The Effects of Mesograzers in Eelgrass Communities

Dissertation

Zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Christian- Albrechts-Universität zu Kiel

vorgelegt von

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Kiel 2006

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Tag der mündlichen Prüfung: 7.6.2007

Zum Druck genehmigt: 7.6.2007

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Summary

Seagrass communities are among the most valuable ecosystems in terms of benefits they provide for society. They harbour a diverse assemblage of invertebrate and fish species, acting as habitat and food source, and nursery area for juvenile stages of commercially important species. The combined ability of seagrass meadows to remove sediment and nutrients from the water column and to abate currents contributes significantly to the protection of coastal areas. Unfortunately, seagrass beds are among the most threatened marine habitats. Growing anthropogenic influence, especially eutrophication caused a dramatic decline in the past decades. Seagrass leaves are colonized by a variety of epiphytic algae. Under high nutrient supply, these epiphytes may overgrow their host with detrimental consequences for seagrass growth. This process is not regulated by nutrients alone, but the effect of mesograzers, small mobile invertebrate species, is assumed to play a relevant role in structuring seagrass-epiphyte systems, too. In this thesis, I analysed the impact of four common mesograzers (the isopod *Idotea baltica*, the amphipod *Gammarus oceanicus*, the gastropods *Littorina littorea* and *Rissoa membranacea*) on processes in eelgrass-epiphyte-systems.

In the laboratory, I tested the effects of increasing mesograzer abundances on eelgrass and epiphyte biomass and productivity in mesocosm experiments. Grazer species identity strongly influenced epiphyte accumulation and eelgrass growth. *Rissoa* was the most efficient grazer and *Gammarus* had the weakest impact. The grazing impact was stronger for gastropods compared to the effect of the crustaceans. The photosynthetic capacity of epiphytes was enhanced by *Littorina* and *Rissoa* via the provision of nutrients probably derived from excretion products.

The effects of mesograzer abundance on epiphyte diversity also varied between the four studied species. Epiphyte diversity showed a unimodal correlation with gastropod abundances as anticipated according to the “intermediate disturbance hypothesis”. *Idotea* had a general negative effect and *Gammarus* showed a constant positive effect on epiphyte diversity. Varying mesograzer selectivity and epiphyte composition are assumed to be relevant factors in determining the impact of mesograzers on algal diversity.

The interaction between top-down and bottom-up effects were tested with intermediate grazing pressure of *Idotea* under three levels of nutrient supply. I found strong and interacting effects of nutrients and grazing on epiphytes. Epiphyte biomass and productivity was enhanced by nutrient enrichment and decreased in the presence of grazers. Nutrient effects were stronger in the absence of grazing and the effect of grazing was more pronounced under high nutrient supply. The effects of grazers and fertilisation on epiphyte composition were antagonistic: chain-forming diatoms and filamentous algae profited from

nutrient enrichment, but their proportions were reduced by grazing. Eelgrass growth was positively effected by grazing and by nutrient enrichment at moderate nutrient concentrations. In contrast, high nutrient supply reduced eelgrass productivity. Field data supported the experimentally found coexistence of bottom-up and top-down control on primary producers in this eelgrass system.

The effect of mesograzer diversity in an eelgrass-epiphyte-microphytobenthos system was analysed in another experiment in the laboratory. Initially, increasing consumer diversity enhanced the grazing efficiency on epiphyte biomass and a cascading diversity effect from the consumer level to the prey level was found. Additionally, strong effects of consumer species identity on taxonomic composition were found in both microalgal assemblages. However, the effects of consumer diversity were not consistent with time. The consequences of high nutrient availability were assumed to have superimposed consumer diversity effects after three weeks.

Furthermore, I used a field survey in the Kiel Fjord to investigate the relevance of epiphytic algae as food source in an eelgrass meadow. Multiple stable isotope and fatty acid analyses were applied to answer this question. Stable carbon isotopic values and fatty acid composition of primary producers and consumers in the studied eelgrass bed strongly supported the assumption of a food web mainly based on epiphytes and sand microflora. Red algae and phytoplankton appear to be of minor importance in this system. The contribution of eelgrass seemed to be negligible.

In conclusion, experiments and field studies indicated that mesograzer can play an important role in structuring eelgrass communities, and thus, this functional group is relevant in maintaining the health and stability of these ecosystems. Nevertheless, the magnitude and directions of mesograzer effects is species-specific and density-dependant. The significance of interaction between grazing pressure, nutrient availability and consumer and prey diversity are emphasised in this study.

Zusammenfassung

Seegrasgemeinschaften gehören hinsichtlich ihres Nutzens für die Allgemeinheit zu den wertvollsten Ökosystemen überhaupt. Sie bieten Lebensraum für eine diverse Gemeinschaft von Invertebraten und Fischen, dienen als Habitat und Futterquelle, sowie als Kinderstube für kommerziel wichtige Arten. Die Fähigkeit von Seegraswiesen Sediment und Nährstoffe aus der Wassersäule zu entfernen und die Stärke von Strömungen abzuschwächen, dient dem Schutz von Küstengebieten. Unglücklicherweise gehören Seegraswiesen zu den am meisten bedrohten Lebensräumen im Meer. Der wachsende menschliche Einfluß, insbesondere die Eutrophierung, haben einen dramatischen Rückgang der Seegrasswiesen in den letzen Dekaden verursacht. Seegrasblätter werden von einer Vielzahl von epiphytischen Algen besiedelt. Unter hoher Nährstoffbelastung können diese Epiphyten die Seegräser überwuchern mit schwerwiegenden Konsequenzen für das Wachstum der Pflanzen. Dieser Vorgang wird nicht nur durch Nährstoffe alleine geregelt, sondern auch der Einfluß von sogenannten „Mesograzern“, kleinen mobilen Invertebraten, spielt ein wichtige Rolle in der Strukturierung von Seegras-Epiphyten Gemeinschaften. In dieser Arbeit habe ich die Auswirkungen von vier häufig vorkommenden Mesograzerarten (die Isopodenart *Idotea baltica*, die Amphipodenart *Gammarus oceanicus*, die Gastropoden *Littorina littorea* and *Rissoa membranacea*) auf Prozesse in Seegras-Epiphyten Systemen untersucht.

Im Labor habe ich den Effekt von zunehmender Mesograzerdichte auf die Biomasse und Produktion von Seegrass und Epiphyten in Mesokosmos-Experimenten untersucht. Die Auswirkungen auf diese Pflanzengemeinschaft varierten zwischen den einzelnen Mesograzerarten, wobei *Rissoa* der effizienteste Grazer war und *Gammarus* den schwächsten Effekt zeigte. Die Gastropoden zeigten generell einen stärkeren Grazing-Effekt als die Crustaceen. Die Produktivität der Epiphyten wurde von *Littorina* und *Rissoa* durch die zusätzliche Versorgung mit Nährstoffen gesteigert. Wahrscheinlich stammten diese Nährstoffe aus den Exkretionsprodukten dieser Arten.

Der Effekt der Mesograzerdichte auf die Diversität der Epiphyten war ebenfalls artspezifisch. Die Diversität der Epiphyten zeigte eine unimodale Korrelation mit der Gastropodendichte wie es aufgrund der „intermediate disturbance hypothesis“ zu erwarten war. *Idotea* hatte einen generell negativen Effekt und *Gammarus* zeigte einen konstant positiven Effekt auf die Diversität der Epiphyten. Variationen in der Selektivität der Grazer und der Zusammensetzung der Epiphytengemeinschaft können als wichtige Faktoren für die unterschiedlichen Auswirkungen von Mesograzern auf die Diversität der Algen angesehen werden.

Die Interaktionen von „top-down“ und „bottom-up“ Effekten wurden bei einem mittleren Fraßdruck von *Idotea* und drei unterschiedlichen Nährstoffkonzentrationen getestet. Ich fand

starke und interaktive Auswirkungen von Nährstoffen und Grazing auf die Epiphyten. Die Biomasse und Produktion der Epiphyten wurde durch die Anreicherung mit Nährstoffen erhöht und durch die Anwesenheit von Grazern erniedrigt. Die Nährstoffeffekte waren stärker in der Abwesenheit von Grazern und der Grazing-Effekt war ausgeprägter unter hoher Nährstoffversorgung. Grazing und Düngung hatten antagonistische Effekte auf die Zusammensetzung der Epiphyten: Diatomeenketten und filamentöse Algen profitierten von der Nährstoffanreicherung, während ihr Anteil an der Gesamtbiomasse durch Grazing reduziert wurde. Das Seegraswachstum wurde positiv durch Grazing beeinflußt und durch mäßige Nährstoffanreicherung. Hohe Nährstoffkonzentrationen hingegen reduzierten die Produktion des Seegrases. Felddaten unterstützten die experimentell gezeigte Koexistenz von „top-down" und „bottom-up" Kontrolle von Primärproduzenten in Seegrassystemen.

