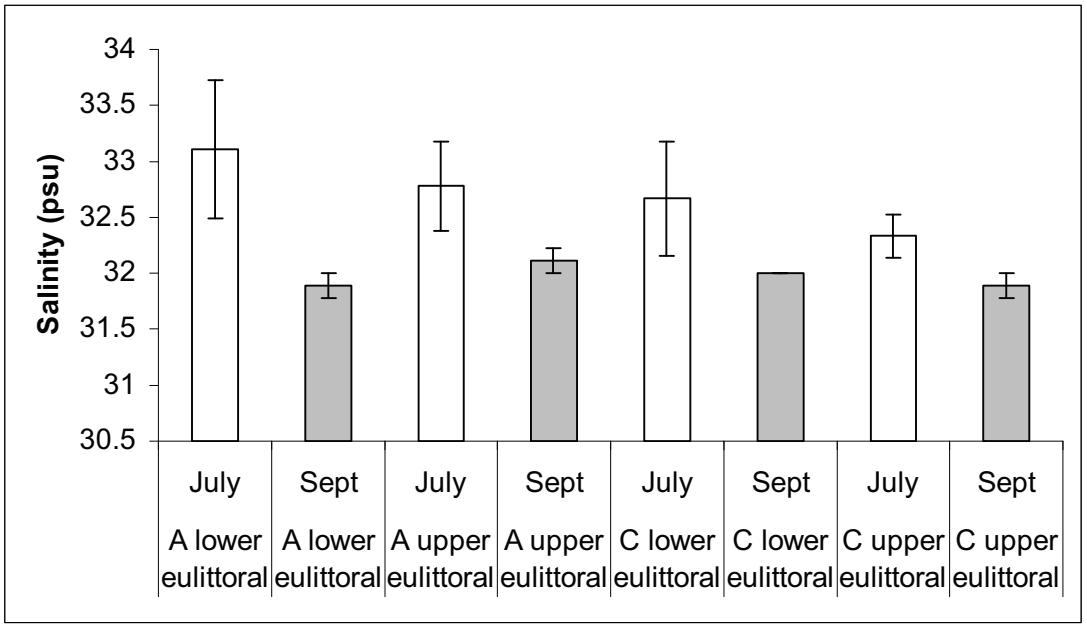

Figure 43: Porewater salinity results of the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effect of tidal height and lugworm exclusion. The results are significant for the factor time. White columns = July 2004; grey columns = September 2004. A = lugworm exclusion plots, c = control plots.

Silicate

The silicate concentrations in the porewater were again higher in July than in September (see fig. 44) and differed between upper and lower intertidal whilst the presence or absence of *Arenicola marina* had no effect.

In July, the silicate concentrations were higher in the lower intertidal than in the upper intertidal. By September the silicate concentrations were generally lower than in July but again the results were higher in the lower intertidal than in the upper intertidal (MANOVA with interaction, tidal height: $F_{3,8} = 9.5595$; $p = 0.0148$; time: $F_{3,8} = 10.0174$; $p = 0.0133$; time x tidal height: $F_{3,8} = 7.8263$; $p = 0.0233$). In September the difference between upper and lower intertidal was much less pronounced.

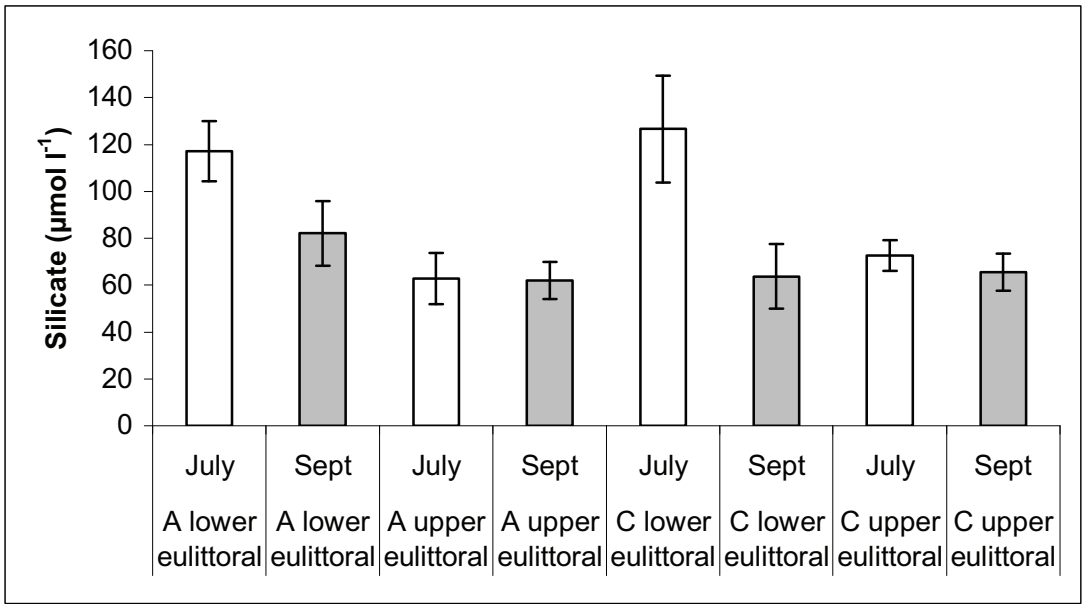

Figure 44: Porewater silicate content in the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effects of tidal height and lugworm exclusion. The results are significant for the factors time, tidal height and interaction between time and tidal height. White columns = July 2004, grey columns = September 2004. A = lugworm exclusion plots, c = control plots.

Phosphate

There was no clear effect of tidal height, time of sampling or lugworm exclusion on the phosphate concentration in the porewater (data not shown). In July, the values ranged from 7.87 to 10.92 µmol l^{-1}. By September 2004 the values ranged from 8.27 to 10.74 µmol l^{-1}.

Ammonium

The ammonium content in the porewater was generally higher in July than in September (see fig. 45). It was also higher in the lower eulittoral plots compared with the upper eulittoral plots (MANOVA with interaction, tidal height: $F_{3,8}$ = 15.4825; p = 0.0043; time: $F_{3,8}$ = 5.4288; p = 0.0482; time x tidal height: $F_{3,8}$ = 8.4356; p = 0.0198).

Even though the presence or absence of the lugworm had no effect on the ammonium concentrations, the interaction between time and tidal height was also statistically significant. In July, there is a clear decrease from the lower to the upper eulittoral plots but in September there is no longer any difference between the results.

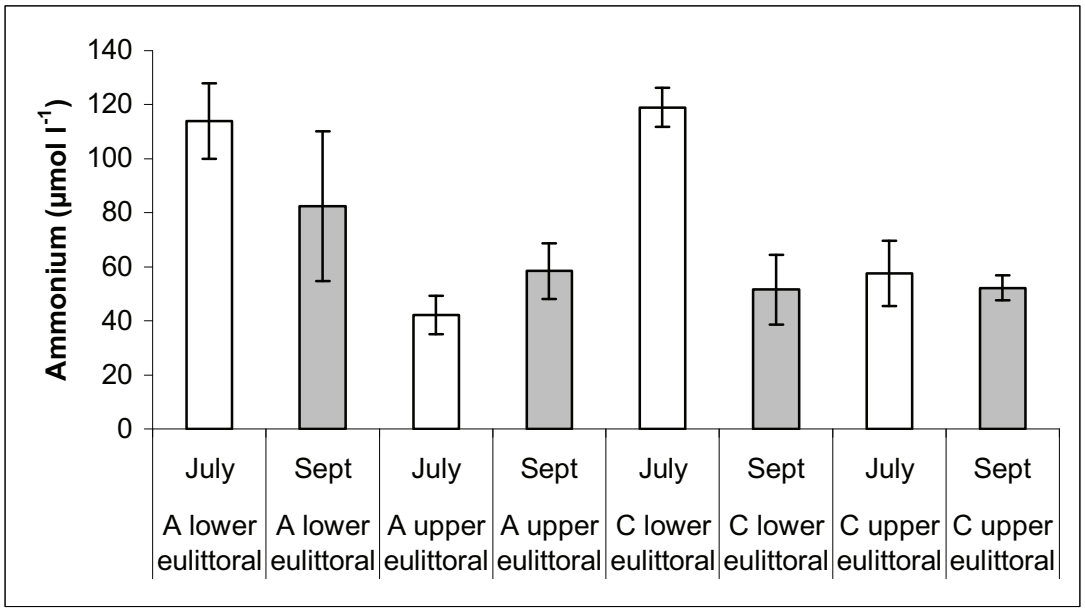

Figure 45: Porewater ammonium content in the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effects of tidal height and lugworm exclusion. The results are significant for the factors time, tidal height and interaction between time and tidal height. White columns = July 2004, grey columns = September 2004. A = lugworm exclusion plots, c = control plots.

3.3.5 Carbon and nitrogen content of *Zostera noltii*

The carbon and nitrogen content and carbon/nitrogen ratio in the seagrass *Zostera noltii* was analysed, investigating if either lugworm exclusion or tidal height have any effect on the carbon or nitrogen concentration in *Z. noltii*. The data was also tested for the effect of current velocity, but only the carbon/nitrogen ratio in the above ground material differed significantly.

Carbon content in above and below ground samples

The above ground samples had generally a higher carbon content than the below ground samples. Tidal height had an effect on carbon content in the below ground samples, but not on the above ground samples.

After 8 weeks on the lugworm exclusion plots, the carbon content in the above ground samples (see fig. 46) ranged from 156.6 to 267.2 µg C mg dry weight^{-1} with no statistically significant difference between them. However, in the below ground samples the carbon content is significantly higher (two-way ANOVA with interaction, tidal height: $F_{1, 11} = 14.4089$; $p = 0.0053$) in the samples from the upper eulittoral plots (158.5 µg C mg dry weight^{-1}) compared with the lower intertidal plots. (114.1 µg C mg dry weight^{-1}).

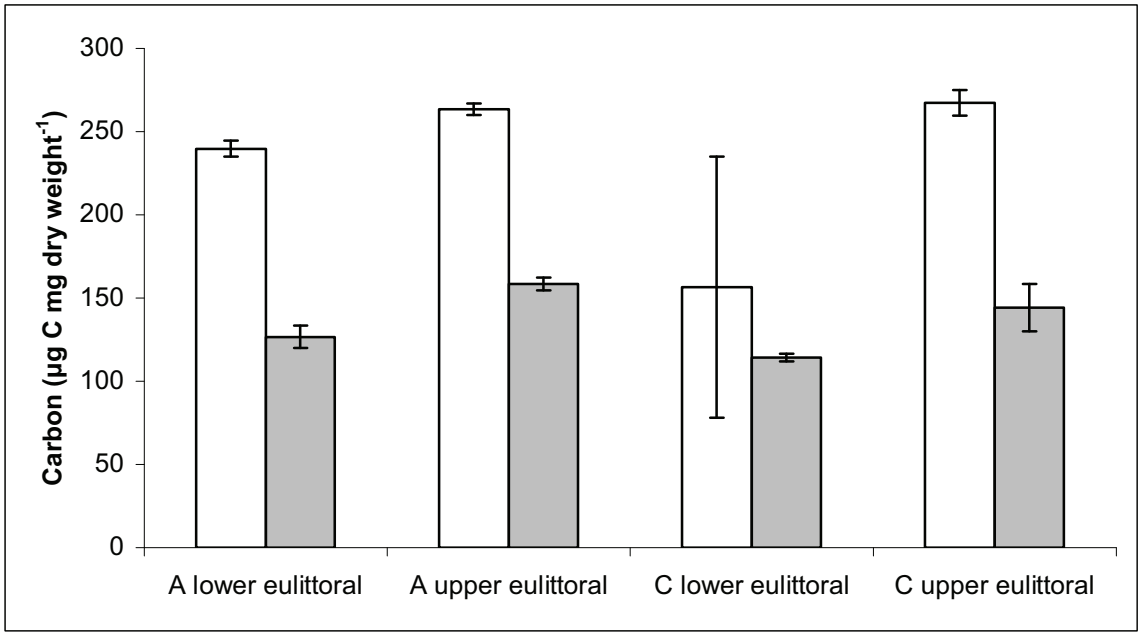

Figure 46: Results for the above ground (AG) and below ground (BG) carbon content of *Zostera noltii* after 8 weeks on the lugworm exclusion plots. The results were significant for the factor tidal height in the below ground samples, no factors were significant in the above ground samples. White columns = AG samples, grey columns = BG samples of *Z. noltii*. A = lugworm exclusion, C = control plot.

Nitrogen content in above and below ground samples

The above ground samples also had a higher nitrogen content than the below ground samples. However, neither the above nor the below ground samples differed significantly amongst themselves. In the above ground samples, the result

ranged from 21.5 to 35.4 µg N mg dry weight^{-1}. The results for the below ground samples ranged from 8.1 to 10.9 µg N mg dry weight^{-1}.

Carbon/Nitrogen ratio (C/N) in the above and below ground seagrass material

The C/N ratio was higher in the below ground samples than in the above ground samples (see fig. 47). Both tidal height and lugworm exclusion had a significant influence on the carbon/nitrogen ratio of the above ground seagrass material (ANOVA with interaction, tidal height: $F_{1, 11} = 10.7089$, $p = 0.0113$; lugworm: $F_{1, 11} = 6.8114$, $p = 0.0311$). The ratio was higher on the lugworm exclusion plots than on the control plots, and also higher on the upper eulittoral than on the lower eulittoral plots.

In the below ground samples, the C/N ratio was highest in the samples from the lugworm exclusion plots in the upper eulittoral (18.58) but the results did not differ statistically significant from each other. The lowest C/N ratio was found in the samples from the control plots in the lower eulittoral.

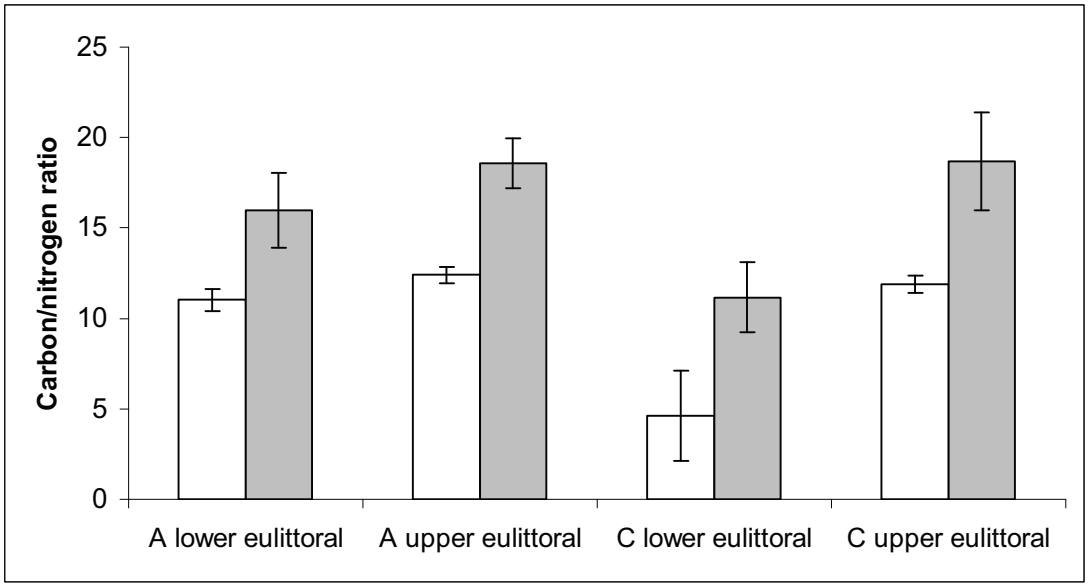

Figure 47: Results for the above ground (AG) and below ground (BG) carbon/nitrogen ratio of *Zostera noltii* after 8 weeks on the lugworm exclusion plots. The results were significant for the factor tidal height and lugworm exclusion in the AG material. White columns = AG samples, grey columns = BG samples of *Z. noltii*. A = lugworm exclusion, C = control plot

Current velocity affecting the carbon/nitrogen in above ground seagrass material

Carbon and nitrogen content in the above and below ground seagrass material did not differ significantly amongst themselves (data not shown). The carbon content ranged from 0 to 236.73 µg C mg dry weight^{-1} in the above and from 128.9 to 483.4 µg C mg dry weight^{-1} in the below ground samples. The nitrogen content ranged from 0 to 21.5 µg N mg dry weight^{-1} in the above ground and from 11.8 to 40.4 µg N mg dry weight^{-1} in the below ground samples.

The carbon: nitrogen ratio is higher in the below ground seagrass material than in the above ground material (see fig. 48), but only differs significantly in the above ground material. Lugworm exclusion resulted in a higher carbon: nitrogen ratio compared with the control plots (2 factorial ANOVA with interaction, $F_{1, 11}$ = 11.2899, p = 0.0099). As expected from the results for carbon and nitrogen content, the highest carbon nitrogen ratio was found on the lugworm exclusion plots exposed to weak current velocities.

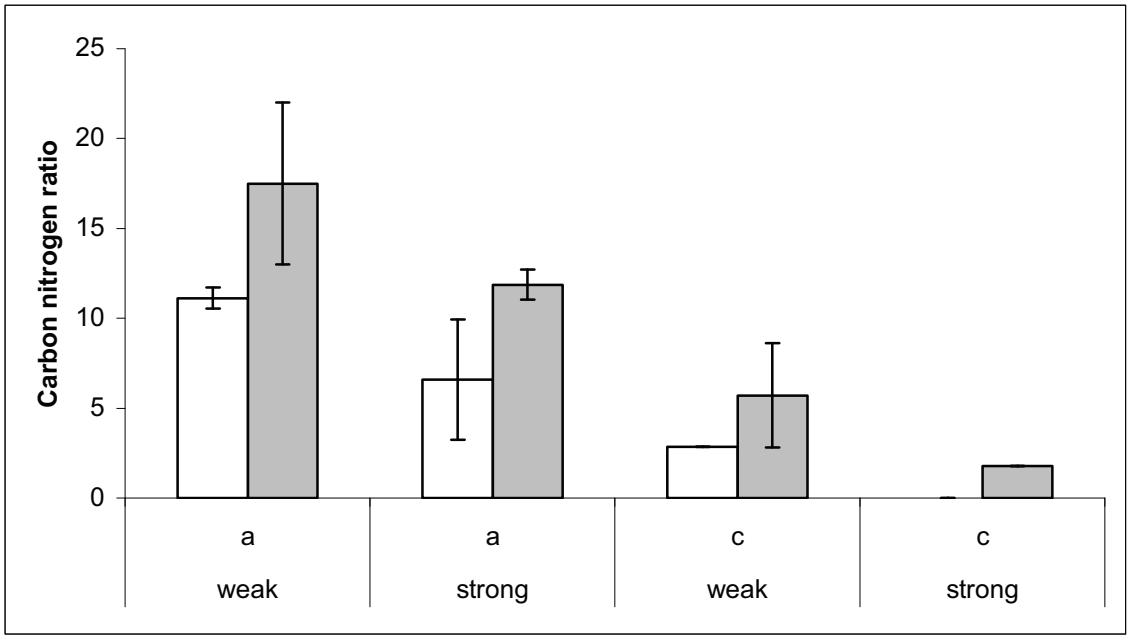

Figure 48: Results for the above ground (AG) and below ground (BG) carbon/nitrogen ratio of *Zostera noltii* after 8 weeks on the lugworm exclusion plots. The results were only significant for the factor lugworm exclusion. White columns = AG samples, grey columns = BG samples of *Z. noltii*. A = lugworm exclusion, C = control plot

3.3.6 Carbon and nitrogen content in the sediment surrounding the seagrass transplants

The sediment surrounding the seagrass cores was analysed looking at the carbon and nitrogen content and carbon: nitrogen ratio. The samples were taken in July and September 2004. The data were also tested for the effect of current velocity, but the carbon and nitrogen content of the sediment was not affected by current velocity. Only the carbon: nitrogen ratio differed significantly in time when looking at the effect of current velocity.

Carbon

The carbon concentration showed similar results for July and September (see fig. 49 below). The presence or absence of *Arenicola marina* made no difference in carbon content. The sediment samples from the lower eulittoral had a higher carbon content than the samples from the upper eulittoral (MANOVA with interaction, tidal height: $F_{1, 8} = 94.4385$; $p =< 0.0001$).