Der Effekt von Mesograzerdiversität auf ein Seegras-Epiphyten-Mikrophytobenthos System wurde in einem weiteren Laborexperiment untersucht. Anfänglich erhöhte die ansteigende Mesograzerdiversität die Grazing-Effizienz auf die Epiphyten und es gab einen positiven Effekt auf die Diversität der Epiphyten. Zusätzlich wurden ein starker Einfluß der einzelnen Mesograzerarten auf die taxonomische Zusammensetzung in beiden Mikroalgengemeinschaften festgestellt. Diese Auswirkungen der Mesograzerdiversität verschwanden nach drei Wochen. Wahrscheinlich hatten die Auswirkungen höher Nährstoffkonzentrationen in diesem Experiment die Effekte der Mesograzerdiversität zu diesem Zeitpunkt überdeckt.

Des weiteren führte ich eine Feldstudie in der Kieler Förde durch, um die Bedeutung von Epiphyten als Nahrungsquelle in Seegrassystemen zu untersuchen. Stabile Isotopen- und Fettsäureanalysen wurden angewandt um diese Frage zu beantworten. Die Werte der stabilen Kohlenstoffisotope und die Fettsäurezusammenstetzung von Primärproduzenten und Konsumenten in der untersuchten Seegraswiese führten zu der Schlußfolgerung, daß das dort vorhandene Nahrungsnetz hauptsächlich Epiphyten und Mikrophytobenthos zur Grundlage hat. Rotalgen und Phytoplankton waren von geringerer Bedeutung in diesem System und das Seegrass selber ist wahrscheinlich als Nahrungsquelle zu vernachlässigen.

Als Schlußfolgerung kann man sagen, daß meine Experimente und Feldstudien die Bedeutung der Mesograzer als strukturierender Faktor in Seegrassystemen hervorheben und Bedeutung dieser funktionalen Gruppe für die Gesundheit und Stabilität dieser Ökosystem bestätigt wurde. Die Größenordnung und Ausrichtung dieser Effekte ist jedoch Abhängig von der Mesograzerart und der Dichte. Der Stellenwert von Interaktionen zwischen Fraßdruck, Nährstoffen und der Diversität von Primärproduzenten und Konsumenten wurde in dieser Studie verdeutlicht.

1. General introduction

1.1. The importance of seagrass systems

Seagrass communities are important components of shallow coastal systems in temperate and tropic regions. Seagrass beds provide habitat and food for a diverse community of invertebrates and fishes, including juvenile life-stages of commercially important fish species (McRoy & Helferich 1977). In tropical regions, they are major food sources for megaherbivores like dugongs, manatees and sea turtles (Valentine & Heck 1999). In northern regions, brent geese and other waterfowl depend on eelgrass as food during their migration (Ganter 2000). Seagrasses reduce suspended sediments and nutrients in the water column and regulate the dissolved oxygen. The roots and rhizomes of seagrasses stabilize bottom sediments and the leaf coverage abates the strength of currents (Short & Neckles 1999). The role of seagrasses is considered as so important that seagrass meadows are regarded as the most valuable ecosystems in terms of benefits they provide for society (Constanza et al. 1997).

In addition, seagrass meadows constitute highly productive ecosystems. They are present only in 0.15% of the planet's ocean surface area, but they are estimated to contribute 12% of the net ecosystem production of the global ocean (Duarte & Cebrian 1996). The primary production of seagrasses and associated macroalgae and epiphytes equals that of many cultivated terrestrial ecosystems (Duarte & Chiscano 1999). Compared to other marine coastal plant ecosystems like salt marshes, mangroves and kelp beds, seagrasses have a much wider geographical range. They have colonized almost all seas with the exception of the extreme high Polar Regions (Green & Short 2003). Extensive seagrass beds can accumulate large amounts of carbon. Some carbon is exported as detritus to deeper regions; some carbon is buried within the seagrass sediments. Therefore, seagrass systems are supposed to be hot spots for the sequestration of carbon in the biosphere (Duarte et al. 2005).

Within the last 30 years a rapid decline of seagrass systems has taken place worldwide, mostly caused by increasing anthropogenic influences in coastal areas. Physical disturbances (e.g. dredging, mooring and use of motorboats in shallow water), aquaculture, invasive species, herbicide runoff, global warming and particularly the increasing input of sediments and nutrients into coastal waters caused seagrass losses at scales up to hundreds of square kilometres (Orth et al. 2006). Seagrasses require considerably higher light intensities compared to macroalgae. This feature renders this plant group especially prone to the consequences of eutrophication. High nutrient supply promotes the growth of planktonic and epiphytic microalgae, which reduce the light reaching the seagrass leaves.

The reported decline of temperate and tropical seagrass systems has increased almost tenfold over the last 40 years. Orth et al. (2006) regard seagrass ecosystem as “coastal canaries”, signalling important deleterious impacts of human influences in coastal systems.
Eelgrass (*Zostera marina*) is the most important seagrass species in northern temperate regions. It has a wide distribution throughout the Atlantic and Pacific, and it is the only seagrass growing up to the Arctic Circle. The Mediterranean Sea constitutes the most southern limit of eelgrass distribution. An outbreak of the “wasting disease”, an infection with the slime mold-like protist *Labyrinthula zosterae*, dramatically decimated eelgrass meadows along the Atlantic coasts of North America and Europe in the 1920s and 1930s (Muehlstein 1989). To date, recovery in European waters has been poor or slow. The small eelgrass (*Zostera noltii*) has partially substituted *Zostera marina* in the Wadden Sea.

1.2. The influence of mesograzers in seagrass systems

Much ecological interest has focused on the interactions of herbivores and their plant prey in marine ecosystems. Several experimental studies in rocky shores and tropical reefs have demonstrated the dramatic effects of herbivores on biomass, species composition and diversity of algal assemblages (Lubchenco & Gaines 1981, Morrison 1988, Paine 1992). These studies concentrated mainly on macroinvertebrates like sea urchins and on fish species as herbivores. The trophic and ecological role of small, mobile herbivore species (mesograzers) in structuring natural plant communities received far less attention.
Marine macroalgae and seagrass systems accommodate a diverse community of small invertebrate species, consisting chiefly of amphipods, isopods, herbivorous crabs and gastropod molluscs. These mesograzers can occur in tremendous numbers and are nearly ubiquitous in coastal macrophyte systems. In temperate regions, where sea urchins and herbivorous fish are usually not abundant, mesograzers are presumed to be the most important consumers of algal biomass (Orth & Montfrans 1984). Mesograzers have high rates of production and thus, may contribute a large portion to total benthic secondary production in many communities (Edgar et al.1994, Taylor 1998). Particularly the crustacean mesograzers are important in the diet of fish and play a crucial role in the transfer of primary production to higher trophic levels (Edgar & Shaw 1995, Bobsien 2006).
The potential importance of mesograzers was chiefly paid attention to in seagrass systems, where mesograzers are assumed to play a key role in maintaining the health of macrophytes, and thus, the functioning of the total community (Jernakoff et al.1996, Heck et al. 2000). Seagrass leaves are colonized by a variety of epiphytes, mostly diatoms and small filamentous algae. Epiphytes are generally competitively superior compared to seagrasses. They can intercept water column nutrients and light and diminish the access of seagrasses to

carbon and oxygen at the leaf surface (Sand-Jensen 1977, Sand-Jensen et al. 1985). Under high nutrient supply, epiphytes can overgrow their host and cause detrimental effects on seagrass growth and biomass. Anthropogenic nitrogen loading to estuaries is supposed to be a major cause of contemporary seagrass decline (Short et al. 1995, Hauxwell et al. 2003). Most mesograzers feed preferentially on epiphytic algae and thus, promote seagrass growth and survival by releasing the plants from competition against epiphytic algae (Brush & Nixon 2002, Hughes et al. 2004). Thus, the detrimental effect of eutrophication on macrophyte communities may partially be mitigated by high mesograzers abundances (Williams & Ruckelshaus 1993, Worm et al. 2000). This hypothesis was addressed in some studies, but the results have been ambiguous (Jernakoff et al. 1996, Hughes et al. 2004).

Traditionally, mesograzers are regarded as a relatively homogenous functional group in terms of structuring effects on plant assemblages. However, recent studies have provided evidence that the species-specific impact of mesograzers is important in benthic macrophyte systems (Duffy & Hay 2000, Duffy et al. 2001). Any functional differentiation is presumably relevant, because of the seasonally and spatially varying mesograzer abundances (Thom et al. 1995).

High abundances of the common mesograzers the isopod *Idotea baltica* and the small gastropod *Rissoa membranacea* had detrimental effects in eelgrass meadows, because these species can graze directly on eelgrass tissue (Duffy et al. 2003, Fredriksen et al. 2004). Thus, the impact of these mesograzers on eelgrass may change from neutral to positive or detrimental in accordance with their density. To my knowledge only one study with the small snail *Lacuna vincta* has so far dealt with the effects of changing mesograzer abundance (Nelson 1997).

Furthermore, there is some evidence for mutualistic effects in herbivore-algae interactions. Few experimental studies have reported an increase of nutrient content of primary producers under grazing pressure (Hunter & Russell-Hunter 1983, Hillebrand et al. 2002). Grazers may mediate the availability of nutrients directly by excretion products containing nitrogen and phosphorus, and by sloppy feeding, or indirectly by removing the overstory of cells, and thus, by destroying the boundary layer, which obstructs nutrient diffusion (McCormick & Stevenson 1991). Although, there are some suggestions that these mechanisms may enhance primary producer photosynthetic capacity, no single study has explicitly verified this hypothesis in marine algal communities.

The effect of grazing on primary producer diversity is supposed to be unimodal (Lubchenco 1978, Sommer 1999, Abrams 2001). Intermediate grazing pressure and thus, intermediate mortality of prey species is assumed to generate the highest diversity in plant communities. High abundances of grazers strongly decrease plant biomass and thereby certain species may be eliminated, causing a reduction in diversity with only the most grazing resistant

species persisting. The superior competitors are supposed to dominate plant assemblages under low grazing pressure. Intermediate grazing effort may prevent the competitive exclusion of inferior species, if the dominant plant species are preferentially consumed (Huston 1979). Some authors regarded this pattern under the framework of the "intermediate disturbance hypothesis" (Connell 1978).

Herbivory and nutrient supply are regarded to play a fundamental role in structuring plant communities. At the moment, two contrasting views try to explain the different trophic structure in ecosystems. The "bottom-up" perspective assumes that ecosystem processes are primarily regulated by abiotic factors such as nutrients and light. The biomass at any trophic level is then controlled by the productivity of its resources. Alternatively, a "top-down" approach focuses on the importance of predation in regulating lower trophic levels. Numerous studies in terrestrial and aquatic systems have lent evidence for both hypotheses (Leibold et al. 1997). Apparently, both forces are not mutually exclusive and the dualism between them is artificial. Recent marine studies have tried to reconcile the two views and focus on the interactions of consumer and resource control in structuring natural communities (Proulx & Mazumder 1998, Hillebrand et al. 2000, Worm et al. 2002, Hillebrand 2003). In seagrass systems, several studies exist that have manipulated grazing or nutrients alone, but so far only one study has simultaneously dealt with bottom-up and top-down forces (Neckles et al. 1993).

The growing concern about the loss of marine habitats and the associated flora and fauna has recently raised interest in the effect of changing consumer diversity on ecosystem processes (see Duffy 2002, Hooper et al. 2005 for reviews). The positive effect of enhanced biodiversity has been well documented in terrestrial plant communities (see Loreau et al. 2002 for an overview), but experiments on the impact of biodiversity in marine systems are scarce. Several considerations suggest that the loss of species on the consumer levels may have drastic consequences. First, consumers often have influences in ecosystems, which are stronger than their abundances may implicate (e.g. keystone species). Second, strong top-down forces are supposed to be relevant in structuring marine ecosystems. Third, species at higher trophic levels face greater risk of extinction (Jackson et al. 2001, Petchey et al. 2004).

Theory predicts that higher biodiversity enhances community resource use, productivity and stability (see Tilman 1999 for review). Two mechanisms are assumed to cause the more efficient resource use in more diverse communities. By chance alone, more diverse assemblages may contain species, which are best adapted to present conditions (the selection effect). Niche partitioning and facilitation allows a stronger exploitation of available resources (the complementary effect). Experiments in marine systems have shown an

increasing resource use by sessile invertebrates (Stachowitz et al. 1999), mobile grazers (Duffy et al. 2003), and ciliates (Gamfeldt et al. 2005).

1.3. Questions

In this thesis, I asked the following questions:

- How do varying densities of mesograzers influence the biomass and productivity of eelgrass and epiphytes?
- Does intermediate grazing pressure enhance microalgal diversity?
- How are grazing effects modified by different nutrient supply?
- Does mesograzer diversity affect grazing efficiency and thus, epiphyte biomass and diversity?
- Are epiphytes a relevant food source for mesograzers in eelgrass communities?

1.4. Approach

To answer these questions, I chose an approach combining laboratory experiments and field surveys. A two-year field study in co-operation with S. Gohse-Reimann in an eelgrass meadow adjacent to Falkenstein Beach in the inner Kiel Fjord provided basic information on biomass and nutrient content of eelgrass and epiphytes, as well as data on mesograzer abundances. In the laboratory, I conducted 3 experiments under summer conditions to test the impact of the four common mesograzers *Idotea baltica*, *Gammarus oceanicus*, *Littorina littorea* and *Rissoa membranacea* in eelgrass-epiphyte systems. Additionally, I analysed stable isotopes and fatty acids of main primary producers and consumers at the study site in June 2002.

1.5. Thesis outline

This thesis is structured in 7 chapters. After the general introduction, I present the results of four experiments, in which I manipulated the densities of the four common mesograzers *Idotea baltica*, *Gammarus oceanicus*, *Littorina littorea* and *Rissoa membranacea*. The effect on eelgrass and epiphyte biomass and productivity are treated in Chapter 2. The consequences for epiphyte composition and diversity are presented in Chapter 3. I discuss the impact of increasing nutrient supply and constant intermediate grazing of *I. baltica* on epiphytes and eelgrass in Chapter 4. The following chapter reveals the influence of mesograzer diversity on epiphyte and microphytobenthos biomass, composition and diversity. A field study in early summer was used to clarify the relevance of epiphytes as food source for mesograzers in an eelgrass bed. A combination of stabile isotope and fatty acid analyses to resolve the food web structure in an eelgrass is presented in Chapter 6. The general conclusions of all studies are summarized in Chapter 7.

2. Effects of mesograzers on epiphyte and eelgrass productivity

2.1. Introduction

Estuarine benthic macrophyte communities are regulated by abiotic conditions, resource availability and food web structure. Small invertebrate consumers, primarily crustacean and gastropod species, are supposed to play a crucial role in controlling and structuring ecosystem processes. These mesograzers are important in the energy transfer of primary production to higher trophic levels including commercially important fish species (Edgar & Shaw 1995, Taylor 1998). Especially in seagrass communities, mesograzers are essential to maintain the fundamental health and functioning of these systems. Most mesograzers preferentially feed on epiphytic algae and thus, promote seagrass growth and survival by releasing the plants from competition for light and nutrients (Brush & Nixon 2002, Hauxwell et al. 2003, Hughes et al. 2004). Thus, the detrimental effect of eutrophication on macrophyte communities may partially be mitigated by high mesograzers abundances (Williams & Ruckelshaus 1993, Hillebrand et al. 2000, Worm et al. 2000).