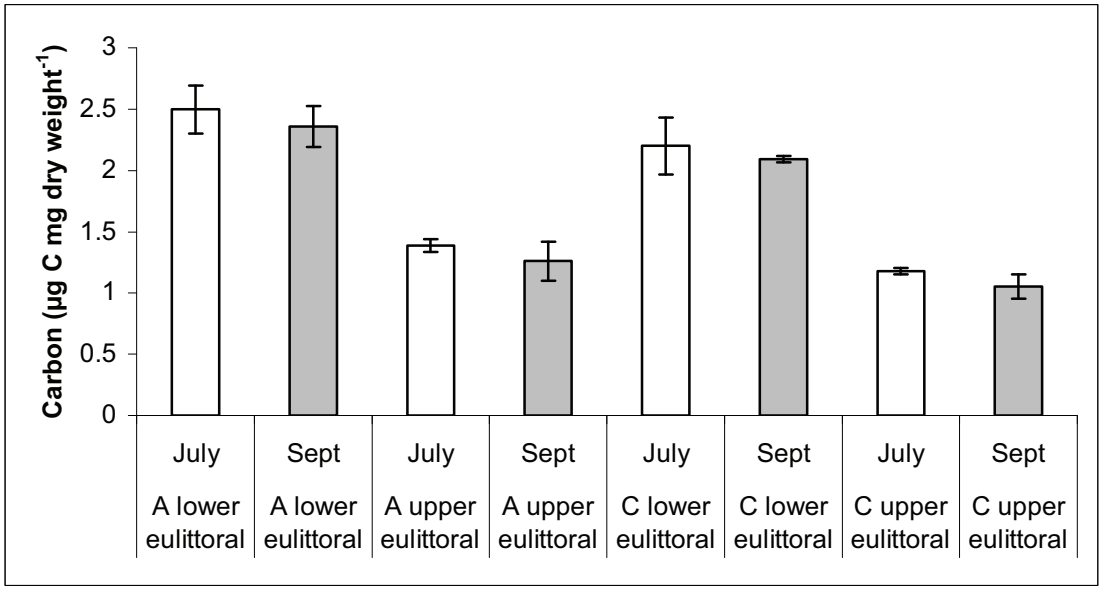

Figure 49: Sediment carbon content of the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effects of tidal height and lugworm exclusion. The results are significant for the factor tidal height. White columns = July 2004, grey columns = September 2004. A = lugworm exclusion plots, c = control plots.

Nitrogen

The amount of nitrogen in the sediment (see fig. 50) was not affected by lugworm exclusion. Instead it was affected by the position of the experimental plots in the upper or lower intertidal and by an interaction between sampling time and tidal height. The nitrogen concentrations were generally higher in the lower eulittoral than in the upper eulittoral both in July and September. (MANOVA with interaction, tidal height: $F_{1, 8} = 94.8376$; $p =< 0.0001$; time x tidal height: $F_{1, 8} = 6.2989$; $p = 0.0364$).

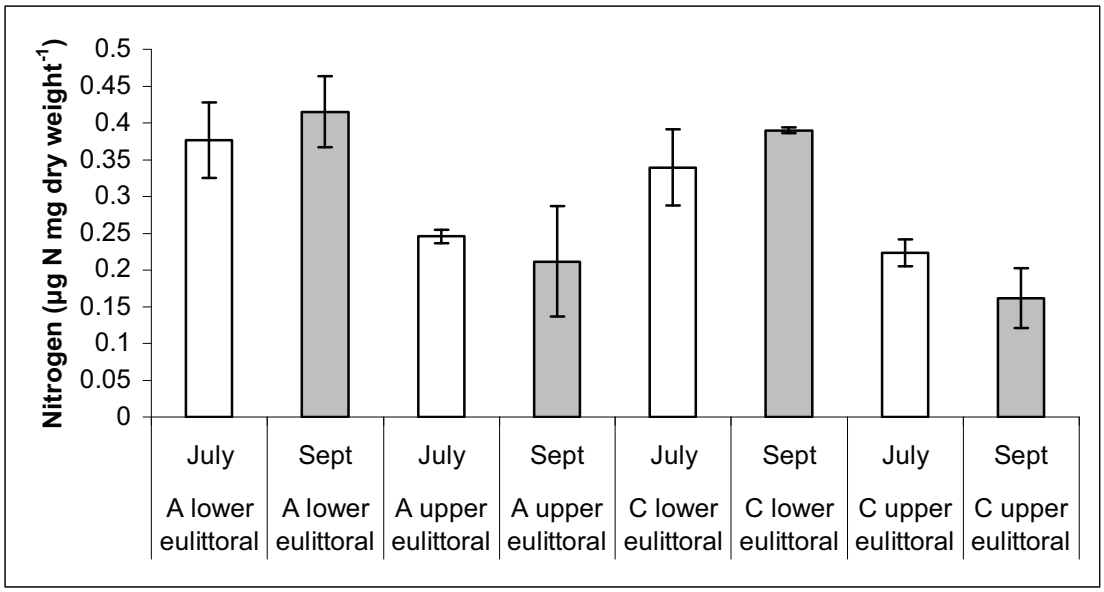

Figure 50: Sediment nitrogen content of the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effects of tidal height and lugworm exclusion. The results are significant for the factors tidal height and interaction between time and tidal height. White columns = July 2004, grey columns = September 2004. A = lugworm exclusion plots, c = control plots.

Carbon/nitrogen ratio

The carbon: nitrogen ratio was not affected by either lugworm exclusion nor tidal height (see fig. 51), but instead by the interaction between tidal height and time (MANOVA with interaction, time x tidal height: $F_{1,8} = 59.3653$; $p =< 0.0001$). The highest carbon: nitrogen ratio was measured in July (6.6) on the lugworm exclusion plots in the lower eulittoral.

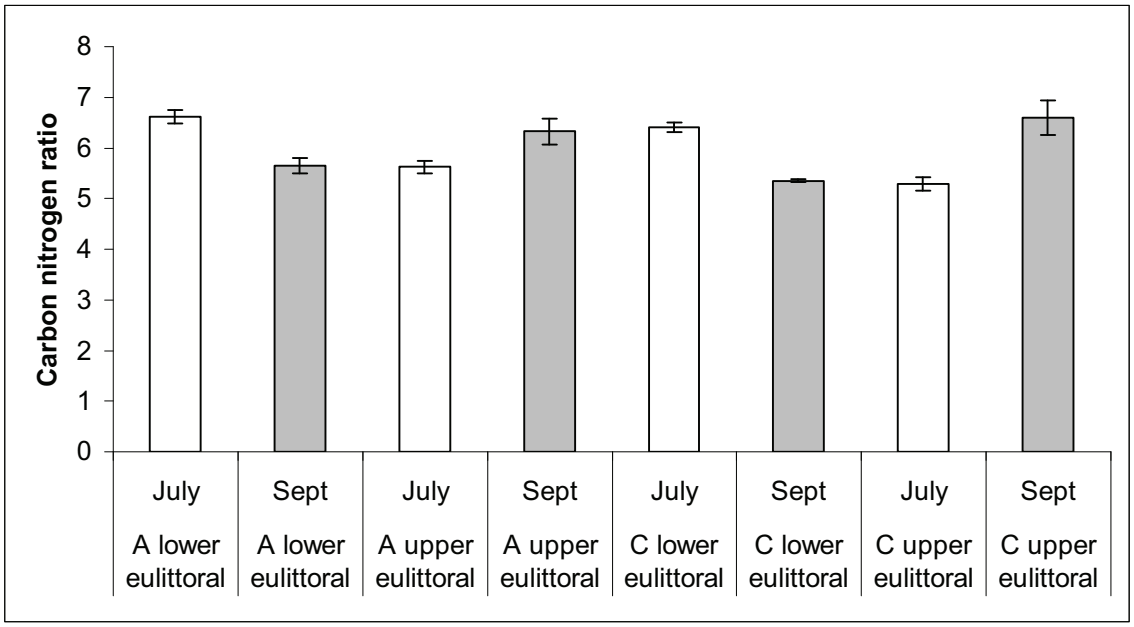

Figure 51: Sediment carbon/nitrogen ratio of the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effects of tidal height and lugworm exclusion. The results are significant for the factor interaction between time and tidal height. White columns = July 2004, grey columns = September 2004. A = lugworm exclusion plots, c = control plots.

Current velocity affecting the carbon: nitrogen ratio (C/N) in the sediment

Neither carbon nor nitrogen content differed significantly between plots that were affected by weak or strong current velocities, lugworm exclusion or in time (data not shown).

In contrast, the carbon: nitrogen ratio is higher in July (see fig. 52) than it is in September (MANOVA with interaction, time: $F_{1,8}$ = 102.5448, p = <0.0001) but again is not influenced by either current velocity or lugworm exclusion.

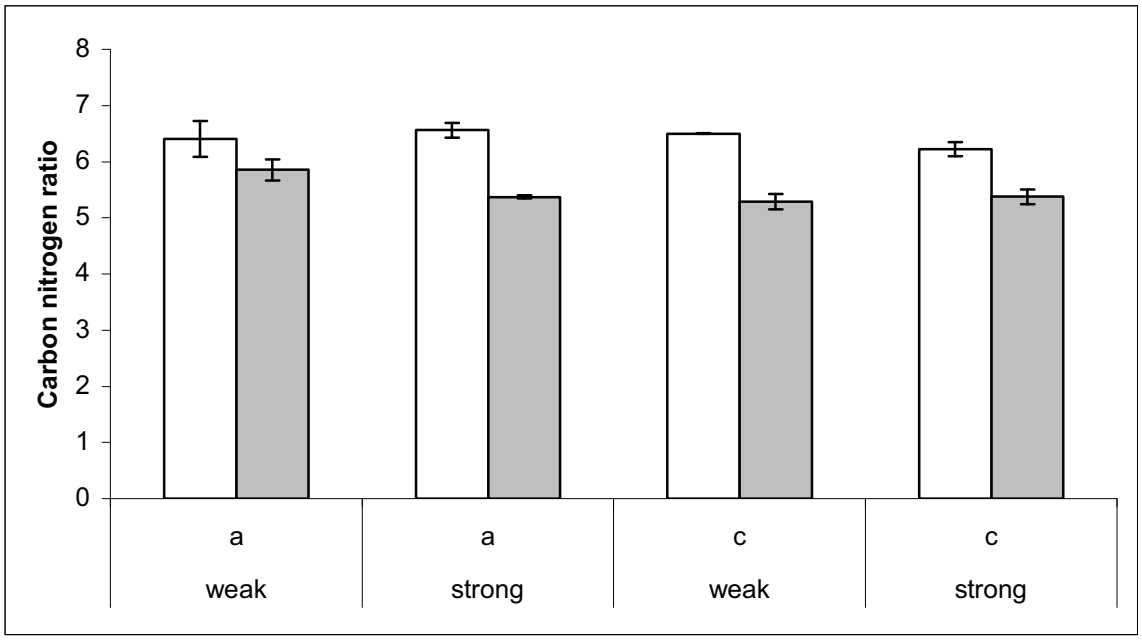

Figure 52: Sediment carbon nitrogen ratio of the C/N experiment on the Möwenberg lugworm exclusion plots investigating the effects of current velocities and lugworm exclusion. The results are significant for the factor time. White columns = July 2004, grey columns = September 2004. A = lugworm exclusion plots, c = control plots.

3.4 Discussion

Adult *Zostera noltii* plants (adult is here defined as plants that were taken in July from the adjacent seagrass meadow) were transplanted from the Möwenberg *Zostera noltii* meadow onto the adjacent lugworm exclusion plots. The carbon and nitrogen content of the seagrass transplants as well as their biomass was analysed. The sediment surrounding the seagrass transplants was analysed for grain size, carbon and nitrogen content and the porewater for nutrient content.

3.4.1 Seagrass biomass

The number of shoots and biomass of the *Zostera noltii* transplants were affected by tidal height, but only the biomass was also affected by the interaction between lugworm exclusion and tidal height. Both the number of shoots and biomass were higher in the upper eulittoral than in lower eulittoral. In the upper intertidal plots, the biomass of the control plots was slightly higher than on the lugworm exclusion plots

but not significantly so. In the lower intertidal, the biomass and number of shoots were higher on the lugworm exclusion plots than on the control plots. These findings correspondent with the results from chapter 2 (see results) where the biomass and number of shoots was also influenced by tidal height and grew better in the upper intertidal. However, it should be noted again that the experimental plots in the upper intertidal were also affected by lower current speeds than in the lower intertidal and that it is difficult to separate these two factors (tidal height and current velocity) completely.

Current velocity also affected the dwarf seagrass. The number of shoots was highest on the lugworm exclusion exposed to low current velocities, but second highest on the lugworm exclusion plots exposed to strong current velocities. This indicates that *Arenicola marina* has a negative effect on the number of shoots of *Zostera noltii*, but also that it benefits from the lower current velocity. The least number of shoots were found on the plots were *A. marina* was present, as well as being exposed to high current velocities, indicating that the combination of both factors (i.e. high current velocity and lugworm present) is worst for the dwarf seagrass.

However, the results for the biomass of *Z. noltii* do not follow the above pattern. Even though the highest biomass can be found again on the lugworm exclusion plot exposed to weak current velocity, the biomass on the control plot exposed to strong current velocity is the second highest biomass. This is unexpected, as the number of shoots on this plot is the lowest.

Irrespective of the slightly different results from biomass and number of shoots, the findings from this study support the findings from both Philippart (1994) and Schanz & Asmus (2003). Philippart (1994) showed that the lugworm had a negative effect on *Zostera noltii* growth, and Schanz and Asmus (2003) found a negative effect of increased hydrodynamics on the dwarf seagrass. The combined effect of both these factors can be seen in the results from this study, where the dwarf seagrass grew best on the lugworm exclusion plots exposed to low current velocities compared with being influenced by either stronger hydrodynamics or lugworm presence.

3.4.2 Grain size

The sediment grain sizes are different in the lower and upper intertidal. The sediment in the lower intertidal was comprised out of more muddy and less sandy sediment, consisted of smaller particles, and could be classed as fine sand according to the Wentworth scale. Conversely, the sediment in the upper intertidal was comprised of more sandy and less muddy sediments, the particles were bigger, and it could be classed as medium sand. Volkenborn (2005) found an increased amount of fine particles on the lugworm exclusion plots in the first surface centimetre, whereas the samples for this study were taken from the first 10 centimetres and no difference was found between lugworm exclusion and control plots. It seems likely that there were no changes in grain size over this depth between treatment and control plots (and indeed this is mentioned by Volkenborn (2005) as well). The medium sand in the upper eulittoral stemed from the adjacent sand dunes and was carried onto the tidal flat by winds (as described by Austen (1994) in his survey of the Königshafen bay).

Schanz & Asmus (2003) mention a difference in sediment median grain size between a sheltered and an exposed site and a similar difference can be observed for at least one of the experimental plots here as well. The plot A1 had the lowest tidal current velocity and also smaller grain particles compared with the other plots. The difference between the sediments from the different plots is not as pronounced as it is in Schanz & Asmus (2003), but the difference in tidal current velocity is much less pronounced as well (at the exposed site, Schanz & Asmus measured 26 cm s^{-1} compared with 15 cm s^{-1} on the Möwenberg tidal flat).

It has been speculated that *Zostera marina* prefers muddy to sandy sediment and that *Z. noltii* grows preferentially on sandy sediments. In the Sylt-Rømø-Bight (and also the Hörnum Deep) these growth patterns can be observed with the naturally occurring seagrass meadows. For example, the monospecific *Zostera marina* meadow on the Gröning tidal flat grows on muddy sediment (see chapter 4) but the monospecific *Zostera noltii* bed on the Möwenberg tidal flat grows on sandy sediment. However, the *Zostera noltii* transplant thrived in the lower intertidal (with the muddier sediment) as long as the tidal current velocities were low and *Arenicola marina* densities were low or the lugworm excluded. The *Z. marina* transplants from

the first experiment also grew well on the sandy substrate of the Möwenberg tidal flat. As the sediment composition and hydrodynamics (tidal current velocity and wave action) are related (i. e. low hydrodynamics lead to accumulation of matter and therefore to more fine particles), the effect of hydrodynamics is superimposed on seagrass sediment preference. The two intertidal seagrass species both grew better at low current velocities, irrespective of sediment composition. From the results of the first and second experiment it can be speculated that should hydrodynamics remain low (up to 8 cm s^{-1}), then both seagrass species would grow better in a muddier sediment than a sandy one because of the higher porewater nutrient content. As it is unlikely that these conditions would arise in nature (accumulation of fine matter coinciding with raised hydrodynamics), this speculation could only be tested in a laboratory.

3.4.3 Porewater

The salinity in the porewater was higher in July than in September, but was not affected by tidal height or lugworm exclusion. A heightened salinity during the summer months is not unexpected, due to less rainfall and higher water evaporation. *Zostera marina* can tolerate salinities ranging between 5 to 42 psu (den Hartog 1970; Giesen *et al.* 1990) in the water column and it is likely that *Zostera noltii* has a similar range, at least in the intertidal areas around Sylt (den Hartog (1970) states 9-10 psu as the lower limit). Increased salinity in the water column stresses seagrass (e.g. van Katwijk *et al.* 1999; Kamermans, Hemminga & de Jong 1999) but neither of those studies looked at the effect of salinity on the root/rhizome system.

The silicate concentration in the porewater was higher in July than September, and was also higher in the lower intertidal compared with the upper intertidal plots. No effect of lugworm exclusion was apparent. Asmus, Sprung & Asmus (2000) state that the incoming tidal water has an annual range between 5.5 – 87.2 µmol l^{-1} in the Königshafen bay, which is lower than the maximum values measured in the porewater in this study. The experimental plots (and the surrounding sand flat) may act as a silicate source for benthic diatoms by releasing silicate into the water column. Whilst no effect of *Arenicola marina* could be found on porewater silicate levels in this study, other authors have found that burrowing infauna may release silicate to the water column due to reworking the sediment (e.g. Asmus, Sprung &

Asmus 2000). Temperature has also been linked with silicate fluxes, with increased fluxes at higher temperatures (Asmus, Sprung & Asmus 2000). This would explain the higher silicate concentrations in July compared to September.

Silicon levels in the water has once be linked with the decline of a *Zostera marina* population in Lake Grevelingen, The Netherlands (Herman *et al.* 1996), but the results could not be repeated in an experimental study (Kamermans, Hemminga & de Jong 1999). The second study showed no increase in biomass or productivity in *Zostera marina* when growing at increased silicon levels (70 or 100 µmol/l). Whilst the presence of silicon could not counteract the negative effects of increased salinity (32 psu), plants growing at low silicon levels (5 µmol/l) but increased salinity performed worse than plants growing at high salinity and high silicon levels. Therefore the presence of silicate in either the water column or porewater may be beneficial to the plants when stressed by increased salinity.

Ammonium and phosphate are used by seagrasses as a source of nitrogen and phosphorus, two of the 15 nutrients required by all plants (Hemminga & Duarte 2000). The global average sediment porewater value of ammonium is 86 µmol l^{-1}, and of phosphate 12 µmol l^{-1} (Hemminga & Duarte 2000). In comparison, the concentrations measured on the lugworm exclusion plots ranged between 42.2 – 119 µmol l^{-1} in ammonium and between 7.87 – 10.74 µmol l^{-1} in phosphate. Phosphate concentrations were not affected by sampling time, tidal height or lugworm exclusion, whereas ammonium concentration was higher in the lower intertidal plots and in July than in September but also not affected by lugworm exclusion. As fine matter accumulated in the lower eulittoral plots it can be assumed that this is the source (e.g. via decomposition of organic material) for the heightened ammonium concentrations in the porewater.

Albrecht *et al.* (1995) also working in the Königshafen bay measured an ammonium concentration of ca. 60 µmol l^{-1} in the porewater of a *Zostera noltii* meadow growing on sandy sediment. The phosphate concentration in the porewater of the same meadow reached ca. 14 µmol l^{-1} and was higher than the phosphate concentrations on the experimental plots. Moriarty & Boon (1989) estimate the concentrations of ammonium in seagrass bed porewater to range between 5 – 100 µmol l^{-1} and for

soluble reactive phosphorus (mostly phosphate) to be less than 20 µmol l^{-1}. The results from this study fall within that range.

Short (1987), working with *Zostera marina* in New Hampshire, USA, has described eelgrass as nitrogen limited (as a temperate seagrass growing on terrigenous sediment). The porewater ammonium concentrations in the substratum of his mesocosms were much lower than the ones found *in situ* at the Möwenberg tidal flats. Moriarty & Boon (1989) estimate that *Z. marina* plants need between 17 – 300 mg N m^{-2} day^{-1}, taken up by leaves and the root/rhizome system. Irrespective of the enhanced nutrient availability, the biomass in the lower eulittoral plots is lower than in the upper eulittoral plots. This is most likely due to the lower tidal current velocity in the upper intertidal and does not reflect the nutrient availability. As the results in chapter 2 shows, the seagrass biomass is higher in the lower intertidal plots when the current velocity is reduced. Phosphate is generally not considered limiting in temperate seagrass beds (Short 1987).