Historically, mesograzers are considered as a homogeneous functional group in many studies (Steneck & Watling 1982, Edgar 1990a). They are thought to feed rather unselectively on epiphytic algae and detritus; this assessment is indirectly corroborated by field experiments demonstrating a rapid compensatory response of mesograzers to manipulation of single grazer species abundances (Edgar 1990b, Edgar & Aoki 1993). However, some experimental studies showed a significant species-specific impact of mesograzers on biomass and taxonomic composition of primary producers in macrophyte assemblages (Jernakoff & Nielsen 1997, Duffy & Harvilicz 2001, Duffy et al. 2001). A meta-analysis approach (Hughes et al. 2004) found mixed evidence for the importance of mesograzers to support seagrass systems, emphasizing the necessity of further studies on the diversity of mesograzer influences. In particular, important invertebrate grazers (e.g. *Idotea baltica*, *Idotea resecata* and *Rissoa membranacea*) potentially feed on both epiphytes and macrophytes, depending on circumstances like food availability and grazer abundance (William & Ruckelshaus 1993, Orav-Kotta & Kotta 2003, Fredriksen et al. 2004). Controlled by their abundance, the effect of mesograzers on macrophytes can be positive, neutral or negative. Therefore, it is necessary to integrate the effect of varying grazer abundances to fully understand the functional characteristics of different mesograzers.

Furthermore, the effect of consumers on periphyton may be not altogether negative. It has been previously shown that grazer can promote nutrient availability to the periphyton community by removing the overstory of cells (McCormick & Stevenson 1991), and thus may enhance the photosynthetic capability. Sloppy feeding and excretion products, containing

nitrogen and phosphorus, may provide additional nutrient sources mediated by grazing (Mulholland et al. 1991, Kahlert & Baunsgaard 1999, Hillebrand et al. 2002).

In this study, mesograzer abundance was manipulated in mesocosm experiments to test for biomass-specific and density-dependent effects on primary productivity in an epiphyte-eelgrass system. The isopod *Idotea baltica* (*Idotea* hereafter), the amphipod *Gammarus oceanicus* (*Gammarus* hereafter) and the gastropods *Littorina littorea* (*Littorina* hereafter) and *Rissoa membranacea* (*Rissoa* hereafter) were stocked in mesocosms that contained eelgrass (*Zostera marina*) according to natural densities in summer, and their impact on epiphyte and eelgrass productivity was measured. All studied species are potentially dominant grazers in coastal waters in temperate regions.

I wanted to answer two questions with this approach:

(1) Are the four studied mesograzer functionally redundant in their impact on the epiphyte-eelgrass assemblage?

(2) How do natural mesograzer abundances influence ecosystem processes?

2.2. Methods

Experimental design

I conducted mesocosm experiments in summer 2002 to test the impact of four common grazer species on primary productivity in an eelgrass-epiphyte system. The experiments took place in temperature controlled room (Fig. 2.1). Six 125 l aquaria were divided into four compartments with a 1 mm metal mesh, resulting in 24 mesocosm units (25 cm x 25 cm x 50 cm). This corresponds to the minimum size recommended for experiments with seagrass (Short et al. 2001). Summer conditions were established concerning light and temperature. The aquaria were illuminated by HQI-lamps with a 16 h day and 8 h night cycle. The light intensity was 100 µmol m^{-2} s^{-1} at the water surface. The temperature in the temperature controlled room was set to 17°C. However, due to a warming-effect of the lamps the water temperature in the aquaria was slightly higher (18.6 °C ± 0.3). Sand-filtered brackish deep water from the Kiel Fjord (salinity: 14.1 PSU ± 2.2) was used and additionally filtered with a 0.8 µm membrane filter to avoid contamination with plankton species. Continuous water circulation was created using pumps and the water was exchanged (up to 90% of the total volume) every day. Periphyton growing on the walls was removed every day before the water exchange.

The mesocosms were filled (5 cm) with 1 mm-sieved homogenized sediment, which consisted mainly of fine sand with low organic content. After 24 h, 20 freshly harvested and washed eelgrass shoots were planted in each mesocosm (320 shoots m^{-2}, average abundance in the Kiel Fjord in summer). Only shoots with at least four leaves were selected and the average length of shoots was 40 cm. On the following day, the mesocosms were

Fig. 2.1. Experimental eelgrass systems

stocked with grazers. All experimental material was collected at Falkenstein Beach in the inner Kiel Fjord, Germany (54°21'/10°9'). The experiment was terminated after ten days. At this time, the eelgrass was harvested, placed in plastic bags and stored frozen until further processing.

Each experiment included four treatments: a grazer-free control and low, mean and high abundances of one grazer species (Table 2.1). Grazer densities were chosen based on summer density data collected within a monitoring program for eelgrass associated macrofauna in the Kiel Bight (1997-2001). Each treatment was replicated in six independent mesocosms in a randomized block-design. All treatments in one aquarium were regarded as one block.

Table 2.1. Grazer abundance in all experiments. Treatments with the same biomass are shown in bold.

Grazer abundance	Density (m^{-2})			Biomass (g AFDM m^{-2})		
	low	mean	high	low	mean	high
Gammarus oceanicus	80	160	320	0.24	0.48	**0.96**
Idotea baltica	128	256	512	0.48	**0.96**	1.92
Littorina littorea	64	128	256	**0.96**	1.92	3.84
Rissoa membranacea	320	640	1280	0.24	0.48	**0.96**

Epiphyte biomass

Epiphyte biomass was measured using chlorophyll *a* as proxy. Six eelgrass shoots were randomly selected from each mesocosm. Epiphytes were carefully scraped from the eelgrass blades using a special plastic scraper and a scalpel and transferred to small amounts of filtered sea water. This suspension was filtered on precombusted (450 ^{o}C, 24 h) Whatmann GF/F filters. Pigment analyses with HPLC, carried out on scraped eelgrass blades and epiphytes, indicated that removal efficiency by scraping was up to 99%. Chlorophyll *a* concentration was calculated according to (Lorenzen 1966). The cleaned eelgrass blades were dried to a constant weight for 48 h at 60^{0}C and subsequently combusted for 8 h at 540^{0}C to determine the ash-free dry mass (AFDM). The eelgrass surface area was calculated using the formula surface (mm^2) = AFDM (g) x 588.88 (R^2=0.97), determined by measuring and weighing 100 eelgrass shoots. All epiphytic chlorophyll concentrations were normalized to unit eelgrass surface area.

Eelgrass growth

Eelgrass leaf production was measured by a variation of the leaf-marking technique (Sand-Jensen 1975). All eelgrass shoots were marked with a needle hole 1 cm above the first node with roots before being planted in the experiment. Six shoots from each mesocosm were cut at the marking and the length and width of new leaves (without hole) and the growth of old leaves were measured. The production of biomass was calculated as AFDM per day using the formula mentioned above.

Eelgrass and epiphyte productivity

Primary productivity estimates, based on ^{14}C-measurements were carried out on the last day of the experiment. Four eelgrass shoots were randomly selected from each mesocosm and the mid section of each shoot (10 cm) was transferred into a transparent Nalgene plastic bottle containing 250 ml seawater (0,2 µm filtrated). After inoculation with 26.4 µCi ^{14}C-Na_2CO_3, three hour incubations (between 10.00 and 14.00 h) were carried out under experimental conditions. One bottle out of each mesocosm was wrapped up in aluminium foil and used as dark incubation. After incubation all eelgrass shoots were placed in plastic bags and stored frozen until further processing. Epiphytes were separated from the eelgrass blades by carefully scraping the blades using a special plastic scraper and a scalpel, and then they were transferred into small amounts of filtered sea water. This suspension was filtered on preweighted membrane filters. The filters and the eelgrass blades were dried for 48 h at 60 ^{0}C and weighted to calculate dry weight. Then the filters were transferred into scintivials containing 10ml Lumagel. Radioactivity was measured in a Liquid Scintillation Counter. The dried eelgrass was wrapped up in Whatman ashless filter paper with a small

amount of starch to promote combustion and compressed into pellets. Combustion took place in a Carbon Oxidiser where the CO_2 was trapped in a scintillating solution. All counts were corrected for background, recovery efficiency after combustion, and counting efficiency.

Productivity was calculated as follows:

$$\text{mg C (g dry wt)}^{-1}\ h^{-1} = \frac{dpm_1 * {}^{12}CO_2 * 1.06}{dpm^2 * wt * t},$$

where dpm_1 is the activity (decay per minute) of the samples minus the activity in the dark incubation as correction for non-photosynthetic uptake of ^{14}C, dmp_2 the activity of the isotope added to the bottles and $^{12}CO_2$ the mg available inorganic carbon. The factor 1.06 is a correction for isotope discrimination. Wt is the dry weight of the epiphyte or eelgrass sample and t the length of the incubation period in hours (Penhale 1977).

Elemental composition

Two eelgrass shoots from each mesocosm were carefully washed in filtered seawater to remove detritus; the epiphytes were removed as described above and filtered on precombusted (450°C, 24h) Whatmann GF/F filters. After drying (24 h, 60°C) the samples were stored in a dissecator until combustion in a CHN-analyser (Fisons, 1500N) to measure C and N content.

Calculation of effect strength of the four grazers

To compare the different impact of the four studied grazer species on processes in the epiphyte-eelgrass system, grazer effects on epiphytes and eelgrass were calculated as the raw difference between control and grazer treatments with the same biomass level (0.96 mg AFDM m^{-2}, Tab. 1).

Statistics

The influence of grazer abundance on epiphytes and eelgrass was initially analysed using randomized block ANOVAs, in which the different abundances were considered fixed factors. The block effect was non-significant in all analyses, therefore the block factor was ignored and the data were reanalysed with a one-factorial ANOVA. Differences between treatments were tested with Tukey`s test. To investigate species-specific effects, one-factorial ANOVAs were conducted.

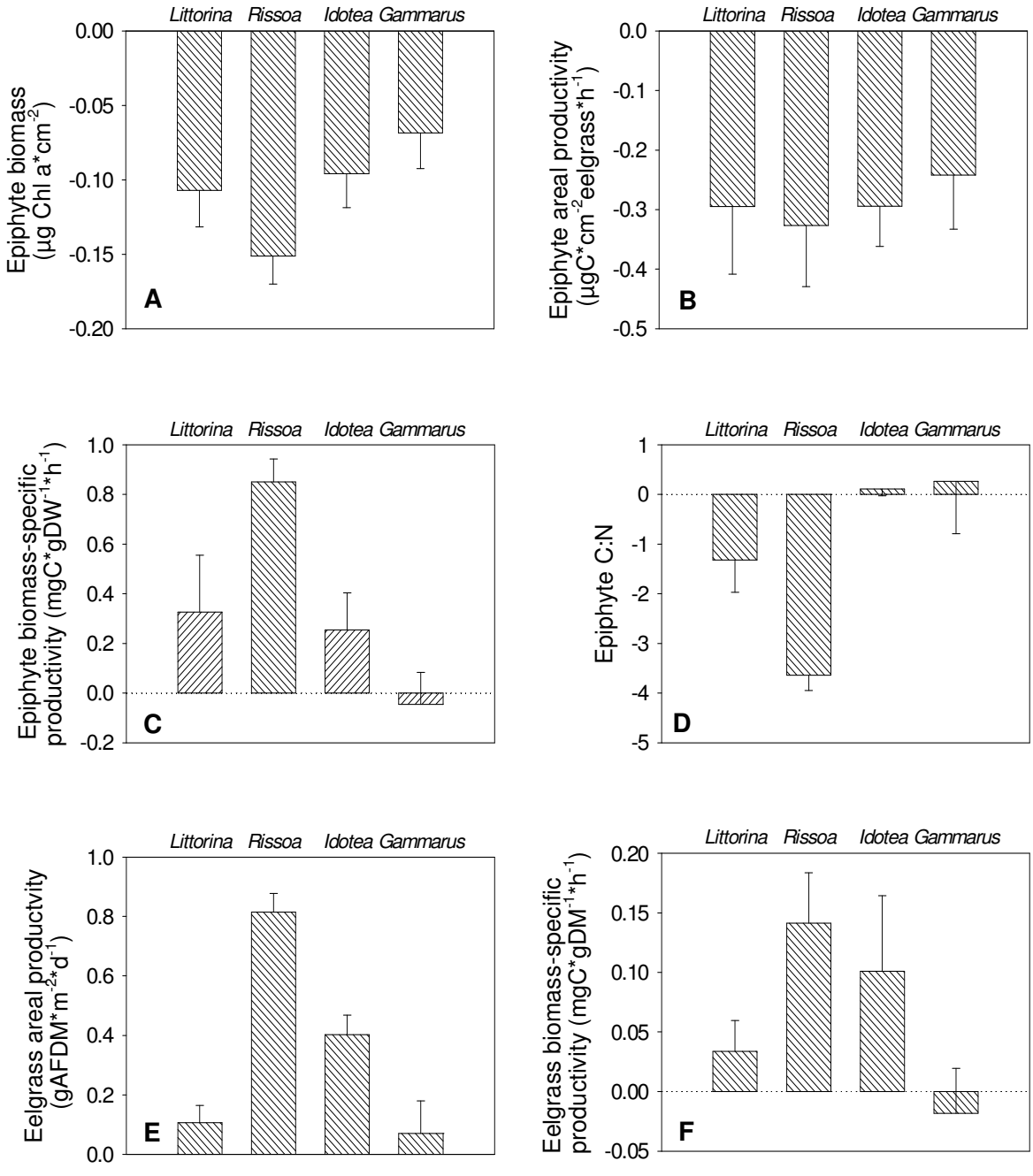

Figure 2.2. Comparative effects of grazer species on epiphyte biomass (A), epiphyte areal productivity (B), epiphyte biomass-specific productivity (C), epiphyte C:N (D), eelgrass areal productivity (E) and eelgrass biomass-specific productivity (F). Shown are the raw, arithmetic differences between grazer treatments and grazer-free controls.

2.3. Results

Comparative effects of the four grazers on epiphytes and eelgrass

The comparison of species-specific effects on epiphytes and eelgrass showed considerable differences among the four grazer species. *Rissoa* reduced epiphyte biomass significantly stronger than the three other species ($p \leq 0.01$) and *Gammarus* had a significantly weaker effect than *Rissoa* and *Littorina* ($p \leq 0.04$) (Fig. 2.2.A).

In contrast to these results, the impact on epiphyte areal productivity was not significantly different, although the same trends as for epiphyte accumulation were found (Fig. 2.2.B).

The effect on epiphyte biomass-specific productivity differed substantially among the four grazers (Fig. 2.2.C). The impact of *Rissoa* enhanced this process significantly compared to the other grazers ($p \leq 0.0002$) and *Idotea* and *Littorina* had a significantly stronger positive effect than *Gammarus* ($p \leq 0.02$).

The positive effect of *Rissoa* ($p \leq 0.0002$) on the nitrogen content of epiphytes was significantly stronger compared to the effect of the other grazer species (Fig. 2.2.D). Furthermore, *Gammarus* and *Idotea* exerted a significantly weaker positive effect than *Littorina* ($p \leq 0.005$).

In accordance with its impact on epiphyte accumulation, *Rissoa* had a significantly stronger positive effect on eelgrass areal and biomass-specific productivity (Fig. 2.2.E+F) than the other three grazer species ($p \leq 0.0002$ and $p \leq 0.03$, respectively), whereas *Gammarus* had the weakest positive effect ($p \leq 0002$) on eelgrass areal productivity and essentially no effect on eelgrass biomass-specific productivity ($p \leq 0.003$). *Littorina*, in contrast, had far less positive effects on both parameters than could be expected from its negative impact on epiphyte accumulation.

Density-dependent effects

All studied grazers had a significant impact on epiphyte biomass compared to the grazer-free controls (Fig. 2.3), but the strength of this effect varied among the different species. *Littorina* affected epiphyte accumulation most strongly; it reduced the epiphyte biomass to 12% of the control values. Epiphytes were virtually eliminated in the high abundance *Littorina* treatment. *Rissoa* and *Idotea* diminished epiphyte biomass to 42% and to 49%, respectively. *Gammarus* exerted the weakest effect, a decrease to 69% of the control values. An interesting difference was found between gastropods and crustaceans: the mean abundances of *Idotea* and the low abundance of *Gammarus* seemed to be a kind of threshold density, regarding their impact on epiphyte biomass. Further increase in animal abundances did not affect epiphyte biomass significantly. *Idotea* reduced epiphyte biomass to a minimum of 0.1 µg chlorophyll cm^{-2} and *Gammarus* to 0.15 µg chlorophyll cm^{-2}. The

gastropods *Littorina* and *Rissoa* reduced epiphyte biomass significantly stronger in the treatments with high abundances. Epiphyte areal productivity showed essentially the same pattern as could be expected from epiphyte biomass.

With the exception of *Gammarus*, epiphyte biomass-specific productivity (based on ^{14}C-measurements) increased significantly with the presence of grazers (Fig. 2.4). *Rissoa* had the strongest effect on epiphyte biomass-specific productivity; even mean abundances of this species significantly enhanced this parameter and high abundances of this species nearly doubled epiphyte productivity compared to controls. *Idotea* and *Littorina* showed significant effects only in the high abundance treatments. Epiphyte biomass-specific productivity increased by 47% and 80% in the high abundance treatments of *Idotea* and *Littorina*. *Gammarus* had no significant impact on epiphyte photosynthetic capacity.