3.4.4 Carbon and nitrogen content in *Zostera noltii*

The organic carbon content in the leaves was not affected by tidal height or lugworm exclusion, but also not by the heightened nutrient availability in the lower eulittoral plots. The carbon content in the roots/rhizome system was higher in the upper eulittoral plots. Atkinson & Smith (1984) have pointed out that the carbon content of the leaves is unlikely to change with the nutritional status of the plants as most of the carbon represents structural components. This has been confirmed by Duarte (1990). It is possible that the decreased hydrodynamics or increased light availability in the upper intertidal allowed the plants to utilise the available nutrients better. The rhizomes act as storage organs for *Z. noltii* (Cabaço & Santos 2007), so as there was less water movement in the upper eulittoral, the seagrass was able to store non structural carbon products, e. g. starch or carbohydrates, in the rhizomes.

There was more nitrogen in the leaves than in the roots and rhizomes of *Zostera noltii*, but there was no influence of tidal height or *Arenicola marina* on the nitrogen content. Pirc & Wollenweber (1988) investigating the nitrogen (N) content of three Mediterranean seagrasses also found a higher N concentration in the leaves compared with the roots/rhizomes. If the *Zostera noltii* transplants were nitrogen limited, an increased biomass in the lower eulittoral would have been expected in

response to the heightened nutrient availability there. As these experiments were conducted in the field, the seagrass transplants were exposed to a range of factors influencing their growth and survival, and it is difficult to single out one factor. One possibility is to look at the nitrogen content in the tissue of the seagrass, because it would be expected to see a small amount of nitrogen in the tissue if the plants were nitrogen limited (Duarte 1990).

Looking at the relationship between carbon: nitrogen (C/N) ratio and the nitrogen content of the seagrass tissue from the experimental plots (see fig. 53 below), it is obvious that leaves and roots/rhizome form two distinct groups. The carbon content in the leaves is low compared with data available in the literature, although not much data is available on carbon and nitrogen content of *Z. noltii* plants (see table 6 below). Duarte (1990) mentions that a C/N ratio of 12:1 in seagrass leaves is the point from which on further nutrient input no longer leads to a lower C/N ratio. Cabaço & Santos (2007), working with *Zostera noltii* growing on sandy substrate in Portugal, report much higher values of carbon in the leaves and roots/rhizomes but the nitrogen content in both tissue types is similar. Another study in the Königshafen area (Albrecht *et al.* 1995, see table 6 below) also reports much higher values *for Z. noltii* leaves, even though the values for the nitrogen content in the roots/rhizomes are comparable. There is also not such a noticeable difference between leaves and roots/rhizomes as reported in this study. Minor variations between locations would have been expected, but the differences reported in this study could be a unique feature of either this *Z. noltii* population or the location.

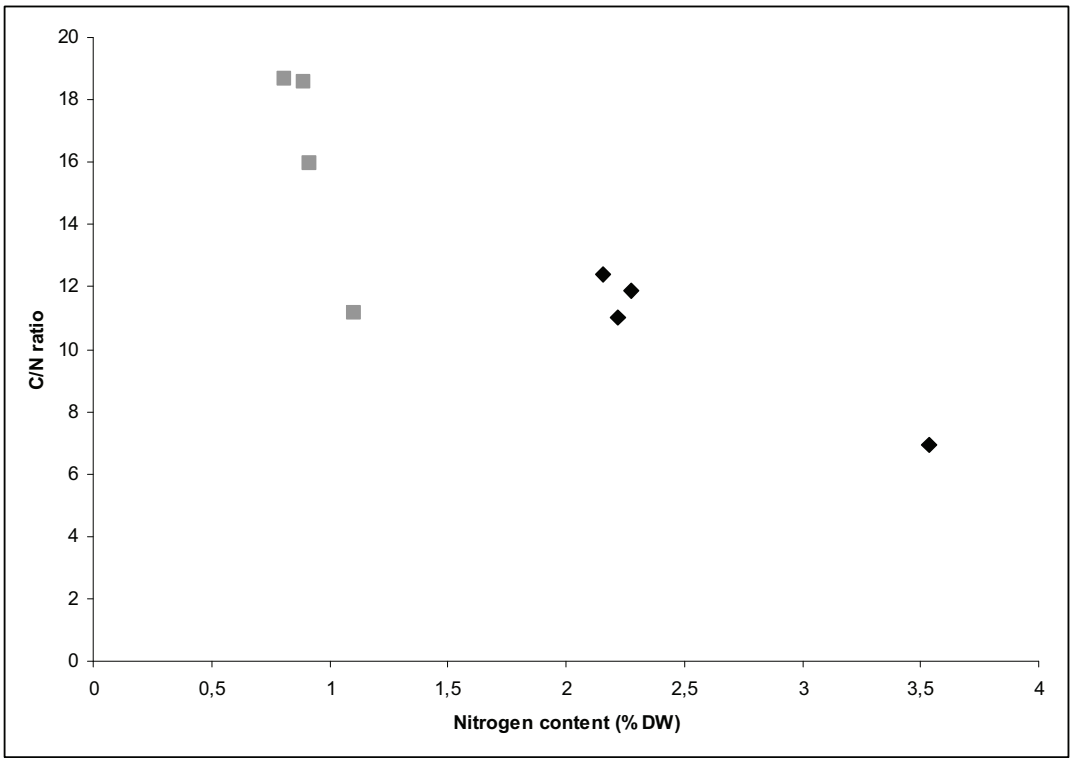

Figure 53. Leaves and roots/rhizome carbon: nitrogen (C/N) ratio in relation to the nitrogen content. Grey squares = roots/rhizome, black diamonds = leaves, DW = dry weight. Outliers in both groups are the values from the control group in the lower intertidal.

The roots/rhizomes had a higher C/N ratio and lower nitrogen content than the leaves, which could indicate a nitrogen limitation (following Duarte 1990). It seems to be a general trend in seagrasses, however, that rhizome and roots have lower nitrogen content and a higher C/N ratio than leaves (see table 6 below, Short 1987; Albrecht *et al.* 1995). Roots are the principal components responsible for ammonium uptake in seagrasses, even though leaves can also take up ammonium (seagrass leaves also take up nitrogen as nitrate from the water column). It has been shown for *Zostera marina* that nitrogen can be transported from the leaves to the roots (basipetal translocation) (Iizumi & Hattori 1982) and also from the roots to the shoots (acropetal translocation) (McRoy & Goering 1974). However, it appears that the leaves continue to take up nitrate even if sediment nitrogen content is high (Thursby & Harlin 1982) whereas the roots reduce their ammonium uptake when the leaves are exposed to increased ammonium concentrations. This could lead to a higher nitrogen concentration in the leaves of *Zostera noltii*, but it is not known if *Zostera noltii* has the same nitrogen uptake kinetics as *Zostera marina*.

Table 6: % carbon, nitrogen and phosphorus content in seagrass tissue from different locations worldwide. DW = dry weight; wt = weight

Seagrass species	% carbon DW	% nitrogen DW	C/N (wt)	% phosphorus DW	Location	Source
Average seagrass values (leaves)	33.6 ± 0.31	1.92 ± 0.05 (median 1.82%)	17.5	0.23 ± 0.011 (median 0.2%)	global	Duarte 1990
Zostera marina (leaves)	34.5 ± 0.8 (mud); 35.5 ± 1.5 (sand)	2.4 ± 0.3 (mud); 1.8 ± 0.2 (sand)	14.38 (mud); 19.72 (sand)	0.47 ±0.06 (mud); 0.39 ± 0.04	New Hampshire, USA (meso-cosms)	Short 1987
Z. marina (rhizome and roots)	36.2 ± 0.5 (mud); 36.6 ± 2.1 (sand)	1 ± 0.3 (mud); 0.6 ± 0.1 (sand)	36.2 (mud); 61 (sand)	0.46 ±0.06 (mud); 0.37 ± 0.08	New Hampshire, USA (mesocosms)	Short 1987
Zostera noltii (leaves)	app. 39 (sand); app. 40 (mud)	app 2.9 (sand); app. 2.8 (mud)	13.45 (sand); 14.29 (mud)		Königshafen	Albrecht *et al.* 1995
Z. noltii (rhizome and roots)	app. 35 (sand); app. 36 (mud)	app. 0.5 (sand); app. 1 (mud)	70 (sand); 36 (mud)		Königshafen	Albrecht *et al.* 1995
Z. marina (leaves)	app. 37 (sand); app. 40 (mud)	app. 2.5 (sand); app. 2.2 (mud)	14.8 (sand); 18.18 (mud)		Königshafen	Albrecht *et al.* 1995
Z. marina (rhizome and roots)	app. 32 (sand); app. 34 (mud)	app. 0.4 (sand); 0.3 (mud)	80 (sand); 113.3 (mud)		Königshafen	Albrecht *et al.* 1995
Z. noltii (leaves)		1 - 2.2	15 - 35		Punta S. Pietro, Ischia	Pirc & Wollenweber 1988
Z. noltii (rhizome and roots)		0.75 – 0.85	30 - 70		Punta S. Pietro, Ischia	Pirc & Wollenweber 1988
Z. noltii (leaves)	37.5 – 38.5 (sand)	2.5 (sand)	15.2 (sand)		Ria Formosa	Cabaço & Santos 2007
Z. noltii (rhizome	36 – 37 (sand)	0.5 – 0.9 (sand)	52.14 (sand)		Ria Formosa	Cabaço & Santos 2007

and roots)					
Z. noltii (leaves)	23.5 – 26.7	2.2 – 3.5	8.66	Königshafen	This study (exp. 2)
Z. noltii (rhizome and roots)	11.5 – 15.8	0.8 – 1.1	14.32	Königshafen	This study (exp. 2)

The carbon: nitrogen (C/N) ratio was influenced in the leaves by tidal height and lugworm exclusion. The C/N ratio was higher in the upper eulittoral plots, indicating a nitrogen limitation, which is confirmed by the heightened nutrient availability in the lower eulittoral plots. The C/N ratio was also higher on the lugworm exclusion plots. Whilst no effect of lugworm exclusion on nutrient load was found in this study, Volkenborn (2005), working on the same experimental plots, showed an increased amount of nutrients and organic material on the lugworm exclusion plots.

The C/N ration in the root/rhizome samples did not differ significantly amongst the plots but was generally lower than in the above ground samples.
Looking at the influence of current velocity and lugworm exclusion on carbon and nitrogen content of *Zostera noltii*, the only effect is on the carbon: nitrogen (C/N) ratio. Again the C/N ratio is higher in the root/rhizome samples than in the leaves. Current velocity has no statistically significant effect, although there is a trend towards a higher C/N ratio on the plots exposed to weak tidal current velocity. However, lugworm exclusion has resulted in higher C/N ratios than on the control plots, even though it is unclear what could have caused such a result.

3.4.5 Carbon and nitrogen content in the sediment

Sediment carbon concentration was not affected by presence or absence of *Arenicola marina* or tidal current velocity, but instead by tidal height. The sediment in the lower intertidal had a higher organic carbon content than the sediment in the upper eulittoral plots, indicating a higher organic load (i.e. more organic components). The carbon content did not differ between July and September. Considering the results of the grain size analysis (i.e. both the control and lugworm exclusion plots had a higher mud than sand proportion in the lower eulittoral), it is

not surprising that the organic carbon content is higher in the lower eulittoral than in the upper eulittoral zone.

The nitrogen content was also higher in the sediments of the lower eulittoral plots, but again was also not affected by lugworm exclusion or tidal current velocity. Furthermore, the nitrogen content increased from July to September in the sediments of the lower intertidal plots, and decreased in the same time in those of the upper eulittoral plots. As with carbon content, it is assumed that higher organic load led to the increase in sediment nitrogen content in the lower intertidal plots. It is not known what caused the increase or decrease from July to September but it is possible that benthic diatom blooms (personal observation) were responsible for the observed pattern. The porewater silicate concentration also decreased from July to September in the lower eulittoral, indicating an uptake by diatoms. In the upper eulittoral, the silicate concentrations remained constant, so it seems likely that benthic diatom uptake is not the reason for the decrease in nitrogen concentrations in the sediment from July to September.

The carbon: nitrogen (C/N) ratio was not affected by tidal height, current velocity or lugworm exclusion, but by the interaction between time of sampling and tidal height. The C/N ratio increased from July to September in the upper eulittoral plots but decreased from July to September in the lower intertidal plots. These findings are confirmed by the tidal current velocity results which also show a reduction of C/N ratio from July to September in the lower eulittoral.

Rusch, Huettel & Forster (2000) report much higher C/ N ratios (between 16 to 42 compared with 5.2 – 6.6 in this study) in the upper 15 cm of a sandy site also located within the Königshafen but less sheltered than the Möwenberg tidal flat. This indicates a much higher organic content in the Möwenberg tidal flat than the study site investigated by Rusch, Huettel & Forster (2000) which lies roughly 2 km to the south east. As the current velocity is higher and the study site in general much more exposed to hydrodynamics, it is not surprising that the organic content in the sediment is much lower. Rusch, Huettel & Forster (2000) report that the hydrodynamic influence in their study site went down to 4 – 8 cm sediment depth

and furthermore that not only porewater but also small particles were removed by hydrodynamic forces.

The sediment values from this study (ranging between 6.2 and 7.7 (atomic ratio)) are very close to the Redfield value of 6.6 for organic seston. This indicates that most of the organic carbon and nitrogen in the sediment derives from microalgae, most likely benthic diatoms.

3.5 Conclusion

Zostera noltii biomass was influenced by tidal height and current velocity, in that low tidal current velocities led to increased biomass. As the current velocity was reduced in the upper eulittoral plots, the biomass increased in the upper intertidal plots compared with the lower intertidal plots. The lugworm *Arenicola marina* had a negative effect on dwarf seagrass growth, and increased the negative effects of strong current velocity. The dwarf seagrass grew best on plots subjected to low tidal current velocities and lugworm exclusion.

The sediment grain size was also influenced by tidal height, with the sediment being muddier in the lower intertidal plots than in the upper intertidal plots which had an input of sand from the adjacent sand dunes. Silicate and ammonium porewater concentrations were higher in the lower intertidal plots, but phosphate was not affected by tidal height.

The organic carbon content of the leaves was higher than in the roots. Small variations were expected as more structural components are situated in the leaves than in the roots, but the leaves had nearly twice as much carbon as the roots/rhizomes. The leaf carbon content was not affected by tidal height, current velocity or lugworm exclusion. It was generally lower here than reported in other studies for the same species or for *Zostera marina*, which often coexists with *Z. noltii*, and the marked difference between leaves and roots/rhizomes has also not been reported before (to this author's knowledge). The roots/rhizomes of *Z. noltii* had less carbon in the lower intertidal plots than in the upper plots, which could be due to reduced water movement or heightened light availability and resulting carbon storage in the rhizomes.

The nitrogen content was also higher in the leaves than in the roots/rhizomes, but was not affected by any other factor in either leaves or roots/rhizomes. It was also generally comparable with the results from other studies.

4. Chapter 4: Comparing the carbon and nitrogen content in the sediment and seagrass material from three different seagrass meadows

4.1 Introduction

In the preceding chapter the carbon and nitrogen content of *Zostera noltii* transplants was investigated. This chapter deals with the carbon and nitrogen content of the seagrass species *Zostera marina* and *Zostera noltii* in naturally occurring meadows, and one mixed meadow from which seedling cores were cut and used in experiment 1 (chapter 2).

Both species occur on the German North Sea island Sylt, either growing monospecifically with few plants of the other species, or in mixed communities. *Z. marina* mostly grows annually from seeds with only a few plants surviving into the next year or growing from rhizome fragments. *Z. noltii* on the other hand reproduces mostly vegetatively and more plants survive into the next year. It is not known if these differences in life cycles also lead to different carbon and nitrogen contents, or if the two seagrasses have similar carbon and nitrogen contents in leaves and roots/rhizomes. Short (1987) reports that *Z. marina* growing on muddy sediment had more nitrogen in its tissue than *Z. marina* growing on sandy sediment. It is possible that sediment composition has a greater influence on carbon and nitrogen content of seagrasses than interspecific differences.

The aims of this experiment were to investigate the differences in carbon and nitrogen content in the leaves and roots/rhizomes of *Z. marina* and *Z. noltii* and the possible causes. Because of the different life cycles adapted it is possible that *Z. noltii* translocates carbon or nitrogen to the rhizomes for storage with the onset of autumn and leaf loss. It would be expected that *Z. marina* has more carbon and nitrogen in the leaves than in the roots/rhizomes due to flowering and seed production.

4.2 Material and Methods

Seagrass and sediment samples from three different seagrass meadows around Sylt (July and October 2004) were taken to compare the carbon and nitrogen content in the sediment and seagrass material. Porewater and grain size samples were taken to allow further comparison.

4.2.1 Study sites

The samples were taken either in the Königshafen bay which lies inside the Sylt - Rømø Bight or in Puant Klent which lies within Hörnum Deep. For a detailed description of both bights and the Möwenberg and Puant Klent meadow please see chapter 2.

Gröning seagrass bed

The Gröning tidal flat is characterised by muddy sediment and also lies within the Königshafen. The intertidal seagrass meadow consists of *Zostera marina* plants which reproduce via seeds (sexually) and occur annually with only a few adult plants surviving the winter. Porewater salinity ranged from 31.6 psu in July to 29.5 psu in October 2004.

4.2.2 Experimental set up

Seagrass, porewater and sediment samples were collected in the Gröning, Möwenberg and Puant Klent tidal flat in July and October 2004. The seagrass samples (n = 6) were taken with a box corer (10 cm by 10 cm), rinsed in freshwater and measurements recorded. The samples were then dried (see chapter 2 for biomass treatment) and a small sub sample (2-6 mg) was taken for the carbon and nitrogen analysis. Sediment (n = 6) and porewater (n = 6) samples were treated as described in chapter 3. Shoot density was measured as described in chapter 2.

Carbon and nitrogen analysis of sediment and seagrass samples

The sediment samples were freeze dried and larger shell particles were removed by hand. Then the samples were pulverised in a swing mill for 30 s to homogenise the sample. The net weight of each sample consisted of 50 – 70 mg. Afterwards each sample was treated with 30 µl fresh H_2O, then 10 µl 6 n HCl was added to

remove limestone. The samples were dried again and then analysed in a Thermo Finnigan Eager 300 (see table 7 for details).

The seagrass samples were also freeze dried and separated into above and below ground samples. Samples were homogenised by hand using mortar and pestle. Net weight consisted of 2 – 6 mg per sample. Each sample was treated with 10 µl fresh H_2O, then 10 µl 4 n HCl was added to remove limestone or epiphytes.

Table 7: The different methodical settings of the C/N analyser for sediment and seagrass samples

Sample type	Temperature left furnace	Gas flow(ml/min)	Injection time	Standard used	Capsule
Sediment	1000°C	Helium 120, Oxygen 300, Reference 100	10 seconds	Aspartic acid	Silver
Seagrass	900°C	Helium 110, Oxygen 300, Reference 100	8 seconds	Acetanilide	Tin

All data were analysed using the JMP software package.

4.3 Results

Three different seagrass meadows (Möwenberg, Gröning and Puant Klent) were studied to see if the carbon and nitrogen content in the plant material differed between *Zostera marina* and *Zostera noltii*, sampling in summer and autumn and on sandy and muddy sediments. The data were analysed for the differences amongst meadows and over time. Puant Klent meadow is a mixed seagrass meadow where both species occur. Gröning meadow is dominated by *Z. marina* and Möwenberg meadow is dominated by *Z. noltii* plants.

4.3.1 Seagrass shoot density and biomass

The shoot density was recorded in each meadow in July and October 2004 (see fig. 54). Even though the *Z. marina* plants from Gröning had the lowest shoot density (MANOVA with interaction, meadow: $F_{2,15} = 67.3388$; $p = <0.0001$; time x meadow: $F_{2,15} = 23.6092$; $p = <0.0001$), they had the highest above ground biomass (see fig. 55) compared with the other two meadows (MANOVA with interaction, meadow: $F_{2,15} = 25.3575$; $p = <0.0001$; time x meadow: $F_{2,15} = 18.2556$; $p = <0.0001$). The

shoot density did not change over time significantly, but the above ground biomass of *Z. marina* decreased from July to October whilst the below ground biomass increased (see fig. 56, Scheirer Ray Hare test, meadow: p = <0.0001).