Initial values of epiphyte C:N ratio ranged from 12.1 to 12.5 indicating a deficiency of nitrogen in summer. Epiphyte C:N values from 7.5 to 8.9 were observed under higher nutrient conditions in spring and autumn. In the experiments with *Idotea* and *Gammarus,* the initial values remained basically unchanged. In contrast, *Littorina* and *Rissoa* had a significant positive effect on the nitrogen content of epiphytes (Fig. 2.5).

Eelgrass areal productivity measured as growth rate increased significantly with increasing abundances of *Idotea*, *Littorina* and *Rissoa* (Fig. 2.5). *Gammarus* had no significant impact on eelgrass productivity which was in accordance with the weak impact of this species on epiphyte accumulation. The highest eelgrass growth rate was found in the high abundance *Rissoa* treatment with 1.9 g AFDM m^{-2} d^{-1}, an increase of 78% relative to control values. The impact of *Idotea* and *Littorina* enhanced eelgrass production by 63% and 72%, respectively.

The presence of grazers significantly increased eelgrass biomass-specific productivity (based on ^{14}C-measurements) in the experiments with *Idotea*, *Littorina* and *Rissoa* (Fig. 2.6). All three grazer species increased eelgrass photosynthetic capacity by 75% relative to control values. *Gammarus* exerted no significant effect on eelgrass biomass-specific productivity.

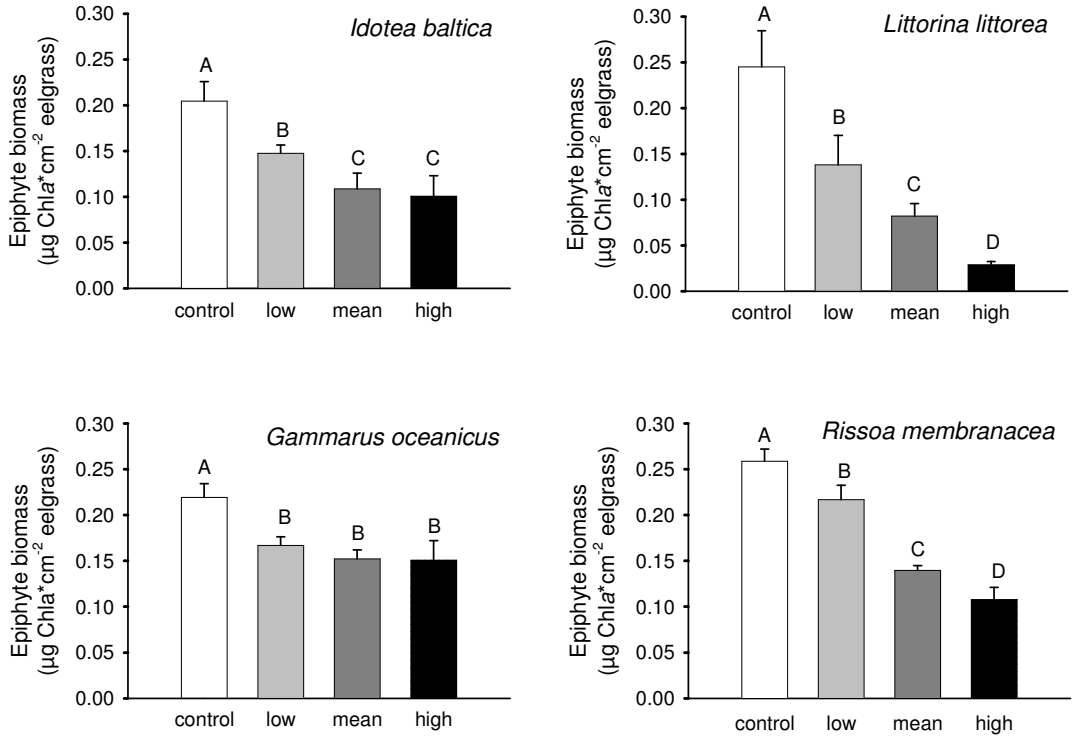

Figure 2.2. Impact of grazer abundance on epiphyte biomass (measured as chlorophyll *a*; mean±SD). Capital letters indicate significant differences between treatments.

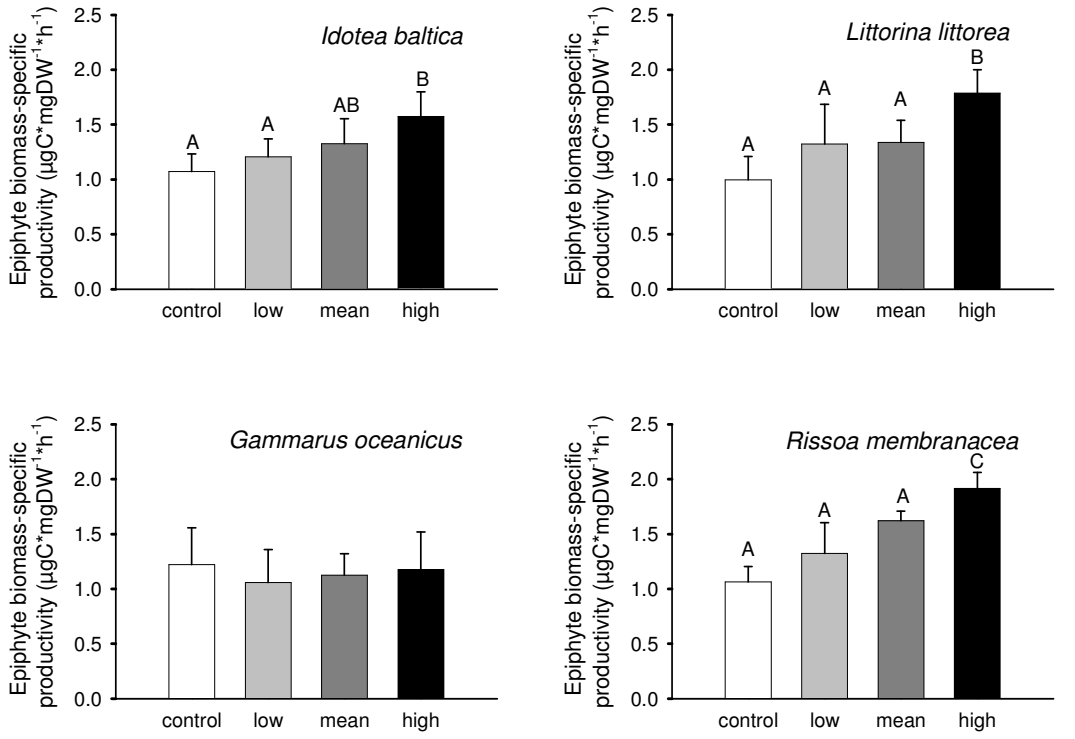

Figure 2.3. Impact of grazer abundance on epiphyte biomass-specific productivity (mean±SD). Capital letters indicate significant differences between treatments.