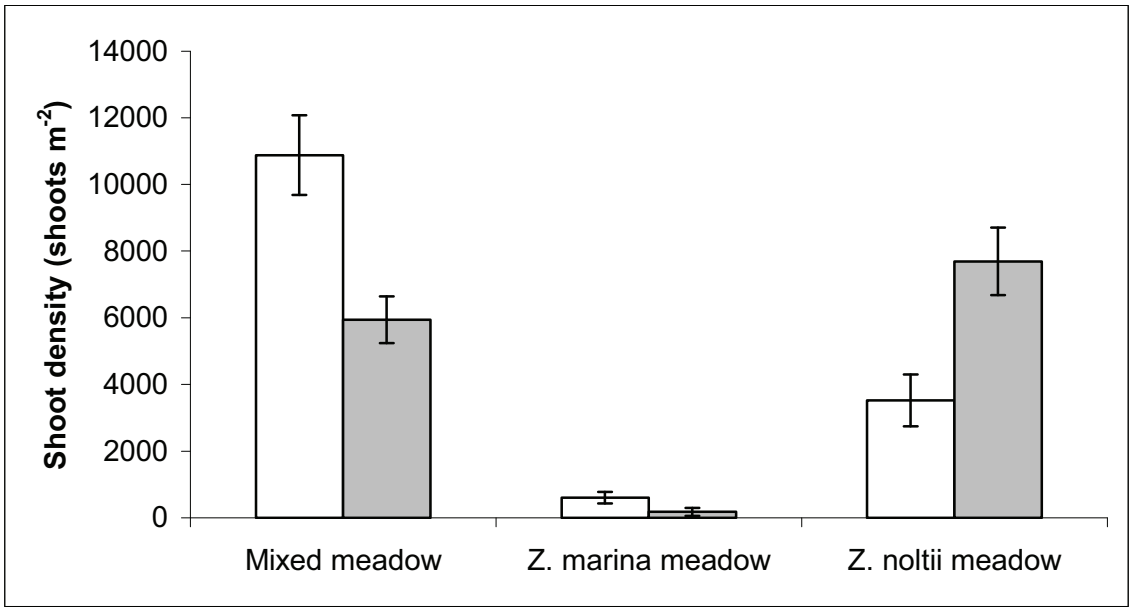

Figure 54: Seagrass shoot density in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factors meadow and interaction between time and meadow. White columns = July 2004, grey columns = October 2004.

The *Z. noltii* plants from the Möwenberg seagrass bed had the second highest shoot density from the three sites both in July and October but the lowest above ground biomass. The shoot density and above ground biomass increased from July to October whilst the below ground biomass did not alter significantly over time.

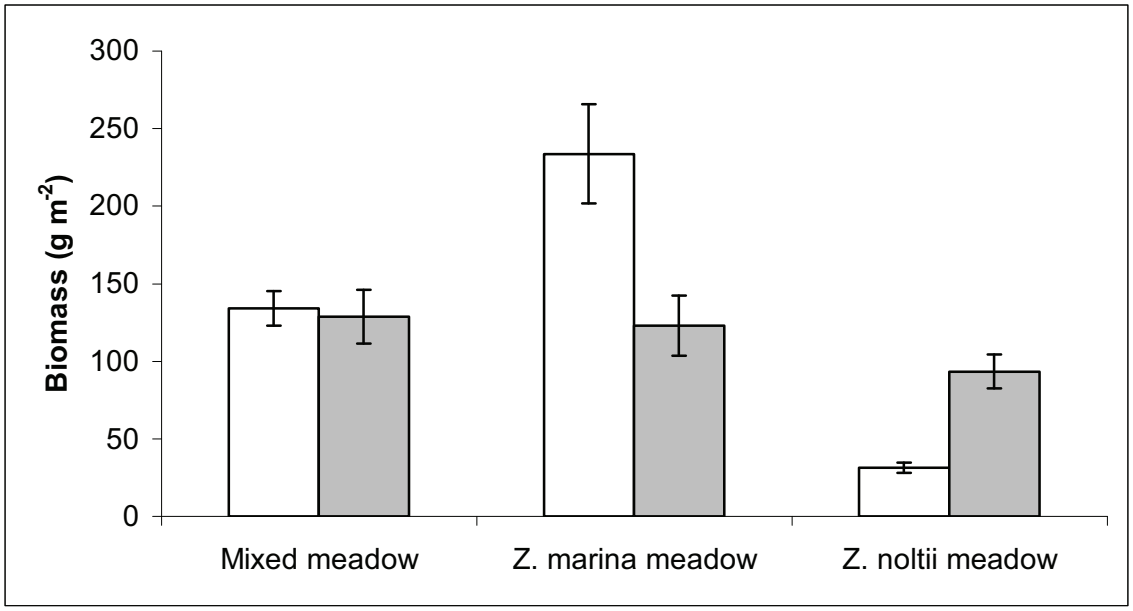

Figure 55: Above ground seagrass biomass in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factors meadow and interaction between time and meadow. White columns = July 2004, grey columns = October 2004.

In the mixed seagrass bed in Puant Klent the shoot density was highest from all three sites, the above ground biomass was second highest after the pure *Z. marina* meadow. The shoot density decreased from July to October, as did the below ground biomass, but the above ground biomass did not change over time.

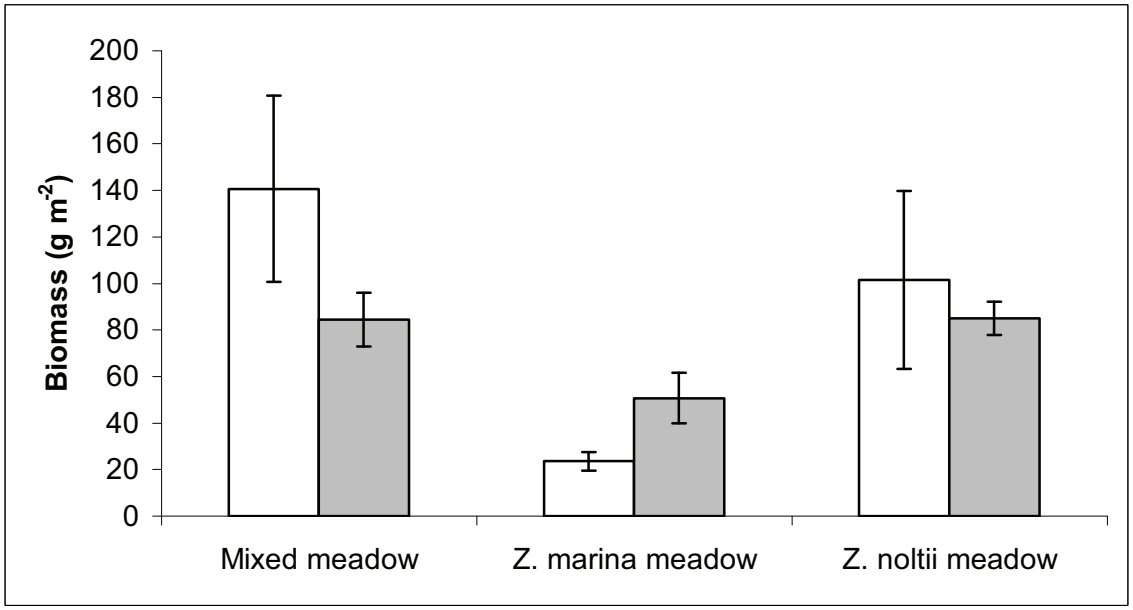

Figure 56: Below ground seagrass biomass in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factor meadow. White columns = July 2004, grey columns = October 2004.

4.3.2 Grain size analysis

Five sediment parameters were analysed in each meadow: grain size median, *phi* value, sorting coefficient QD, percent sand (sandy fraction, > 63 µm < 1,000 µm) and percent mud (fine fraction, < 63 µm). The samples were all taken in July 2004 (see table 8).

Table 8: Grain size results of the C/N comparison in three seagrass meadows around the island of Sylt, studying the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factor meadow.

Meadow	Median (µm)	*Phi*	Sorting coefficient QD	% Sand	% Mud
Puant Klent	270.59	1.89	1.59	81.56	18.44
Gröning	36.79	4.97	3.56	34.77	65.23
Möwenberg centre	240.56	2.06	1.89	77.95	22.05
Möwenberg edge	234.26	2.1	1.69	79.23	20.77
Oneway ANOVA ($F_{3, 20}$ value)	219.2151	78.5702	9.2231	54.6486	54.6486
p value	<0.0001	<0.0001	0.0005	<0.0001	<0.0001

The sediment in the Puant Klent meadow has the largest amount of sand and the least amount of mud (fine fraction) from the three meadows. It has the biggest particles (see median) and the lowest *phi* value. The sediment would be classed as medium sand (see material and methods in chapter 3 for classification) and has the most well sorted sediment from these three meadows even though it has a relatively high sorting coefficient.

In comparison, the sediment from the Gröning meadow has the largest amount of mud and the least amount of sand. Correspondingly, it has the smallest median and a high *phi* value. The sediment is poorly sorted and the least sorted from the three meadows. It would be classed as very coarse silt.

In the Möwenberg meadow, the samples were taken in the centre and at the edge of the meadow. The results for edge and centre are similar but not identical. The results show that the Möwenberg meadow is more similar to Puant Klent than to the Gröning meadow, despite being geographically much closer to Gröning. The centre has a high sand and small mud fraction, even though it is lower than the Puant Klent meadow. The median is high, if lower than it is in Puant Klent, but the *phi* value is higher than in Puant Klent. The sediment would be classed as fine sand

both at the edge and the centre. The sediment from the edge has more sand and less mud than the centre samples has. The median value from the edge is slightly lower and the *phi* value slightly higher. The sorting coefficient is lower at the edge than in the centre, suggesting a better-sorted sediment at the edge than at the centre, but is higher at both sampling stations than in the Puant Klent meadow.

4.3.3 Porewater analysis

Porewater samples were taken in all three meadows in July and October 2004. The porewater samples were analysed for salinity, silicate, phosphate and ammonium concentration.

Salinity

Salinity was generally higher in July at a time of comparably warm weather than in October (see fig. 57 below). It also differed between meadows, being highest in Puant Klent (Repeated measurements MANOVA, meadow:
$F_{2,15} = 9.2886$; $p = 0.0024$; time: $F_{2,15} = 55.6322$; $p = < 0.0001$).

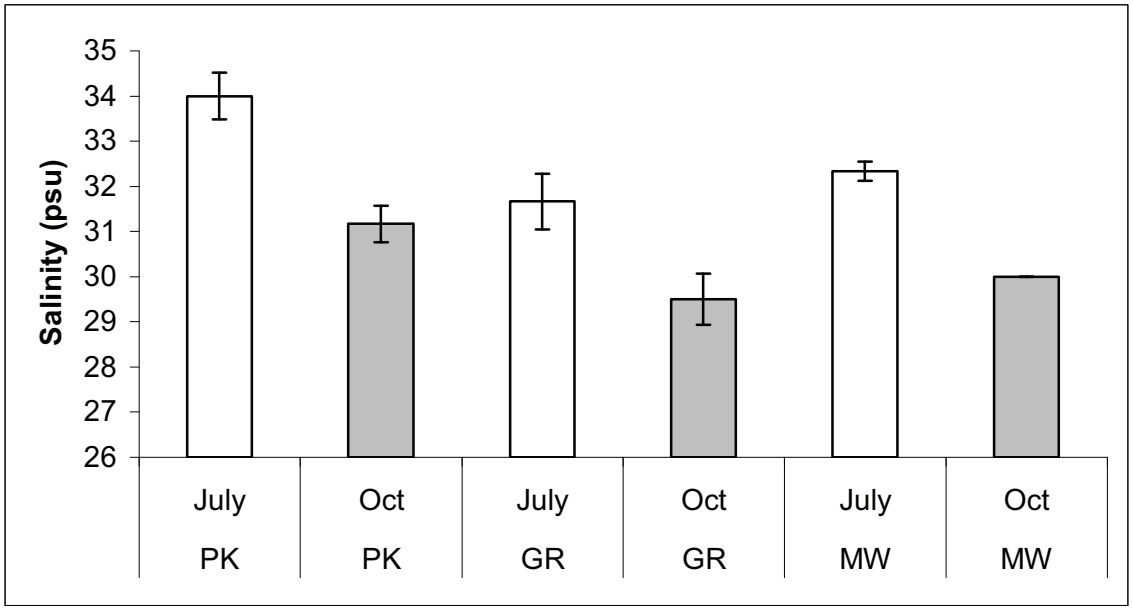

Figure 57: Porewater salinity results for the C/N comparison experiment in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factors meadow and time. White columns = July 2004, grey columns = October 2004. PK = Puant Klent; GR = Gröning, MW = Möwenberg.

Silicate and phosphate

The silicate concentration differed significantly among the meadows (repeated measurements MANOVA, meadow: F 2,15 = 24.7445; p = <0.0001), being highest in October in the Gröning meadow (see fig. 58 below). It increased in the Gröning

meadow from July to October but remained at the same level in Puant Klent and Möwenberg.

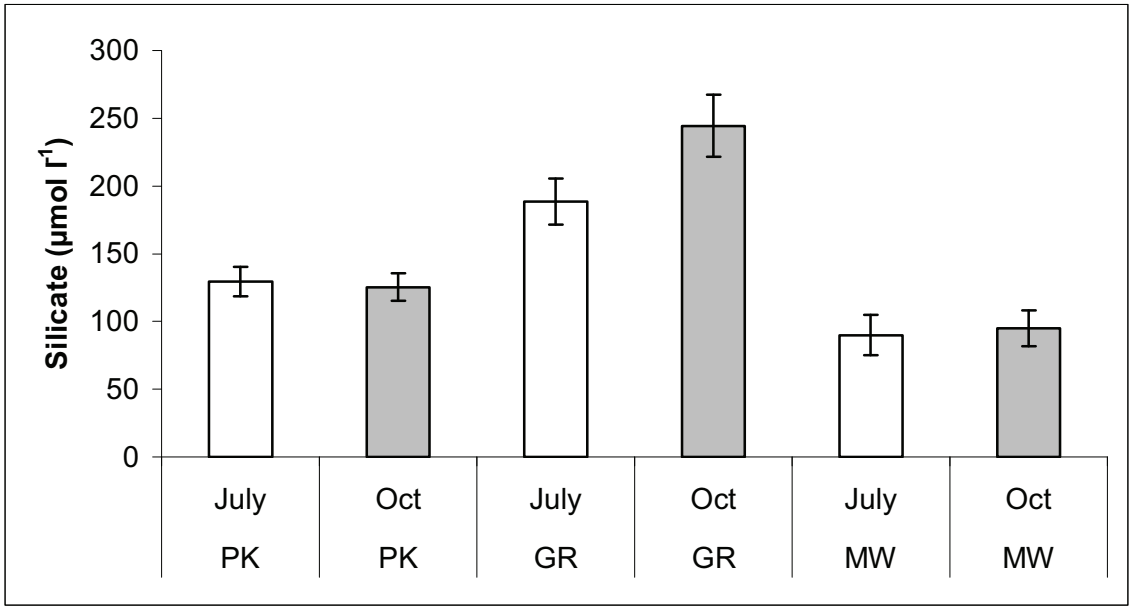

Figure 58: Porewater silicate content of the C/N comparison experiment in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factor meadow. White columns = July 2004, grey columns = October 2004. PK = Puant Klent; GR = Gröning, MW = Möwenberg.

The phosphate concentration (see fig. 59) on the other hand was affected by the interaction between meadow and time (Repeated measurements MANOVA, meadow x time: $F_{2,15} = 7.0742$; $p = 0.0069$). Whilst it increased from July to October in both Puant Klent and Gröning meadow, it decreased in the Möwenberg meadow during the same period.

Spearman's rank order correlation indicates that there was a strong negative association between porewater phosphate levels and seagrass biomass in July, but not in October 2004 (see table 18 in the appendix). Below ground biomass is not correlated with phosphate levels either in July or October.

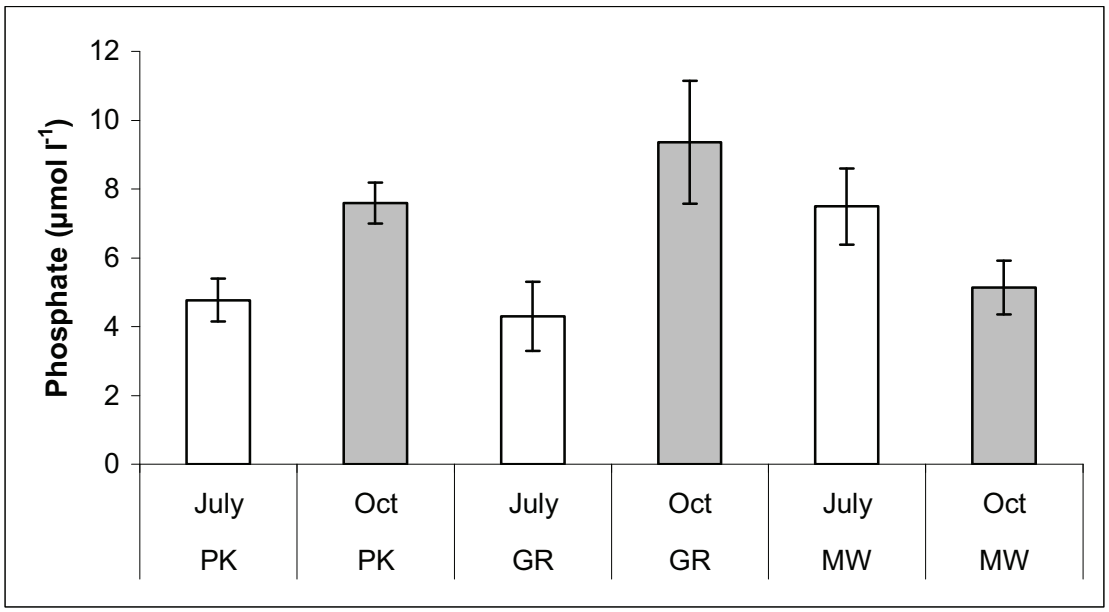

Figure 59: Porewater phosphate content of the C/N comparison experiment in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factor interaction between meadow and time. White columns = July 2004, grey columns = October 2004. PK = Puant Klent; GR = Gröning, MW = Möwenberg.

Ammonium

As for silicate and phosphate, the highest ammonium amount was found in the Gröning meadow in October (see fig. 60 below) whilst the lowest ammonium amount was found in Puant Klent (Repeated measurements MANOVA, meadow: $F_{2,15} = 6.8741$; $p = 0.0076$; time: $F_{2,15} = 44.9310$; $p = <0.0001$). In July, the ammonium concentration was much lower in all three meadows compared with October.

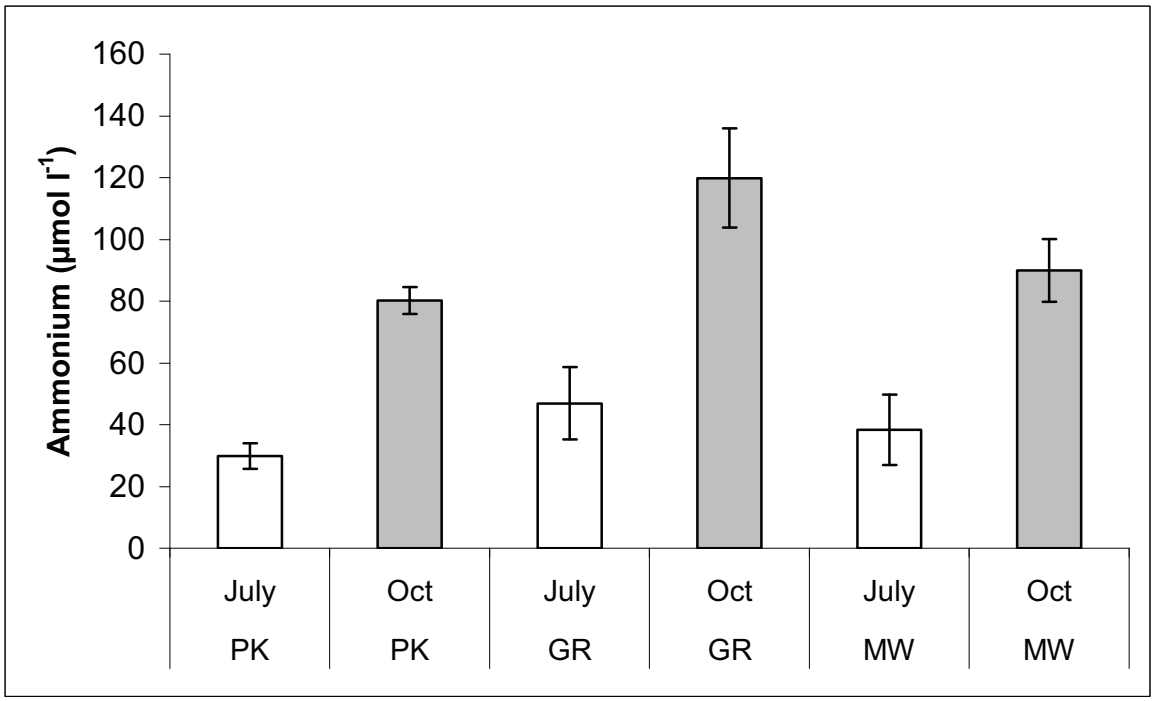

Figure 60: Porewater ammonium content of the C/N comparison experiment in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factors meadow and time. White columns = July 2004, grey columns = October 2004. PK = Puant Klent; GR = Gröning, MW = Möwenberg.

4.3.4 Carbon and nitrogen content in seagrass samples from three different meadows

The carbon and nitrogen content and the carbon/nitrogen ratio in the two seagrass species *Zostera noltii* and *Z. marina* (samples were collected in three different meadows) were analysed, investigating if the carbon and nitrogen content in the plant material differed between *Zostera marina* and *Zostera noltii*, sampling in summer and autumn and sandy and muddy sediments. The plant material was separated into above and below ground samples and samples were collected in July and October 2004.

Carbon content in the above and below ground samples

The carbon content in the above ground samples (see fig. 61 below) differed amongst the seagrass meadows and over time, and there was also interaction between meadow and time. The carbon content was higher in October than in July in *Z. marina* (Gröning meadow) and also in the mixed species meadow (Puant Klent), but it remained similar in *Z. noltii* from July to October (repeated measurements MANOVA, time: $F_{1,15} = 11.1494$; $p = 0.0045$). Whilst the carbon content was higher in *Z. noltii* in July than in *Z. marina* or the mixed meadow, by October it was only slightly higher in *Z. noltii* than in *Z. marina*. It was highest in the mixed meadow (repeated measurements MANOVA, meadow: $F_{2,15} = 4.1915$; $p = 0.0358$; interaction: $F_{2,15} = 9.3360$; $p = 0.0023$).

The carbon content of the below ground samples (see fig. 62 below) differed between the three meadows (repeated measurements MANOVA, meadows: $F_{2,15} = 4.7817$; $p = 0.0247$). Whilst it did not differ in time significantly for either *Z. marina* or *Z. noltii*, it increased from July to October in the mixed meadow. In contrast to the above ground samples, there was no significant difference between the two seagrass species. The carbon content was slightly higher in *Z. noltii* both in July and in October, but not significantly so.

Nitrogen content in the above and below ground samples

Zostera marina had significantly less nitrogen in its above ground material in July than in October (see fig. 63 below) and the same is true for the above ground material from the mixed meadow. *Zostera noltii* on the other hand had nearly the same amount of nitrogen in its above ground material at both times (repeated measurements MANOVA, time: $F_{1,15} = 40.7548$; $p = <0.0001$; meadow: $F_{2,15} = 11.2804$; $p = 0.001$; interaction between time and meadow: $F_{2,15} = 9.2816$; $p = 0.0024$).

The below ground material from *Z. marina* was again lower in nitrogen in July than in October (see fig. 64), but not significantly so. The mixed seagrass material also showed this trend, but the difference was also not significantly different. However, the below ground material from *Zostera noltii* had more nitrogen in July than in October and this resulted in an interaction between time of sampling and

meadow/species (repeated measurements MANOVA, interaction between time and meadow: $F_{2,15} = 10.3583$; $p = 0.0015$).

Carbon/nitrogen ratio in the above and below ground samples

The C/N ratio was much higher in the below ground biomass than in the above ground biomass. The above ground biomass also differed significantly between the species and over time.

The above ground biomass of the mixed meadow (see fig. 65) had the highest C/N ratio in July and October (15.35 and 12.05, respectively). *Z. noltii* had the lowest C/N ratio and *Z. marina* the second highest. In October, *Z. marina* had the lowest C/N ratio and *Z. noltii* the second highest (Scheirer Ray Hare test, meadow: $p = 0.02$; time: $p = 0.0001$).

The C/N ratio in the below ground material (see fig. 66) did not differ between meadows or time, or was affected by any significant interaction of the two. The highest C/N ration was measured during July in the mixed meadow (27.79), values ranged between 27.29 and 17.63.

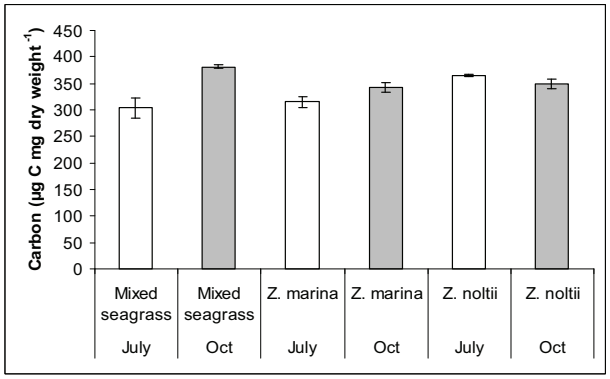

Figure 61: Above ground seagrass carbon content of the C/N comparison experiment in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factors time, meadow, and interaction between time and meadow. White columns = July 2004, grey columns = October 2004.

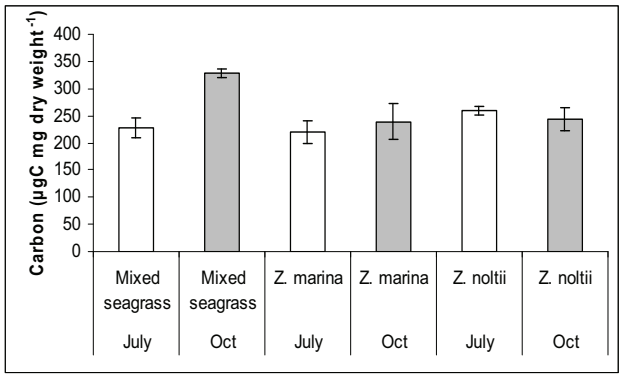

Figure 62: Below ground seagrass carbon content of the C/N comparison experiment. The results are significant for the factor meadow.

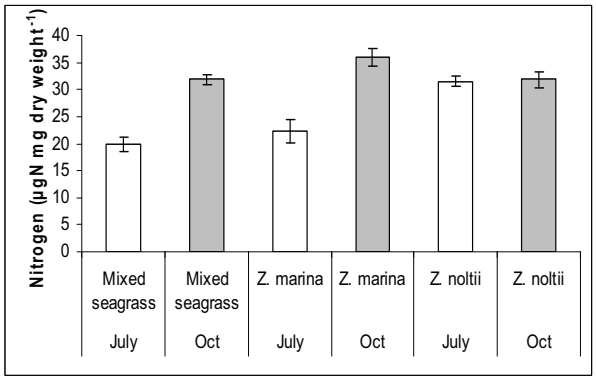

Figure 63: Above ground seagrass nitrogen content of the C/N comparison experiment. The results are significant for the factors meadow, time and their interaction.

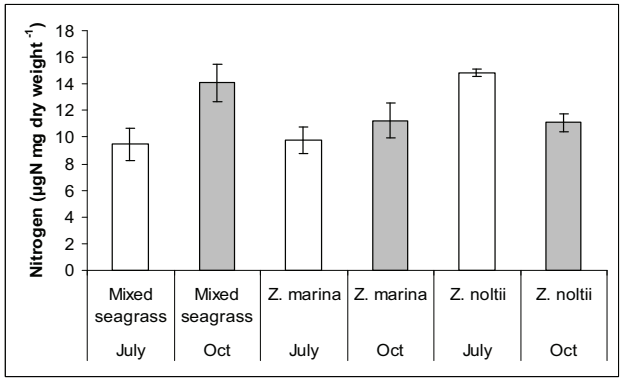

Figure 64: Below ground seagrass nitrogen content of the C/N comparison experiment. The results are significant for the interaction between meadow and time.

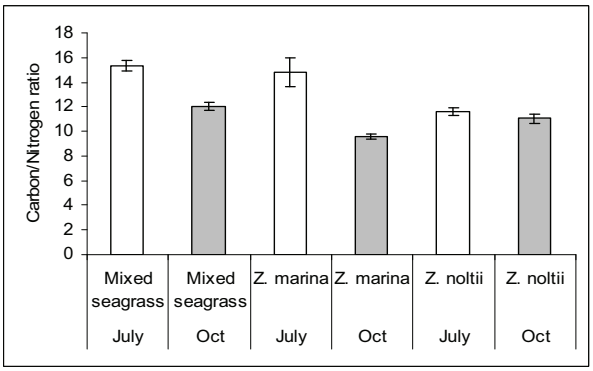

Figure 65: Above ground seagrass carbon/nitrogen ratio of the C/N comparison experiment. The results are significant for the factors meadow and time.

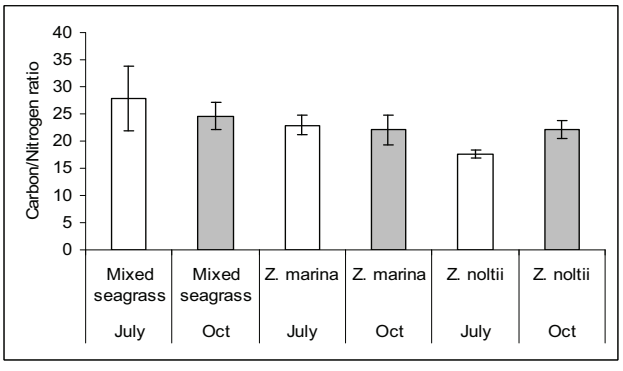

Figure 66: Below ground seagrass carbon/nitrogen ratio of the C/N comparison experiment. The results are not significant for any factor.

4.3.5 Carbon and nitrogen content in the sediment surrounding seagrasses

The carbon (C) and nitrogen (N) content and the carbon/nitrogen (C/N) ratio in the sediment around seagrass plants from Puant Klent, Gröning and Möwenberg were analysed and investigated for the same aims as above. Samples were collected in July and October 2004.

The carbon content increased significantly from July to October in all three meadows (see fig. 67 below, repeated measurements MANOVA, time: $F_{1,15} = 11.6299$; $p = 0.0039$). At both times it was highest in the sediment from the *Zostera marina* meadow with not much difference between the mixed and the *Z. noltii* meadow (repeated measurements MANOVA, meadow: $F_{2,15} = 33.6536$; $p = <0.0001$; meadow x time: $F_{2,15} = 5.4814$; $p = 0.0163$).

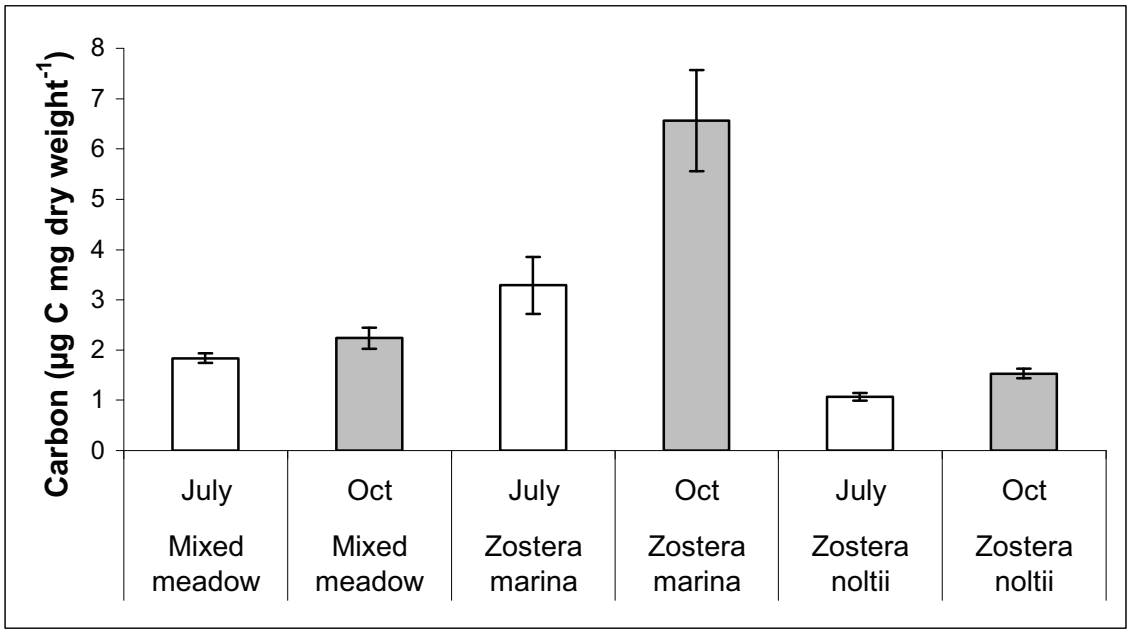

Figure 67: Sediment carbon content of the C/N comparison experiment in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factors meadow, time and interaction between time and meadow. White columns = July 2004, grey columns = October 2004.

The amount of nitrogen in the sediment (see fig. 68) was also highest in the *Z. marina* meadow, both in July and October. As with carbon content, the nitrogen content was higher in October in all three locations (Scheirer Ray Hare test, meadow: p = <0.0001, time: p = 0.009).

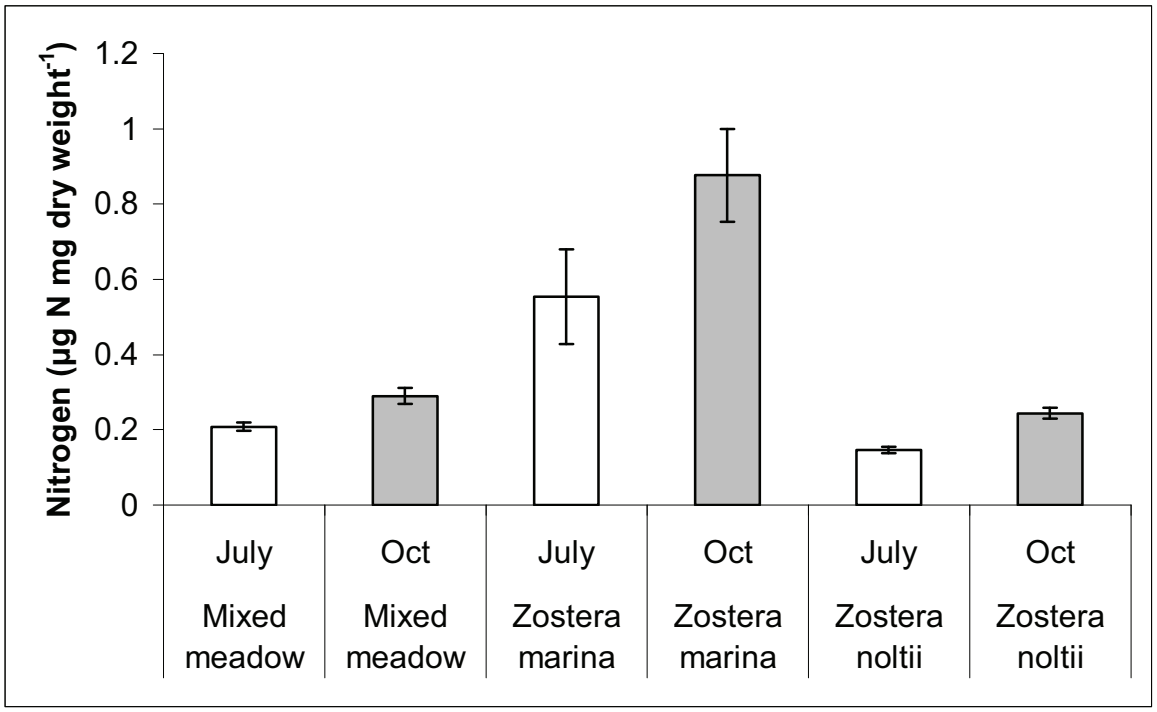

Figure 68: Sediment nitrogen content of the C/N comparison experiment in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factors meadow and time. White columns = July 2004, grey columns = October 2004.

The carbon/nitrogen ratio differed significantly amongst the three sites (see fig. 69, repeated measurements MANOVA, meadow: $F_{2,15} = 14.6794$; $p = 0.0003$; meadow x time: $F_{2,15} = 7.8758$; $p = 0.0046$). It was highest in both July and October in the sediment from the mixed meadow, but decreased from July to October. Whilst the carbon nitrogen ratio increased slightly from July to October in the sediment from the *Zostera marina* meadow, it decreased slightly over the same time period in the *Z. noltii* meadow.

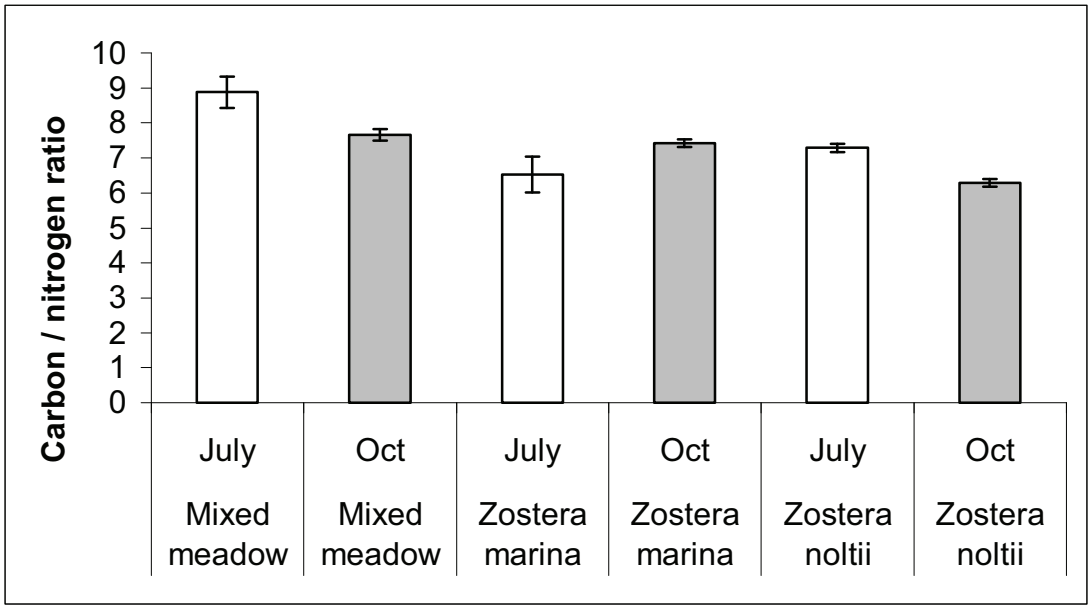

Figure 69: Sediment carbon/nitrogen ratio of the C/N comparison experiment in three meadows around the island of Sylt investigating the effects of species, season and sediment on carbon and nitrogen content. The results are significant for the factors meadow and interaction between time and meadow. White columns = July 2004, grey columns = October 2004.

4.4 Discussion

4.4.1 Seagrass development in three different meadows

Seagrass shoot densities and biomasses were monitored in two monospecific and one mixed seagrass meadow. Shoot density decreased in the mixed meadow as well as the *Z. marina* meadow from July to October, but increased in the *Z. noltii* meadow. The mixed seagrass meadow had the highest shoot density of all three meadows in July, but not the highest above ground biomass. Whilst the monospecific *Z. marina* plants had the lowest shoot density in July and October, they had the highest above ground biomass in July. By October 2004, the above ground biomass of the three meadows showed no significant differences any longer, because the shoot density and above ground biomass of the monospecific *Z. noltii* had increased. The development of the below ground biomass showed a

different pattern, with a decrease in below ground biomass from July to October in the mixed and the *Z. noltii* meadow, but an increase in below ground biomass in the *Z. marina* meadow.

These findings suggest that a) the growth maximum of *Zostera marina* was in July 2004, which was also true for the mixed seagrass meadow, but the monospecific *Zostera noltii* meadow had the highest biomass at a later point, probably August – September 2004 and b) the shoot density and above ground biomass of the mixed seagrass bed consisted mainly of *Zostera marina* plants (it followed the pattern of the monospecific *Z. marina* population), but the below ground biomass belonged mainly to *Z. noltii* plants.