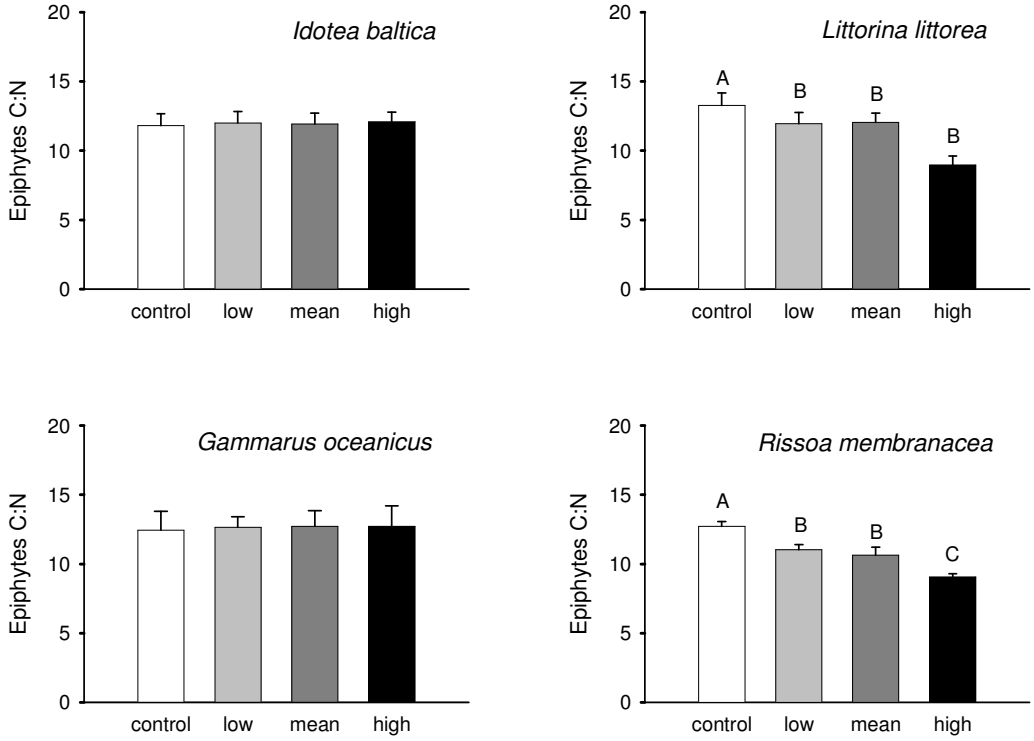

Figure 2.4. Impact of grazer abundance on epiphyte C:N (mean and standard deviation are given). Capital letters indicate significant difference between treatments.

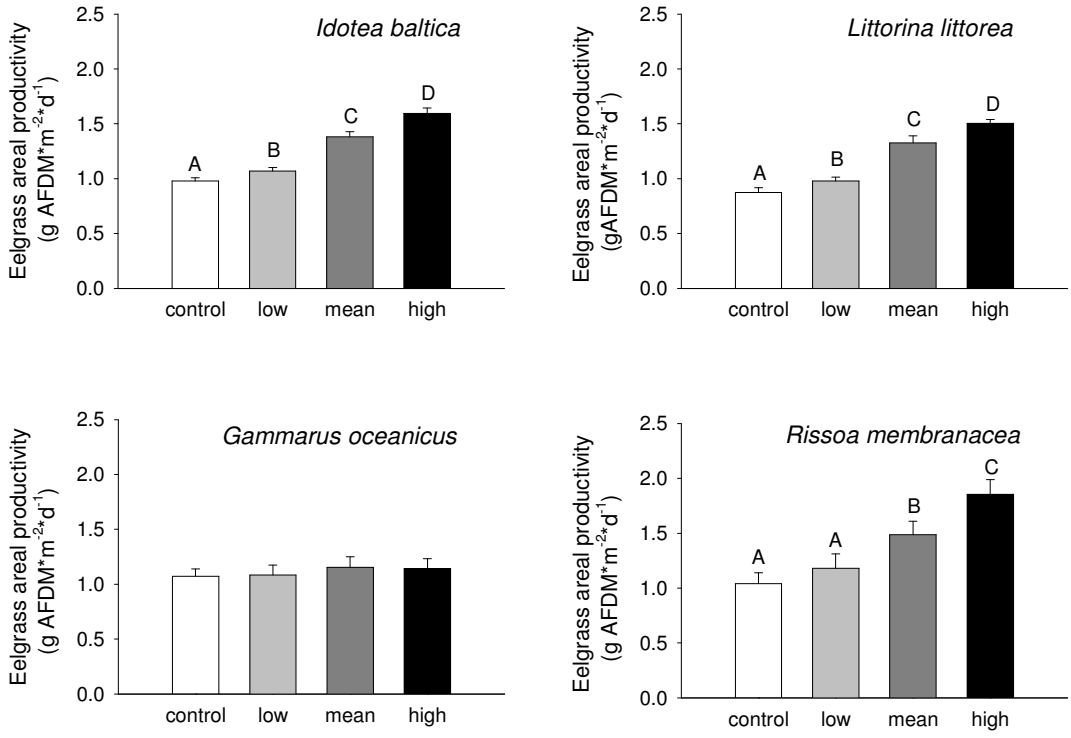

Figure 2.5. Impact of grazer abundance on eelgrass areal productivity (mean±SD). Capital letters indicate significant differences between treatments

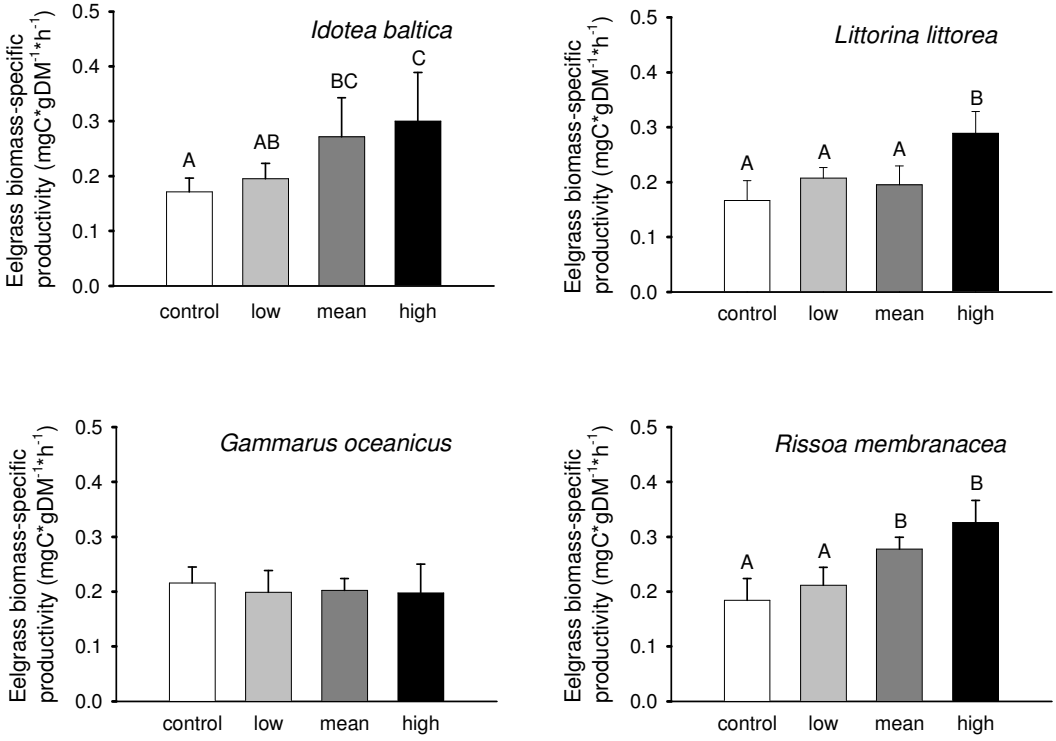

Figure 2.6. Impact of grazer abundance on eelgrass biomass-specific productivity (mean±SD). Capital letters indicate significant differences between treatments.

2.4. Discussion

Grazer functional diversity and its impact on ecosystem processes

The four mesograzers had significant impacts on the ecosystem processes studied, but the effects varied considerably between the different species and the response variable. Important ecosystem properties like epiphyte biomass, productivity and nitrogen content were differently affected just as eelgrass productivity.

The results confirmed previous conclusions that mesograzers can exert strong top-down control on the fouling community in seagrass systems (Orth & van Montfrans 1984, Duffy et al. 2001, Hughes et al. 2004). However, marked differences in the species-specific impact were found. First of all, the gastropods *Rissoa membranacea* and *Littorina littorea* exerted a stronger negative per biomass effect on epiphyte accumulation than the crustaceans *Idotea baltica* and *Gammarus oceanicus*: the impact of *Rissoa* was the strongest and that of *Gammarus* the weakest. Our experiments confirmed the conclusion of Jernakoff and Nielsen (1997), that gastropods are more efficient grazers than amphipods. Earlier studies also found a pronounced impact of various gastropods on epiphyte assemblages in seagrass systems (Klumpp et al. 1992, Philippart 1995, Fong et al. 2000). The evidence on grazing effects of

amphipods is inconclusive and species-specific (Howard 1982, Duffy & Harvilicz 2001), but *Gammarus mucronatus* affected epiphyte biomass much less than two isopod mesograzers in another experimental eelgrass system (Duffy et al. 2001).