4.4.2 Grain size and porewater content

The mixed seagrass meadow growing on the Puant Klent tidal flat had the most sand and the least mud, and would be classed as medium sand. In contrast, the sediment of the *Zostera marina* meadow in the Gröning tidal flat consisted mostly of mud and had the least sand, and would be classed as very coarse silt. The sediment of the Möwenberg seagrass bed had less sand and more mud than the Puant Klent tidal flat, and would be classed as fine sand.

The porewater salinity decreased in all three locations from July to October, and also differed between meadows. It was highest in the Puant Klent tidal flat, and lowest in the Gröning tidal flat. The Puant Klent meadow is situated in the Hörnum Deep which has more access to the North Sea than the Königshafen bay, in which the other two seagrass meadows are located. It seems likely that any decreases in salinity due to freshwater input (e.g. rainfall, runoffs from sand dunes, sewage plants) will be compensated by North Sea water much faster in Puant Klent than in the Königshafen which only has one tidal inlet. The salinity values fall within the salinity tolerance range for both species.

Volkenborn (2005) found silicate levels in the porewater of the upper eight centimetres (on the undisturbed 'ambient' plots) ranging between 45 – 100 µmol l^{-1} in July 2004 in the Möwenberg tidal flat. The silicate levels found in the porewater on the lugworm exclusion plots extracted in July and September 2004 (see preceding experiment, chapter 3) ranged between 55 – 120 µmol l^{-1}, which is

confirmed by the silicate levels from this experiment in the Möwenberg *Zostera noltii* meadow. Interestingly the silicate levels differ amongst the three meadows and are higher for the other two meadows. In Puant Klent, the silicate levels do not change from July to October and remain at 125 µmol l^{-1}. The highest silicate concentration can be found in the Gröning meadow, also showing an increase from July to October. The concentrations range from 185 to 250 µmol l^{-1}. These high levels of silicate are due to the accumulation of particles in the sheltered location. Increased levels of silicate could help the seagrasses to cope with high salinity levels (Kamermans, Hemminga & de Jong (1999)) and would thus be advantageous, but the meadow with the highest silicate levels also had the lowest salinity. It may help *Z. marina* cope with extreme high salinity events, but no such events were observed during this study. It is not known if increased silicate levels would also be advantageous for Z*. noltii* plants.

The phosphate concentration also showed a varied response in the three meadows. Whilst there was an increase from July to October in both Puant Klent and Gröning seagrass beds, there was a decrease in the Möwenberg seagrass beds. This would indicate that phosphate was taken up in greater amounts by the monospecific *Zostera noltii* meadow which would be supported by the above ground growth maximum in August – September 2004. The opposite development can be seen in the monospecific *Z. marina* bed in the Gröning tidal flat, where the phosphate concentration in the porewater is lower in July when the *Z. marina* plants are still growing than in October when biomass is decreasing. As there is also a strong negative correlation between seagrass growth and phosphate levels, it seems likely that phosphate levels are reduced in the porewater because of seagrass growth.

The ammonium concentration on the other hand shows an increase from July to October in all three meadows. It was also higher in the Gröning *Z. marina* meadow than in the other two meadows. The increase of ammonium levels in the porewater from July to October coincides with the above ground growth maximum for *Zostera marina* (i.e. high biomass in July and therefore uptake of ammonium to support growth) but not with *Z. noltii*. This would suggest that the growth of *Z. noltii* is not limited in any way by ammonium (nitrogen) levels in the porewater but could be

influenced by the available phosphate (see above). While the ammonium levels increase with the decreasing biomass of *Z. marina*, they do not decrease with the increasing biomass of *Z. noltii*. The phosphate concentration on the other hand increases with decreasing *Z. marina* biomass, and decrease in the *Z. noltii* meadow as the maximum above ground biomass is reached later in the year.

The highest concentration of nutrients (silicate, phosphate and ammonium) was found in the porewater of the monospecific *Zostera marina* meadow. This is reflected in the highest above ground biomass which was also in the *Z. marina* bed. Therefore it seems unlikely that the *Zostera marina* meadow on the muddy sediment is nutrient limited. The two seagrass beds growing on the sandy substrates have fewer nutrients available in the porewater. The phosphate concentrations are indeed reduced by growing *Z. noltii* plants, and seem low compared to the global average of 12 µmol/l (Hemminga & Duarte 2000). Holmer, Carta & Anderson (2006) report a P limitation at a sandy *Zostera marina* site compared with a muddy *Z. marina* location because most of the available P is bound to calcium and thus not available to the plants.

Phosphate can be taken up by either the leaves or the roots/rhizomes of the seagrass plants (Brix & Lyngby 1985). Brix and Lyngby (1985) found that *Zostera marina* takes up most of the required phosphorus through its leaves and takes up phosphorus from the sediment only when it is no longer available in the water column. In contrast, Pellikaan & Nienhuis (1988) have demonstrated that in *Z. marina* the uptake of the porewater phosphate is more important than the water column uptake. Furthermore, Holmer, Carta & Anderson (2006) found the dissolved inorganic phosphorus (P) concentration in the water column was not high enough to support seagrass growth, whereas the sedimentary phosphorus pools exceed the amount required by *Zostera marina*. Therefore it seems likely that porewater phosphorus is the main source of phosphorus in the seagrass meadows around Sylt as well. Unfortunately the phosphorus (P) concentration in the seagrass samples was not measured so it cannot be ascertained if the levels were above those reported by Duarte (1990) as the critical limit for growth.

Ammonium can also be taken up by either the roots/rhizomes or the leaves, but it is still not clear if the leaves or the roots/rhizomes take up the most ammonium. It seems that seagrasses are able to switch and that it will depend on environmental factors which form of uptake is more important. Irrespective of that, porewater generally has the higher concentrations of phosphate and ammonium compared with the water column (Hemminga 1998), even though not all of that will be readily available to the seagrasses.

If seagrass growth is limited by either of the main nutrients, phosphate limitations seems more likely. The amount of porewater phosphate follows the growth pattern of the seagrass in the three meadows monitored here, whereas the ammonium concentrations are not linked. Furthermore, Holmer, Carta & Anderson (2006) have proven that phosphorus is limiting *Z. marina* growth on sandy substrate and the same mechanism could be possible here. This hypothesis is further supported by the strong negative correlation between seagrass growth and porewater phosphate levels.

4.4.3 Carbon and nitrogen content of *Zostera marina* and *Z. noltii*

One of the aims of this PhD study was to find out if seagrasses transport carbon or nitrogen from the leaves to the roots/rhizomes with the onset of autumn to help them survive the winter. It has also been shown by Short (1987) that seagrasses growing on muddy substrate have a higher carbon content than those growing on sandy substrate. Therefore it would be expected to see the most carbon and nitrogen in the *Z. marina* leaves and roots/rhizomes.

The carbon content in the leaves of *Z. noltii* growing on sandy sediment (fine sand) was higher than that of *Z. marina* growing on muddy sediment (very coarse silt). This was unexpected as it was assumed that more nutrients would lead to more carbon in the leaves. The greatest increase in carbon content in the leaves from July to October was in the samples from the mixed meadow growing on medium sand.

In the root/rhizome tissue the greatest increase of carbon content can again be seen in the samples from the mixed meadow. The root/rhizome carbon content does not differ significantly between the two species growing monospecifically.

In general, the leaves had a higher carbon content in all three meadows compared with the roots and rhizomes. The carbon content in the leaves of *Z. marina* was higher in October than in July, whereas it remained constant in *Z. noltii*. This could be the result of producing flowers and fruits which could result in less carbon being available for the non flowering leaves. After the seeds have been released, more carbon can be invested in the leaves again. It would have been expected to see an internal translocation of carbon from the leaves to the roots in autumn to prepare for the loss of leaves (e.g. by grazing of water fowl), but this was not the case in either species. Whilst the carbon content remains constant in the *Z. noltii* meadow, there is an increase in both the leaves and roots/rhizomes in the mixed and an increase in the leaves in the *Z. marina* meadow. There is no indication that either species translocates carbon or nitrogen from the leaves to the roots/rhizomes for storage.

Table 9: Carbon, nitrogen, phosphorus and C/N ratio in *Zostera marina* and *Z. noltii* populations worldwide

Seagrass species	**% carbon DW**	**% nitrogen DW**	**C/N (wt)**	**% phosphorus DW**	**Location**	**Source**
Average seagrass values (leaves)	33.6 ± 0.31	1.92 ± 0.05 (median 1.82%)	17.5	0.23 ± 0.011 (median 0.2%)	global	Duarte 1990
Zostera marina (leaves)	34.5 ± 0.8 (mud); 35.5 ± 1.5 (sand)	2.4 ± 0.3 (mud); 1.8 ± 0.2 (sand)	14.38 (mud); 19.72 (sand)	0.47 ±0.06 (mud); 0.39 ± 0.04	New Hampshire, USA (mesocosms)	Short 1987
Z. marina (rhizome and roots)	36.2 ± 0.5 (mud); 36.6 ± 2.1 (sand)	1 ± 0.3 (mud); 0.6 ± 0.1 (sand)	36.2 (mud); 61 (sand)	0.46 ±0.06 (mud); 0.37 ± 0.08	New Hampshire, USA (mesocosms)	Short 1987
Z. marina (leaves)	app. 37 (sand); app. 40 (mud)	app. 2.5 (sand); app. 2.2 (mud)	14.8 (sand); 18.18 (mud)		Königshafen	Albrecht et al. 1995
Z. marina (rhizome and roots)	app. 32 (sand); app. 34 (mud)	app. 0.4 (sand); 0.3 (mud)	80 (sand); 113.3 (mud)		Königshafen	Albrecht et al. 1995
Z. noltii		1 - 2.2	15 -		Punta S.	Pirc &

(leaves)			35		Pietro, Ischia	Wollenweber 1988
Z. noltii (rhizome and roots)		0.75 – 0.85	30 - 70		Punta S. Pietro, Ischia	Pirc & Wollenweber 1988
Z. marina (green leaves)	36.3	2.33	15.58	0.38	Tomales Bay, USA	Fourqurean et al. 1997
Z. marina (leaves)	35.78	2.82 ± 0.07	12.69	0.28 ± 0.01	Zandkreek	Stapel & Hemminga 1997
Z. marina (leaves)	35.18	1.98 ± 0.11	17.77	0.27 ± 0.01	Veerse Meer	Stapel & Hemminga 1997
Z. capricorni (leaves)	40.5	2.1	19.29		Moreton Bay, Australia	Grice et al. 1996
Z. noltii (leaves)	34.9 – 36.5 (sand)	3.1 – 3.2 (sand)	10.9 – 11.8 (sand)		Königshafen, Germany	This study (exp. 3)
Z. noltii (rhizome and roots)	24.3 – 26 (sand)	1.1 – 1.5 (sand)	17.3 – 22.1 (sand)		Königshafen, Germany	This study (exp. 3)
Z. marina (leaves)	31.5 – 34.2 (mud)	2.2 – 3.6 (mud)	9.5 – 14.3 (mud)		Königshafen, Germany	This study (exp. 3)
Z. marina (rhizome and roots)	22 – 24 (mud)	1 – 1.1 (mud)	21.8 – 22 (mud)		Königshafen, Germany	This study (exp. 3)
Mixed seagrass leaves	30.4 – 38.2 (sand)	2 – 3.2 (sand)	11.9 – 15.2 (sand)		Königshafen, Germany	This study (exp. 3)
Mixed seagrass roots/rhizomes	22.9 – 32.8 (sand)	0.9 – 1.4 (sand)	23.4 – 25.4 (sand)		Königshafen, Germany	This study (exp. 3)

Comparing carbon contents reported in the scientific literature with the results from this experiment (see table 9), it becomes apparent again that both in summer and autumn the carbon content of the leaves is much higher than in the roots/rhizomes. The carbon content of the leaves (of both species) is at the lower range of the reported values, but such a marked difference between leaves and roots/rhizomes has not been reported before (as far as this author is aware). In chapter 3 of this thesis, the same phenomenon has been described for transplanted *Z. noltii* cores.

This would suggest that the difference in carbon content is a general feature of these three populations which is maintained over considerable time (3 months).

As with carbon, the leaves had a higher nitrogen content than the roots and rhizomes. Whilst it is again surprising to see these differences within the same plants, it is consistent with what other authors have reported (see table 9).

In July, the leaves of *Z. noltii* had the highest nitrogen content of all three meadows and it remained constant. In contrast, the nitrogen content of the *Z. marina* leaves increased from July to October, as it did with the material from the mixed seagrass meadow. By October the nitrogen content in the leaves of *Z. marina* was higher than that of *Z. noltii*. It would have been expected to find the highest nitrogen content in the *Z. marina* leaves growing on the muddy sediment at both times of the year, as described by Short (1987), but this was not the case. Instead the *Z. noltii* plants growing on a sandy substrate had the highest nitrogen content in July, but by October there was no longer a significant difference between the three meadows.

The roots and rhizomes of *Zostera marina* also increased in nitrogen content from July to October, but there is a decrease in nitrogen content of the roots and rhizome of *Z. noltii* from July to October. Whilst the same cannot be seen in the leaves, this decrease in nitrogen content of the below ground biomass happened at the same time as an increase in plant biomass of *Z. noltii*. It seems likely that nitrogen was translocated from the roots to the meristems to support the increasing above ground biomass.

It is possible that any internal translocation of either carbon or nitrogen would occur later than October. However, at this point in time seagrass leaves are already being lost and grazing by water fowl has already started. Therefore the hypothesis that either the perennial *Z. noltii* or the annual *Z. marina* populations transfer carbon and/or nitrogen to their root/rhizomes has to be discarded.

The carbon:nitrogen (C/N) ratio in the leaves does not change in *Zostera noltii* from July to October. Duarte (1990) states that nutrient limited seagrasses should have a high C/N ratio and the tissue should be depleted of nitrogen. He also states that a

C/N ratio of 12 indicates a stable state at which further nutrient enrichment no longer leads to a lower C/N value. By October 2004, the leaves from all three seagrass meadows range around the C/N value 12, but in July both the values from the *Z. marina* meadow and the mixed meadow are higher. This would indicate that in July these two meadows were nutrient limited (they also had the highest above ground biomass then), whereas the *Z. noltii* meadow with its later peak in leaf biomass was not nutrient limited in July or October.

The below ground C/N ratio is higher in all three meadows than the above ground C/N. However, the root and rhizome C/N ratio did not differ between species or time. It is not clear if the higher C/N ratio in the below ground tissue is the result of nutrient limitation in any way (for example, because more nutrients are translocated from the roots to the leaves than the reverse) or a result of other processes in the leaves resulting in more carbon and nitrogen in the leaves.

4.4.4 Carbon and nitrogen in seagrass sediment

As expected, the highest carbon and nitrogen content was found in the muddy sediment of the *Zostera marina* meadow. The sediments of the other two meadows, being classified as sandy sediments, had a much lower carbon and nitrogen content. In all three meadows, carbon and nitrogen content increased from July to October. The largest increase was also found in the *Z. marina* meadow. As the leaf and root/rhizome biomass decreased at the same time in the meadow, it is possible that the increased amounts of carbon and nitrogen in the sediment stem from decomposing seagrass material of this meadow. The slight increase in carbon and nitrogen in the *Z. noltii* sediment could also be due to decomposing biomass, but as the biomass peak was reached later in the year (August/September) the impact of decomposing seagrass material might manifest at a later time. Increased hydrodynamics may also mean that more seagrass material is washed away than decomposing in the meadow where it originated.

The carbon/nitrogen ratio also differed between the three locations, being highest in the mixed meadow sediment. C/N decreased in the mixed meadow from July to October, as it did in the *Z. noltii* meadow, but it increased in the *Z. marina* meadow.

4.5 Conclusion

Neither *Z. marina* nor *Z. noltii* translocated carbon or nitrogen from their leaves to their roots and rhizomes in preparation for the cold season. Seasonal differences in carbon and nitrogen content are due to different growth maxima, with *Z. noltii* reaching maximum biomass at a later time in the year than *Z. marina*. Therefore the hypothesis that either seagrass species stores carbon or nitrogen in their roots/rhizomes to better survive the winter has to be rejected.

Seagrass growing on muddy substrate did not exhibit higher carbon or nitrogen content in either leaves or roots/rhizomes than those growing on sandy substrate. In fact the opposite was true for the populations studied here. In July *Z. noltii*, growing on sandy substrate, had more carbon and nitrogen in its above and below ground tissue than *Z. marina* or the mixed meadow. By October there was no longer a difference in the monospecifically growing plants. In the mixed meadow, the leaves had the highest carbon and second highest nitrogen concentration, and the roots/rhizomes had the highest carbon and nitrogen concentration. As this population also had a high density of seedlings, the carbon and nitrogen content in the tissue does not appear to be linked to life cycles.

No seagrass population studied here appears to be nitrogen limited, as porewater nitrogen (ammonium) concentrations do not fall or rise with biomass development. Phosphorus (phosphate) may be limiting seagrass growth as it appears to be linked to seagrass growth.

5. Chapter 5: Concluding remarks

The aim of this PhD study was to investigate the influence of the lugworm *Arenicola marina* and tidal height on seedlings of the two North European seagrass species *Zostera marina* and *Zostera noltii*. It became apparent during the experiment that the experimental lugworm exclusion plots were exposed to different tidal current velocities, so current velocity was also included as a factor influencing the seagrass development.

To compliment the findings of experiment 1, adult *Zostera noltii* plants were also transplanted onto the experimental plots. The influence of tidal height, tidal current velocities and lugworm exclusion on the carbon and nitrogen content in the seagrass tissue and surrounding sediment was investigated. The porewater nutrient content was also looked at to see if the dwarf seagrass was nutrient limited or benefited from raised nutrient concentrations on lugworm exclusion plots.

Contrasting the manipulated results (manipulated in the sense that the plants were grown on created lugworm free plots) of experiment 2, samples were taken in three naturally occurring seagrass meadows growing in different locations around Sylt and consisting of either monospecific seagrass stands or a mixed seagrass stands. Two of these seagrass beds also grow on different types of sediment (sandy and muddy) and furthermore the influence of season on carbon and nitrogen content in seagrass tissue was investigated.

Summarising the findings of the first experiment (see chapter 2) on the Möwenberg tidal flat, it becomes clear that current velocity and position on shore (i.e. tidal height) play a major role in growth and survival of the mixed seagrass transplants. Unfortunately, the effect of tidal height cannot be separated from the influence of tidal current velocity as the current velocity was reduced higher up the shore. The sediment was made up out of medium sand in the upper eulittoral and out of fine sand in the lower eulittoral which is due to the aeolian input from the adjacent sand dunes (Austen 1994). When current velocity was low (e.g. on the western plots in

the lower intertidal, see fig. 2 in chapter 2), then the conditions in the lower intertidal were more suitable for both seagrass species.

The lugworm *Arenicola marina* did influence seagrass growth, and in the most part negatively, but had less influence than current velocity. It should be noted, however, that in 2004 lugworm abundances were generally low, and lugworm bioturbation and indirect effects could be more influential when the *A. marina* is more abundant. As the effects were more pronounced with *Z. noltii* than with *Z. marina*, it would be expected to see this influence even more clearly with higher lugworm abundances. It also seems likely that dwarf seagrass was more susceptible to lugworm influence than eelgrass because of its smaller growth form. Furthermore, the seedlings of both species would be more susceptible than the adult plants or ramets (especially if these were still connected via their rhizomes to other ramets). As the seedlings of *Z. marina* emerged earlier at the sediment surface than those of *Z. noltii*, the eelgrass seedlings might also benefit from low lugworm abundances or activity earlier in the year.

Contrary to expectations Z. *noltii* turned out to be the more robust of the two seagrass species, as it managed to withstand the negative effects of high current velocity in the lower intertidal for longer. Bouma *et al.* (2005) have described *Z. noltii* as a plant that avoids hydrodynamic stress by bending and thus minimising drag forces at the cost of having a lower capacity to reduce wave energy than e.g. *Spartina anglica*. It is possible that *Z. noltii* shoots also have less stiffness than intertidal *Z. marina* shoots, which would allow it to withstand hydrodynamic stress longer than the eelgrass. The other possibility is that with its shorted and narrower shoots the dwarf seagrass has less area which would be affected by drag forces, and is therefore better able to withstand hydrodynamics compared with *Z. marina*.

The findings from the second experiment (see chapter 3) show that *Zostera noltii* biomass was influenced by tidal height and current velocity, in that low tidal current velocities led to increased biomass. As the current velocity was even lower in the upper eulittoral plots, the biomass increased in the upper intertidal plots compared with the lower intertidal plots. The lugworm *Arenicola marina* had a negative effect on dwarf seagrass growth, and increased the negative effects of strong current

velocity. The dwarf seagrass grew best on plots subjected to low tidal current velocities and with lugworm exclusion.

Silicate and ammonium porewater concentrations were higher in the lower intertidal plots, but phosphate concentrations did not differ between the two different tidal heights. Even though porewater nutrients were higher in the lower eulittoral, the negative influence of stronger hydrodynamics prevented the dwarf seagrass from exploiting the higher nutrient availability. Whilst it is likely that the higher hydrodynamics led to the washing out of porewater nutrients in the upper centimetre of the sediment (e.g. Rusch, Huettel & Forster 2000), the porewater concentration over the seagrass rhizosphere (the upper 8 centimetre of substrate) were not diminished. As the nutrient concentration was in fact higher in the lower intertidal, the increased biomass in the upper eulittoral was not linked to nutrient concentration (and thus an indirect effect of hydrodynamics) but instead can be linked directly to the direct effect of hydrodynamic forces on the seagrass plants.

The organic carbon content of the leaves was higher than of the roots. Small variations between leaves and roots were expected as more structural components are situated in the leaves than in the roots and carbon is one of the main components of these structures, but the leaves had nearly twice as much carbon as the roots/rhizomes.

The leaf carbon content was not affected by tidal height, current velocity or lugworm exclusion. It was generally lower here than reported in other studies (see table 6 in chapter 3) for the same species or for *Zostera marina*, which often co exists with *Z. noltii*, and the marked difference between leaves and roots/rhizomes has also not been reported before (to this author's knowledge). The roots/rhizomes of *Z. noltii* had less carbon in the lower intertidal plots than in the upper plots. This could be due to reduced water movement in the upper intertidal or heightened light availability and resulting carbon storage in the rhizomes.

The nitrogen content was also higher in the leaves than in the roots/rhizomes, but was not affected by any other factor. It was also generally comparable with the results from other studies.

Neither *Z. marina* nor *Z. noltii* translocated carbon or nitrogen from their leaves to their roots and rhizomes in preparation for the cold season. Seasonal differences in carbon and nitrogen content are due to different growth maxima, with *Z .noltii* reaching maximum biomass at a later time in the year than *Z. marina*. Therefore the hypothesis that either seagrass species stores carbon or nitrogen in their roots/rhizomes to better survive the winter has to be rejected.

Seagrass growing on muddy substrate did not exhibit higher carbon or nitrogen content in either leaves or roots/rhizomes than those growing on sandy substrate. In fact the opposite was true for the populations studied here. In July *Z. noltii*, growing on sandy substrate, had more carbon and nitrogen in its above and below ground tissue than *Z. marina* or the mixed meadow. By October there was no longer a difference in the monospecifically growing plants. In the mixed meadow, the leaves had the highest carbon and second highest nitrogen concentration, and the roots/rhizomes had the highest carbon and nitrogen concentration. As this population also had a high density of seedlings, the carbon and nitrogen content in the tissue does not appear to be linked to life cycles (i.e. the perennially growing *Z. noltii* storing carbon and/or nitrogen in its rhizomes to better survive the winter)

No seagrass population studied here appears to be nitrogen limited, as porewater nitrogen (ammonium) concentrations do not fall or rise with biomass development. Phosphorus (phosphate) may be limiting seagrass growth as it appears to be linked to seagrass growth

It has been suggested by Jacobs (1982) that low below ground biomass is an indicator of annual *Z. marina* populations as the rhizomes are mostly used for anchorage and there is limited branching and vegetative reproduction. This theory is supported by the findings from this study as indeed the roots/rhizome biomass is lower than in the above ground biomass, and the plants grew from seeds. However, the same does not appear to apply to *Z. noltii*. Even though the *Z. noltii* plants in the transplants grew mostly from seeds and not from rhizome fragments, there is no difference between the above and below ground biomass. However, seedlings have been rarely observed for this species and it is possible that even though the plants

studied here from seeds, more energy was transferred to maintenance (and thus into the rhizomes) and less into the production of flowering shoots which resulted into a balanced above and below ground growth.

In conclusion it can be said that the best environmental conditions for both seagrass species were found in the upper eulittoral. The increased nutrient availability in the lower intertidal did not result in a higher biomass. The current velocity measurements in July 2005 showed that current velocity was stronger in the lower intertidal, so while more nutrients were available in the lower intertidal, the stronger hydrodynamics here led to a reduced biomass.

These findings highlight yet again that hydrodynamics are the major factor influencing seagrass growth and survival, in particular as the impact of hydrodynamics is superimposed over every other factor influencing seagrass growth in the intertidal. Therefore the hydrodynamic regime of a potential restoration site should be carefully investigated before transplanting the seagrass.

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References

- Ackermann, J. D. (1986) Mechanistic implications for pollination in the marine angiosperm *Zostera marina*. *Aquatic Botany*, **24**, 343 – 353.
- Albrecht, A., Asmus, H., Asmus, R. M., Berger, J., Drebes, G., Lackschewitz, D., Reise, K., Schories, D., Schubert, F., Wille, A. (1995) *Sylter Wattenmeer Austauschprozesse Abschlussbericht: Produktion und Stoffaustausch des Benthos*, pp 111, unpublished.
- Alexandre, A., Cabaço, S., Santos, R & Serrão, E. A. (2006) Timing and success of reproductive stages in the seagrass *Zostera noltii*. *Aquatic Botany*, **85**, 219 - 23.
- Asmus, H., Asmus, R. M. (2000) Material exchange and food web of seagrass beds in the Sylt-Rømø Bight: how significant are community changes at the ecosystem level? *Helgoland Marine Research*, **54**, 137 – 150.
- Asmus, R. M., Sprung, M., Asmus, H. (2000) Nutrient fluxes in intertidal communities of a South European lagoon (River Formosa) – similarities and differences with a northern Wadden Sea bay (Sylt-Rømø Bay). *Hydrobiologia*, **436**, 217 – 235.
- Asmus, R. M., Asmus, H. (1998) Bedeutung der Organismusgemeinschaften für den bentho-pelagischen Stoffaustausch im Sylt- Rømø Wattenmeer. In: Gätje, C.; Reise, Karsten (editors) (1998) *Ökosystem Wattenmeer. Austausch-, Transport- und Stoffumwandlungsprozesse.* Springer Verlag, Berlin.
- Atkinson, M. J., Smith, S. V. (1984) C:N:P ratios of benthic marine plants. *Limnol. Oceanogr.*, **28**, 568 – 74.
- Austen, I. (1994) The surficial sediments of Königshafen – variations over the past 50 years. *Helgoland Marine Research*, **48**, 163 – 171.
- Backhaus, J., Hartke, D., Hübner, U., Lohse, H., Müller, A. (1998) Hydrography and Climate of the List Tidal Basin. In: Gätje, C., Reise, K. (editors) (1998) *Ökosystem Wattenmeer. Austausch-, Transport- und Stoffumwandlungsprozesse.* Springer Verlag, Berlin.

- Baird, D., Asmus, H., Asmus, R. M. (2004) Energy flow of a boreal intertidal ecosystem, the Sylt-Rømø Bight. *Marine Ecology Progress Series*, **279**, 45 – 61.
- Beukema, J. J. (1976) Biomass and species richness of the macro-benthic animals living on the tidal flats of the Dutch Wadden Sea. *Netherlands Journal of Sea Research*, **10**, 236 - 61.
- Bouma, T. J., de Vries, M. B., Low, E., Peralta, G., Tánczos, C., van de Koppel, J., Herman, P. M. J. (2005) Trade-offs related to ecosystem engineering: a case study on stiffness of emerging macrophytes. *Ecology*, **86**, 2187 – 2199.
- Brix, H., Lyngby, J. E. (1985) Uptake and translocation of phosphorus in eelgrass (*Zostera marina*). *Marine Biology*, **90**, 111 – 116.
- Cabaço, S., Santos, R. (2007) Effects of burial and erosion on the seagrass *Zostera noltii*. *Journal of Experimental Marina Biology and Ecology*, **340**, 204 – 212.
- Cadée, G. C. (1976) Sediment reworking by *Arenicola marina* on tidal flats in the Dutch Wadden Sea. *Netherlands Journal of Sea Research*, **10**, 440 – 460.
- Churchill, A. C., Nieves, G., Brenowitz, A. H. (1985) Flotation and dispersal of eelgrass seeds by gas bubbles. *Estuaries*, **8**, 352 – 354.
- Cock, A.W. A. M. de (1980) Flowering, pollination and fruiting in *Zostera marina* L.. *Aquatic Botany*, **9**, 201 – 229.
- Coops, H., Boeter, R., Smith, H. (1991) Direct and indirect effects of waves attack on halophytes. *Aquatic Botany*, **41**, 333 – 352.
- De Jonge, V.N. & Ruiter, J.F. (1996) How subtidal were the ‘subtidal beds’ of *Zostera marina* L. before the occurrence of the wasting disease in the early 1930s? *Netherlands Journal of Aquatic Ecology*, **30**, 99-106.
- den Hartog, C. (1970) *The Sea-Grasses of the World*. North-Holland Publishing Company, Amsterdam.
- Duarte, C. M. (1990) Seagrass nutrient content. *Marine Ecology Progress Series*, **67**, 201 – 207.
- Dytham, C. (1999) *Choosing and Using Statistics*. Blackwell Science Ltd, London.

- Ewanchuk, P. J., Williams, S. L. (1996) Survival and re-establishment of vegetative fragments of eelgrass (*Zostera marina*). *Canadian Journal of Botany*, **74**, 1584 – 1590.
- Fish, J.D. & Fish, S. (1995, 2nd edition) *A Student's Guide to the Seashore*. Cambridge University Press, New York.
- Fonseca, M. S., Bell, S. S. (1998) Influence of physical setting on seagrass landscapes near Beaufort, North Carolina, USA. *Marine Ecology Progress Series*, **171**, 109 – 121.
- Fonseca, M. S., Fisher, J. S., Zieman, J. C., Thayer, G. W., (1982) Influence of the seagrass, *Zostera marina* L., on current flow. *Estuarine, Coastal and Shelf Science*, **15**, 351 – 364.
- Fonseca, M. S., Kenworthy, J. (1987) Effects of currents on photosynthesis and distribution of seagrasses. *Aquatic Botany*, **27**, 59 – 78.
- Fonseca, M. S., Kenworthy, W. J., Colby, D. R., Rittmaster, K. A., Thayer, g. W. (1990) Comparisons of fauna among natural and transplanted eelgrass *Zostera marina* meadows: Criteria for mitigation. *Marine Ecology Progress Series*, **65**, 251 – 264.
- Fonseca, M. S., Zieman, J. C., Thayer, G. W., Fisher, J. S. (1983) The Role of Current Velocity in Structuring Eelgrass (*Zostera marina* L.) Meadows. *Estuarine, Coastal and Shelf Science*, **17**, 367 – 80.
- Gätje, C., Reise, K. (editors) (1998) *Ökosystem Wattenmeer. Austausch-, Transport- und Stoffumwandlungsprozesse.* Springer Verlag, Berlin.
- Giesen, W. B. J. T., van Katwijk, M. M., den Hartog, C. (1990) Eelgrass condition and turbidity in the Dutch Wadden Sea. *Aquatic Botany*, **37**, 71 – 85.
- Goubin, C., Loquès, F. (1991) Germinating *Zostera noltii* Hornemann found in the Etang de Diana, Corsica. *Aquatic Botany*, **42**, 75 – 79.
- Grasshoff, K.; Ehrhardt, M., Kremling, K. (editors) (1983) *Methods of seawater analysis.* Verlag Chemie, Weinheim. 419 p.
- Harrison, P. G. (1993) Variations in demography of *Zostera marina* and *Z. noltii* on an intertidal gradient. *Aquatic Botany*, **45**, 63 – 77.
- Hedtkamp, S. I. C. (2005) Shallow Subtidal sand: Permeability, nutrient dynamics, microphytobenthos and organic matter. PhD thesis, Kiel university, 2005.

- Hemminga, M. A. (1998) The root/rhizome system of seagrasses: an asset and a burden. *Journal of Sea Research*, **39**, 183 – 196.
- Hemminga, M. A., Duarte, C. M (2000) *Seagrass Ecology*. Cambridge University Press, Cambridge.
- Hemminga, M. A., Koutstaal, B. P., Soelen, J. van, Merks, A. G. A. (1994) The nitrogen supply to intertidal eelgrass (*Zostera marina*). *Marine Biology*, **118**, 223 – 227.
- Herman, P. M., Hemminga, M. A., Nienhuis, P. H., Verschuure, J. M., Wessel, E. G. (1996) The wax and wane of eelgrass (Zostera marina L.) and water column silicon levels. *Marine Ecology Progress Series*, **144**, 303 – 307.
- Holmer, M., Carta, C., Andersen, F. O. (2006) Biogeochemical implications for phosphorus cycling in sandy and muddy rhizosphere sediments of *Zostera marina* meadows (Denmark). *Marine Ecology Progress Series*, **320**, 141 – 151.
- Hootsmans, M. J. M., Vermaat, J. E. & van Vierssen, W. (1987) Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L. and *Zostera noltii* Hornem. *Aquatic Botany*, **28**, 257 - 285.
- Hughes, R. G., Lloyd, A., Ball, L., Emson, D. (2000) The effects of the polychaete *Nereis diversicolor* on the distribution and transplanting success of *Zostera noltii*. *Helgoland Marine Research*, **54**, 129 – 36.
- Hüttel, M. (1990) Influence of the lugworm *Arenicola marina* on porewater nutrient profiles of sand flat sediments. *Marine Ecology Progress Series*, **62**, 241 – 248.
- Iizumi, H., Hattori, A. (1982) Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific coast of Japan. III. The kinetics of nitrogen uptake. *Aquatic Botany*, **12**, 245 – 256.
- Jacobs, R. P. W. M. (1982) Reproductive strategies of two seagrass species (*Zostera marina* and *Z. noltii*) along West European Coasts. In: Symoens, J.J., Hooper, S.S. & Compère, P. (1982) *Studies on Aquatic Vascular Plants*. Royal Botanical Society of Belgium, Brussels.
- Jones, C. G., Lawton, J. H., Shachak, M. (1994) Organisms as ecosystem engineers. *Oikos,* **69**, 373 – 386.

- Kamermans, P., Hemminga, M. A., de Jong, D. J. (1999) Significance of salinity and silicon levels for growth of a formerly estuarine eelgrass (*Zostera marina*) population (Lake Grevelingen, The Netherlands). *Marine Biology*, **133**, 527 – 539.
- Kautsky, L. (1987) Life-cycles of three populations of *Potamogeton pectinatus* L., of different degrees of wave exposure in the Asko area, northern Baltic proper. *Aquatic Botany*, **27**, 177 – 186.
- Kellermann, A., Eskildsen, K. (2005) Reference Areas. *Wadden Sea Ecosystem*, **19**, 22 – 25.
- Kirchner, D (2005) Räumliche Verteilungsmuster des Makrobenthos in Seegraswiesen des Sylt-Romo-Bucht. Diplomarbeit, Bielefeld university, 134 pages.
- Kirkman, H., Kuo, J. (1990) Pattern and processes in southern Western Australian seagrasses. *Aquatic Botany*, **37**, 367 – 382.
- Koch, E. W., (2001) Beyond light: Physical, geological, and geochemical parameters as possible submerged aquatic vegetation requirements. *Estuaries*, 24, 1 - 17.
- Koch, E. W., Beer, S. (1996) Tides, light, and the distribution of *Zostera marina* in Long Island Sound, USA. *Aquatic Botany*, **53**, 97 – 107.
- Levington, J.S. (1995) *Marine Biology. Function, Biodiversity, Ecology*. Oxford University Press, New York.
- Loques, F., Caye, G. & Meinesz, A. (1990) Germination in the marine phanerogam *Zostera noltii* Hornemann at Golfe Juan, French Mediterranean. *Aquatic Botany*, **38**, 249 – 260.
- McRoy, C. P., Goering, J. J. (1974) Nutrient transfer between the seagrass *Zostera marina* and its epiphytes. *Nature*, **248**, 173 – 174.
- Moore, K. A., Orth, R. J. & Nowak, J. F. (1993) Environmental regulation of seed germination in *Zostera marina* L. (eelgrass) in Chesapeake Bay: effects of light, oxygen and sediment burial. *Aquatic Botany*, **45**, 79 – 91.
- Moriarty, D. J., Boon, P. I. (1989) Interactions of seagrasses with sediment and water. In: Larkum, A. W. D (Ed.) (1989) *Biology of Seagrasses. A treatise on the biology of seagrasses with specific reference to the Australian region.* Elsevier, Amsterdam.

- Orth, R. J., Harwell, M. C., Bailey, E. M., Bartholomew, A., Jawad, J. T., Lombana, A. V., Moore, K. A., Rhode, J. M., Woods, H. E. (2000) A review of issues in seagrass seed dormancy and germination: implications for conservation and restoration. *Marine Ecology Progress Series*, **200**, 277 – 288.
- Paramor, O. A. L., Hughes, R. G. (2004) The effects of bioturbation and herbivory by the polychaete *Nereis diversicolor* on loss of saltmarsh in south-east England. *Journal of Applied Ecology*, **41**, 449 – 63.
- Pellikaan, G. C., Nienhuis, P. H. (1988) Nutrient uptake and release during growth and decomposition of eelgrass, *Zostera marina* L., and its effects on the nutrient dynamics of Lake Grevelingen. *Aquatic Botany*, **30**, 189 – 214.
- Peralta, G., Brun, F. G., Hernández, I., Vergara, J. J., Pérez-Loréns, J. L. (2005) Morphometric variations as acclimation mechanisms in *Zostera noltii* beds. *Estuarine, Coastal and Shelf Science*, **64**, 347 - 356.
- Peralta, G., Pérez-Lloréns, J. L., Hernández, I., Vergara, J. J. (2002) Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem. *Journal of Experimental Marine Biology and Ecology*, **269**, 9 – 26.
- Philippart, C. J. M. (1994) Interactions between *Arenicola marina* and *Zostera noltii* on a tidal flat in the Wadden Sea. *Marine Ecology Progress Series*, **111**, 251 - 257.
- Philippart, C. J. M. (1995) Effects of shading on growth, biomass and population maintenance of the intertidal seagrass *Zostera noltii* Hornem. In the Dutch Wadden Sea. *Journal of Experimental Marine Biology and Ecology*, **188**, 199 – 213.
- Pirc, H., Wollenweber, B. (1988) Seasonal changes in nitrogen, free amino acids, and C/N ratio in Mediterranean seagrasses. *Marine Ecology*, **9**, 167 – 179.
- Polte, P., Asmus, H. (2006) Intertidal seagrass beds (Zostera noltii) as spawning grounds for transient fishes in the Wadden Sea. *Marine Ecology Progress Series*, **312**, 235 – 243.
- Probert, R. J., Brenchley, J. L. (1999) The effect of environmental factors on field and laboratory germination in a population of *Zostera marina* L. from southern England. *Seed Science Research*, **9**, 331 – 339.

- Reise, K., Herre, E. & Sturm, M. (1989) Historical changes in the benthos of the Wadden Sea around the island of Sylt in the North Sea. *Helgoländer Meeresuntersuchungen*, **43**, 417-433.
- Reise, K., Riethmüller, R. (1998) The Sylt-Rømø Bight in the Wadden Sea: An Overview. In: Gätje, C., Reise, K. (editors) (1998) *Ökosystem Wattenmeer. Austausch-, Transport- und Stoffumwandlungsprozesse.* Springer Verlag, Berlin.
- Riisgård, H. U., Banta, G. T. (1998) Irrigation and deposit feeding by the lugworm *Arenicola marina*, characteristics and secondary effects on the environment. A review of current knowledge. *Vie Milieu*, **48**, 243 – 257.
- Rusch, A., Huettel, M., Forster, S. (2000) Particulate organic matter in permeable marine sands – dynamics in time and depth. *Estuarine, Coastal and Shelf Science*, **51**, 399 – 414.
- Schanz, A (2003) Impact of hydrodynamics on intertidal seagrass beds in the northern Wadden Sea. PhD Thesis, Bremen University, 142 pages.
- Schanz, A., Asmus, H. (2003) Impact of hydrodynamics on development and morphology of intertidal seagrasses in the Wadden Sea. *Marine Ecology Progress Series*, **261**, 123 – 134.
- Schanz, A., Polte, P., Asmus, H. (2002) Cascading effects of hydrodynamics on an epiphyte-grazer system in intertidal seagrass beds of the Wadden Sea. *Marine Biology*, **141**, 287 – 297.
- Seddon, S., Connolly, R. M. & Edyvane, K. S. (2000) Large-scale sea grass dieback in northern Spencer Gulf, South Australia. *Aquatic Botany*, **66**, 297 – 310.
- Short, F. T. (1987) Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. *Aquatic Botany*, **27**, 41 – 57.
- Short, F. T., Wyllie – Echeverria, S. (1996) Natural and human-induced disturbance of seagrasses. *Environmental Conversation*, **23** (1), 17 – 27.
- Thursby, G. B., Harlin, M. M. (1982) Leaf-root interaction in the uptake of ammonia by *Zostera marina*. *Marine Biology*, **72**, 109 – 112.
- Touchette, B. W., Burkholder, J. M (2000) Review of nitrogen and phosphorus metabolism in seagrasses. *Journal of Experimental Marine Biology and Ecology*, **250**, 133 – 167.

- van Katwijk, M. M., Hermus, D. C. R. (2000) Effects of water dynamics on *Zostera marina*: transplantation experiments in the intertidal Dutch Wadden Sea. *Marine Ecology Progress Series*, **208**, 107 – 118.
- van Katwijk, M. M., Schmitz, G. H., W., Gasseling, A. P., van Avesaath, P. H. (1999) Effects of salinity and nutrient load and their interaction on *Zostera marina*. *Marine Ecology Progress Series*, **190**, 155 – 165.
- Volkenborn, N. (2005) Ecosystem engineering in intertidal sand by the lugworm *Arenicola marina*. PhD Thesis, Bremen University, 137 pages.
- Wohlenberg, E. (1935) Beobachtungen über das Seegras, *Zostera marina* L., und seine Erkrankung im nordfriesischen Watttenmeer. *Beiträge zur Heimatforschung in Schleswig-Holstein*, Hamburg und Lübeck, **11**, 1-19.

Appendices

Appendix 1: Statistical tables

In this appendix the results of the t tests are compiled. It was necessary to conduct this series of t tests as due to unfavourable weather conditions two sets of seagrass cores were transplanted and therefore two data sets were obtained.

T test results for experiment 1 (chapter 2)

Table 11: T test results for combined number of shoots per experimental unit . The shoots from both seagrass species were counted. Pair 1 has not enough values from the second set to do a t test.

Pair	Date	p-value	Significance
Pair 1	30.3. / 6.4.		Not sufficient data
Pair 2	10.4. / 23.4.	0.7734	Not significant
Pair 3	10.5. / 3.6.	0.1721	Not significant
Pair 4	16.6. / 22.6.	0.8574	Not significant
Pair 5	16.7. / 19.7.	0.6766	Not significant
Pair 6	10.8. / 10.8.	0.5708	Not significant

Table 12: T test results of the two transplantation data sets for number of shoots per experimental unit. The number of shoots was counted for each seagrass species separately.

Pair	Date	p-value	Significance
Pair 4 *Z. marina*	16.6./22.6.	0.6654	Not significant
Pair 5 *Z. marina*	16.7./19.7.	0.5560	Not significant
Pair 6 *Z. marina*	10.8./10.8.	0.1214	Not significant
Pair 4 *Z. noltii*	16.6./22.6.	0.2683	Not significant
Pair 5 *Z. noltii*	16.7./19.7.	0.6384	Not significant
Pair 6 *Z. noltii*	10.8./10.8.	0.4994	Not significant

Table 13: Shoot length t test results of the two transplantation data sets. Apart from Pair 2, all data sets can be combined. Tested was the pooled shoot length of each experimental unit.

Pair	Date	p-value	Significance
Pair 1	03.04./08.04.	0.7657	Not significant
Pair 2	10.04./14.04.	0.0115	*Significant*
Pair 3	20.04./23.04.	0.1231	Not significant
Pair 4	28.04./05.05.	0.1371	Not significant
Pair 5	14.05./18.05.	0.3918	Not significant
Pair 6	25.05./03.06.	0.0956	Not significant
Pair 7	16.6./22.6.	0.9791	Not significant
Pair 8	16.7./19.7.	0.3015	Not significant
Pair 9	10.8./10.8.	0.6331	Not significant

Table 14: SER t test results of the two transplantation data sets testing the combined data of the two seagrass species.

Pair	Date	p-value	Significance
Pair 1	10.04./14.04.	0.009	*Significant*
Pair 2	20.04./23.04.	0.1855	Not significant
Pair 3	28.04./05.05.	0.9503	Not significant
Pair 4	14.05./18.05.	0.3565	Not significant
Pair 5	25.05./03.06.	0.0704	Not significant
Pair 6	16.6./22.6.	0.4635	Not significant
Pair 7	16.7./19.7.	0.7965	Not significant

Table 15: Biomass t test of the two transplantation data sets. The above ground and below ground biomass were combined for each species. The seagrass cores were harvested in August 2004.

Species	Date	p-value	Significance
Z. noltii	10.8./10.8.	0.5202	Not significant
Z. marina	10.8./10.8.	0.5830	Not significant

Table 16: Number of leaves per shoot t test results of the two transplantation data sets. The numbers of leaves were combined for each species. From Pair 4 onwards the data were analysed for current velocities and treatment as there was an obvious west - east effect in the data.

Pair	Date	p-value	Significance
Pair 1	20. / 23. 4.	0.1475	Not significant
Pair 2	28. 4. / 4. 5.	< 0.0001	*Significant*
Pair 3	14.5. / 18.5.	0.6256	Not significant
Pair 4	25.5. / 3.6.	0.2342	Not significant
Pair 5	16.6. / 22. 6.	0.6049	Not significant
Pair 6	16.7. / 19.7.	0.2984	Not significant
Pair 7	10.8. / 10.8.	0.2810	Not significant

Table 17: LAR t test results of the two transplantation data sets testing the combined data of the two seagrass species. Apart from Pair 3 and 4, all data pairs can be combined. Pair 3 and 4 were therefore analysed split into two separate data sets.

Pair	Date	p-value	Significance
Pair 1	28.04./05.05.	0.4147	Not significant
Pair 2	14.05./18.05.	0.9560	Not significant
Pair 3	25.05./03.06.	0.0248	*Significant*
Pair 4	16.6./22.6.	0.0240	*Significant*
Pair 5	16.7./19.7.	0.8107	Not significant

Statistical tables for experiment 3 (chapter 4)

Table 18: Statistical values of the Spearman's rank order correlation indication a strong negative association between seagrass growth and porewater phosphate levels

	July above ground	July all biomass	October above ground	October all biomass
r_S	-0.73565217	-0.67130435	0.0712	-0.0382
d.f.	23	23	17	17
p	p = < 0.0001	p = 0.0003	0.7789	0.8804

Appendix 2: Weather data

Table 19: Weather data was collected from March – October 2004. Data were obtained from the "Bundesamt für Seeschifffahrt und Hydrographie" and were measured in List harbour (Sylt).

Date	Wind force (Bft)	Wind direction	Water temperature (°C)	Water height (m) low tide	Air temperature (°C)
30.3.2004		O			
31.3.2004	4.5	O		-0.5 – 0.75	
1.4.2004	6	O		-0.5 – 0.75	
2.4.2004	6.5	O		-0.5 – 0.75	
3.4.2004	4.5	SSO	6.8	-0.1	8
4.4.2004	5.5	WSW	7	0.4	7.3
5.4.2004	5	SW	6.5	-0.2	5.8
6.4.2004	1	W	7	0	4.5
7.4.2004	3.4	NO	7	-0.2	2.7
8.4.2004	5	N	7.2	-0.3	5.7
9.4.2004	5	WNW	8	-0.2	6.7
10.4.2004	5	N	7.9	0	6
11.4.2004	5.5	NW	8	-0.3	8.2
12.4.2004	4	NW	8	0	7.2
13.4.2004	2.5	NW	8	-0.3	7.1
14.4.2004	3	SSW	8.1	0	7
15.4.2004	2	SSO	9	-0.1	10.9
16.4.2004	3	SO	9	-0.1	11.3
17.4.2004	2.5	OSO		-0.1	9.8
18.4.2004	4.5	SSW	9.6	0	9.3
19.4.2004	4	SW	10	-0.1	9.1
20.4.2004	3.5	W	10.1	-0.3	9.7
21.4.2004	3.5	O	9.9	-0.3	7.2
22.4.2004	1	SSW	10.5	0	12.7

23.4.2004	4	NNW		0.1	9.5
24.4.2004	2.5	NNW		-0.1	10.7
25.4.2004	3	WNW		0	12
26.4.2004	2	W	11.1	0	10.5
27.4.2004	3.5	SO		0.1	9.4
28.4.2004	5	O	12	0.2	10.2
29.4.2004	6	OSO		0.2	10.3
30.4.2004	5	O	11.4	0	13.3
1.5.2004	4.5	O	11.1	0.2	17.4
2.5.2004	3	NNW	11.5	-0.3	13.9
3.5.2004	4	SW	11.6	-0.2	9.5
4.5.2004	4	SSW	11	-0.2	9.6
5.5.2004	2.5	SSO	11.8	-0.1	12.5
6.5.2004	4.5	NNW	11.8	-0.2	9.9
7.5.2004	4.5	ONO	12	-0.2	
8.5.2004					
9.5.2004	3.5	NO		0	18.9
10.5.2004	5	NNW	13	-0.1	13.9
11.5.2004	3.5	NW	13.6	0	10.7
12.5.2004	4.5	NW	13.4	0	10.8
13.5.2004	2	SO	13.2	-0.2	10.4
14.5.2004	4	W	12.4	0	10.6
15.5.2004	6	NW	12.6	0	12.6
16.5.2004	5	NW		-0.2	11.9
17.5.2004	3	NW	12.7	-0.2	9.2
18.5.2004	4	W	13	-0.1	12.6
19.5.2004	6	WNW	13.5	0.1	13.4
20.5.2004	4.5	NW		-0.2	11.1
21.5.2004	5	NNW	13.6	-0.1	8.2
22.5.2004					
23.5.2004	5.5	NNW		-0.1	10.7
24.5.2004	5.5	NW	12	0	10.5
25.5.2004	5.5	NW	12.4	0.3	10.9
26.5.2004					
27.5.2004	3	NW		0.2	13.3
28.5.2004	4.5	WNW	12.7	0	10.6
29.5.2004	3.5	SSW	12.5	0	14.3
30.5.2004	3	SSO		-0.3	20.1
31.5.2004	5.5	OSO		0.1	19
1.6.2004	4.5	O		-0.2	18.5
2.6.2004	3	NO		-0.1	19.4
3.6.2004	2.5	SO	15.3	-0.4	18.5
4.6.2004	3	W	15.4	-0.3	13.9
5.6.2004	4	N	15.9	-0.3	15.3
6.6.2004	4.5	WSW		-0.3	14.3
7.6.2004	5.5	SW	16.4	-0.2	17.2
8.6.2004	4	NNW	16.2	-0.1	15.1
9.6.2004	4	SO	17	-0.2	16.7

10.6.2004					
11.6.2004	5	W		0.4	16.5
12.6.2004	4	WNW	16.7	0.3	16.1
13.6.2004	4.5	WNW	16.8	0.1	14.1
14.6.2004	5	W	17.1	-0.1	15.2
15.6.2004	7	NW	16.7	0.3	15.7
16.6.2004					
17.6.2004					
18.6.2004	5	NW	15.3	-0.2	13.1
19.6.2004					
20.6.2004	5	SW		0.1	12.8
21.6.2004					
22.6.2004	3.5	W		-0.2	15.2
23.6.2004	5.5	OSO	16	-0.3	14.5
24.6.2004	6	SW		1	13.6
25.6.2004	6	W	15	0.75 - 1	13.9
26.6.2004	5	WNW	15.2	0.1	13.8
27.6.2004	3	SO	15	0	13.5
28.6.2004	6	WNW	15	0.5	
29.6.2004	5	W	15.3	0.1	16.2
30.6.2004	4	SSW	16	-0.1	17.7
1.7.2004					
2.7.2004	4.5	WSW	16.3	-0.2	17.2
3.7.2004	5	SW		0.1	13.3
4.7.2004	5	W		-0.1	14.8
5.7.2004	4	WNW		-0.2	15.8
6.7.2004	5	WNW	16.8	-0.4	15.1
7.7.2004	3.5	NNW	16.8	-0.3	15.1
8.7.2004	4	SO	17	0.2	18
9.7.2004	2	W	17.1	0.1	17.5
10.7.2004	5	WNW		0.4	15.3
11.7.2004	3.5	W		0.5	15
12.7.2004	6	WNW	16.6	0.75	14.3
13.7.2004	6	NW	16.2	0.4	12.8
14.7.2004	3.5	SW	15.8	0.2	15.5
15.7.2004	4.5	WNW	15.7	0.3	15.5
16.7.2004	3.5	NW		0	16.9
17.7.2004	3.5	SO	16.5	-0.1	21.1
18.7.2004	3.5	WSW	17.2	0	16.4
19.7.2004	3.5	W	17.6	-0.1	17.1
20.7.2004	3	SO	18	-0.2	17.3
21.7.2004	3	NO	18	-0.3	16.7
22.7.2004	2.5	NNW	18.1	-0.2	19.5
23.7.2004	4	O	18.2	-0.1	20.4
24.7.2004	4	W	18	0.1	18
25.7.2004	5	W		0.2	14.8
26.7.2004	4	WNW	18	0.3	15.7
27.7.2004	4	NNW	18.3	0.1	16.2

28.7.2004	3	NW	18.5	0	17.6
29.7.2004	2	SO	18	-0.3	20.8
30.7.2004	2.5	OSO	18	-0.3	23.8
31.7.2004	1	NW		-0.3	20.7
1.8.2004	3.5	NNW	19.7	-0.4	22.1
2.8.2004	3	N	20.3	-0.3	21.9
3.8.2004	4	NW	20.7	-0.4	21.5
4.8.2004	2.5	SSO	21.2	-0.2	23.2
5.8.2004	4	O	21.7	-0.2	24.3
6.8.2004	4	O		-0.2	19.6
7.8.2004	4	OSO		0	27.6
8.8.2004	4	OSO		-0.1	23
9.8.2004	4.5	OSO	22.2	0	27.2
10.8.2004	5	O		0.1	25.8
11.8.2004	5	O	22.3	0.3	21.3
12.8.2004	2.5	OSO	21.8	0.2	23.2
13.8.2004					
14.8.2004	3	WNW	21.3	0.2	22.2
15.8.2004	5	WNW	21.5	-0.1	18.5
16.8.2004	2.5	S	21	-0.1	18.1
17.8.2004	5	SW	20.5	-0.1	20.3
18.8.2004	4	OSO	20	-0.1	18.5
19.8.2004	6.5	SSW	20.5	0.2	19.8
20.8.2004	5.5	SW	19.9	0.2	19.5
21.8.2004	4.5	NW		0.1	18.3
22.8.2004	5	WNW		-0.2	16.8
23.8.2004	4.5	OSO	18	-0.3	17.2
24.8.2004	3.5	OSO		0	15.7
25.8.2004	1	SSW	17.8	0.4	15
26.8.2004	3	WNW		0.4	17.4
27.8.2004	6	SW	18	0.3	16.3
28.8.2004	5	W		0.4	17.5
29.8.2004	5	S	17.6	-0.1	17.2
30.8.2004	4.5	SW	17.6	0.2	15.3
31.8.2004					
1.9.2004	6	W		-0.1	15.1
2.9.2004	2.5	SO	16.8	-0.3	18.6
3.9.2004	3.5	SO		-0.2	19.9
4.9.2004	2	SSO		-0.1	19.3
5.9.2004	1	NW	17.8	-0.1	19.6
6.9.2004	5	N	18.3	0.1	19.1
7.9.2004	3	N		-0.1	19.2
8.9.2004					
9.9.2004	3	S		0	14.2
10.9.2004					
11.9.2004					
12.9.2004	8	W	17.6	1.25	15.2
13.9.2004	6.5	SSW	17.5	0.4	16.9

14.9.2004	6.5	SSW	16.4	0.4	16.3
15.9.2004	5.5	WNW	16	0.8775	13.8
16.9.2004	4	W	15.3	-0.1	15.6
17.9.2004	5	S	15.2	0	16.2
18.9.2004	6	SSW	15.4	0.1	16.9
19.9.2004	5	W		0.3	15.7
20.9.2004	7.5	SSW	15.6	1.25	13.8
21.9.2004	6	WNW	15	2	13.5
22.9.2004	6.5	WNW	14.3	1	12.9
23.9.2004	4.5	NW	14.4	0.3	13.2
24.9.2004	5	NW	13.9	0.8775	14.2
25.9.2004	4.5	NW		0.1	12.2
26.9.2004	5	WNW		-0.1	15.4
27.9.2004	6	WNW	13.6	0.3	13.2
28.9.2004	2.5	ONO		0.2	
29.9.2004	4	N		-0.1	14.4
30.9.2004	2	O	13.3	-0.2	13.3
1.10.2004	4	OSO	13.6	-0.4	14.3
2.10.2004	5	SW	13.5	0.2	15.8
3.10.2004	4	SW	13.6	0.4	14.6
4.10.2004	7	S		0.4	14.8
5.10.2004					
6.10.2004	6.5	SW	13.4	1.25	14.9
7.10.2004	5	WSW	13.4	1	11.6
8.10.2004	5	NW		0.5	13.6
9.10.2004	1	O	12.5	0	12.2
10.10.2004	3.5	NO	12.4	-0.3	11.8
11.10.2004					
12.10.2004	5.5	OSO	11.2	-1.25	6.7
13.10.2004	5	SO	10.5	-0.2	8.8

Appendix 3: Veränderungen zur Gutachterversion

Die folgenden Veränderungen wurden gegenüber der Gutachterversion dieser Arbeit im Einvernehmen mit den Gutachtern vorgenommen. Hierbei handelt es sich um geringfügige formale Korrekturen, es wurden keinerlei inhaltliche Veränderungen vorgenommen.

Rechtschreibfehler:

- S.5 ‚not connected to'
- S. 7 ‚europäischer Seegrasarten'

Einfügungen:

- S. 21 ‚(see fig. 3)' und ‚(see fig. 4)'
- S. 22 '(see fig. 5)'
- S. 23 '(see fig. 6)'
- S. 25 '(see fig. 7)'
- S. 26 '(see fig. 8, 10, 12)' und '(see fig. 9, 11, 13)'
- S. 28 '(see fig. 14)' zweimal
- S. 29 '(see fig. 15)' und '(see fig. 16)'
- S. 31 '(see fig. 17)'
- S. 32 '(see fig. 18)'
- S. 33 '(see fig. 19)'
- S. 35 '(see table 2)'
- S. 37 '(see fig. 21, 23, 25)'
- S. 38 '(see fig. 21)'; '(see fig. 23)'; '(see fig. 25)'; '(see fig. 22, 24, 26)'; '(see fig. 24)'
- S. 39 '(see fig. 26)'
- S. 41 '(see fig. 27)' zweimal
- S. 42 '(see fig. 28)' dreimal
- S. 43 '(see fig. 29)' und '(see fig. 30)'
- S. 44 '(see fig. 30)' dreimal
- S. 45 '(see fig. 32)'
- S. 48 '(see fig. 34)'
- S. 71 '(see fig. 35)'
- S. 72 '(see fig. 36)'
- S. 73 '(see fig. 37, …)'

- S. 74 ‘(see fig. 38)’
- S. 75 ‘(see fig. 39)’ und ‘(see fig. 40 below)’
- S. 76 ‘(see fig. 41)’
- S. 78 ‘(see fig. 42)’ und ‘(see table 5)’
- S. 80 ‘(see table 5 above)’ und ‘(see fig. 43)’
- S. 81 ‘(see fig. 44)’
- S. 82 ‘(see fig. 45)’
- S. 83 ‘(see fig. 46)’
- S. 84 ‘(see fig. 47)’
- S. 85 ‘(data not shown)’ und ‘(see fig. 48)’
- S. 87 ‘(see fig. 50)’
- S. 88 ‘(see fig. 51)’
- S. 95 Absatz ‘The roots/rzizomes…’ auf S.96 verschoben
- S. 104 ‘(see fig. 54)’ und ‘(see fig. 55)’
- S. 105 ‘(see fig. 56, …)’
- S. 107 ‘(see table 8)’
- S. 109 ‘(see fig. 59)’
- S. 113 ‘(see fig. 65)’ und ‘(see fig. 66)’
- S. 116 ‘(see fig. 68)’
- S. 117 ‘(see fig. 69)’