Epiphyte consumption by mesograzers can generate a positive cascading effect on seagrasses, promoting the growth and survival of the foundation species of these systems, because epiphytes and seagrass compete for light and nutrients (Orth & van Montfrans 1984, Brush & Nixon 2002, Hauxwell et al. 2003, Hughes et al. 2004). In good accordance with their impact on epiphyte biomass, per biomass effects of *Rissoa* on eelgrass productivity were strongly positive and the ones of *Idotea* were moderately positive. *Littorina* and *Gammarus* exerted weaker effects on eelgrass productivity than could be expected from their impact on the epiphyte assemblages. These results are in accordance with previous studies that found a strong positive effect of gastropods and isopods on the growth and survival of seagrasses (Philippart 1995, Duffy et al. 2001, Schanz et al. 2002). *Gammarus* species are known to have no relevant impact on eelgrass productivity (Duffy & Harvilicz 2001, Duffy et al. 2001). Although *Gammarus* had a significant albeit weak impact on epiphyte biomass in this study, the effect on eelgrass was essentially zero.

Assuming that the competition for light is more eminent than the competition for nutrients, because seagrasses can obtain nutrients from both the water column and sediment pore water (Touchette & Burkholder 2000), the relationship between epiphyte accumulation and light attenuation could be a plausible explanation for this result. The reduction in irradiance, reaching the eelgrass leaves with higher epiphyte load, is best described by a negative hyperbolic equation levelling off to a constant (Brush & Nixon 2002). The grazing impact must exceed a certain critical level to have a positive effect on eelgrass productivity by the means of increasing availability of light. Obviously, grazing of *Gammarus* was not adequate in removing sufficient epiphyte biomass to generate this positive effect.

In contrast, *Littorina* exerted a strong grazing pressure on epiphytes, but only a weak positive effect on eelgrass productivity was found. Potentially, *Littorina* is capable to feed on macrophyte tissue (Steneck & Watling 1982, Norton et al. 1990). In our study, it was the only species that reduced the bottom layer of adnate diatoms (mostly *Cocconeis scutellum*) significantly (see Chapter 3) and completely freed the eelgrass leaves from epiphytes. This grazer species may have incidentally destroyed the chloroplast-rich epidermis of the eelgrass while feeding on the epiphytes and thus, confined eelgrass productivity.

Grazer effects on epiphyte biomass-specific productivity were strongly positive for *Rissoa,* intermediate for *Littorina* and *Idotea* and essentially zero for *Gammarus*. The photosynthetic capacity of epiphytes can be enhanced by the presence of grazers in different ways. A grazed and, thus thinner epiphytic community may have an increased access to water column nutrients and light. It has previously been assumed that grazers can influence the

photosynthetic capacity of biofilms in a positive way by removing the overstory of cells and by destroying the boundary layer which impedes nutrient diffusion (McCormick and Stevenson 1991). Furthermore, nutrient availability might be increased by sloppy feeding and by excretion products containing nitrogen and phosphorus (Mulholland et al. 1991, Kahlert & Baunsgaard 1999, Hillebrand et al. 2002).

The strong negative impact of *Rissoa* and *Littorina* on epiphyte C:N ratio and the finding that *Idotea* and *Gammarus* do not affect the C:N ratio of epiphytes support the assumption that, in contrast to the tested crustaceans, gastropods enhanced the photosynthetic capacity of epiphytes via excretion in our study. Especially, in the *Rissoa* treatments faecal pellets have been observed, which adhered to the biofilm. Apparently, this had immediate consequences for the nutrient availability in adjacent algal patches. A positive effect of grazers on nutrient content of microalgae has been previously reported for freshwater and one intertidal periphyton community (Hunter & Russell-Hunter 1983, Rosemond et al. 1993, Hillebrand et al. 2004). All studies used gastropods including *Littorina* as grazers which live in a close association with their food source. The more mobile crustacean grazers have the potential to supply a large amount of nitrogen to the plant community (Taylor & Rees 1998), but experimental evidence on the importance of nutrient recycling via grazing so far exists only for slow moving or sessile organisms like bryozoans and barnacles (Hurd et al. 1994, Williamson & Rees 1994).

The difference in the impact on epiphyte photosynthetic capacity between *Idotea* (positive effect) and *Gammarus* (no effect) strengthens the conjecture that the removal of the biofilm's canopy layer also played an important role in the enhancement of the photosynthetic capacity of epiphytes by mesograzers. *Idotea* fed on diatom chains, macroalgae and stalked diatoms; whereas *Gammarus* is only capable to remove filamentous growth forms (see Chapter 3). The stronger impact of *Idotea* on the structure of the algal assemblage might have mediated the availability of nutrients and light to the epiphyte community.

Even in a short-term experiment, grazer species composition strongly influenced processes in the studied eelgrass-epiphyte system. The co-occurring mesograzers varied substantially in their effect on epiphyte and eelgrass productivity. A combination of qualitatively and quantitatively different grazing behaviour of the studied consumers created this effect.

The impact of mesograzers in natural abundances on ecosystem processes

Most studies on the interaction of grazing organisms and ecosystem processes in seagrass systems are restricted to testing the presence and the absence of grazers (Williams & Ruckelshaus 1993, Philippart 1995, Jernakoff & Nielsen 1997, Fong et al. 2000). Experiments investigating density-dependent effects like this study are scarce (Nelson 1997).

The mesograzers tested in this study decreased epiphyte biomass and areal productivity even at low densities. However, I found species-specific differences with increasing grazer activity. The gastropods *Rissoa* and especially *Littorina* were more effective in reducing epiphyte accumulations on eelgrass leaves than the crustaceans *Idotea* and *Gammarus*. Furthermore, the impact of the gastropods increased continuously with increasing grazer abundance, whereas the impact of the isopod seemed to level of to a threshold value of epiphyte biomass than could not be under-run. In contrast, the amphipod showed no density-dependent effects at all. Another gastropod *Lacuna vincta* has been found to exert a similar effect on epiphytes as the gastropods in this study (Nelson 1997).

The four studied mesograzers are known to consume a diverse array of micro- and macroalgae (Warén 1996, Norton et al. 1990, Duffy & Harvilicz 2001, Orav-Kotta & Kotta 2003). The actively swimming, omnivorous *Idotea* and *Gammarus* are in general considered to reduce the microalgal community homogenously ("lawn-mower" type of grazer), whereas the slow moving, predominantly herbivorous *Littorina* and *Rissoa* produce a feeding trail by scraping the surface with their radula ("bulldozer" type of grazer) (Sommer 1999a). The taenioglossan radula of the studied gastropods enables theses species to feed in a rasping mode that is especially useful for the grazing of microalgae and filamentous algae (Steneck & Watling 1982), and taenioglossan gastropods have the ability to completely remove the epiphytic layer on eelgrass leaves (van Montfrans et al. 1982).

The epiphyte assemblage on eelgrass consisted of a basic monolayer of prostrate, strongly adhering diatoms, mostly *Cocconeis scutellum*, stalked forms like genus *Licmophora* and diatom chains. Tube-living diatoms and filamentous algae were of minor importance. Analyses of taxonomic composition of epiphytes in this study showed that *Littorina* uniformly reduced all growth forms and *Rissoa* diminished mostly stalked and chain-forming diatoms (see Chapter 3). This indicated that *Littorina* removed the epiphytic matrix completely and unselectively in its feeding trail and therefore this species had the strongest impact on the epiphyte assemblage, whereas the *Cocconeis* crust remained virtually unaffected by *Rissoa*, resulting in a slightly weaker grazing effect. The feeding activity of *Idotea* was further restricted mainly to chain-forming diatoms with a weak impact on stalked forms, whereas *Gammarus* only had a negative impact on diatom chains and filamentous algae. The difference in the functional morphology of their mouthparts (molluscan radula vs. crustacean mandibles) and different feeding behaviour presumably are responsible for the diminished impact of the crustacean grazers.

These results supported the hypothesis that top-down forces can influence the fitness of eelgrass, the structuring species of this system. The positive effect on eelgrass productivity increased with growing grazer activity. *Rissoa* increased eelgrass growth up to 78%. *Littorina* showed a less positive effect than could be expected by its strong impact on epiphyte

biomass. This effect could have been caused by the earlier mentioned potentially disruptive effect of the periwinkle on eelgrass tissue. Direct grazing on living eelgrass is known for *Idotea* and *Rissoa* (Duffy et al. 2001, Fredriksen et al. 2004). Grazing scars on eelgrass were only found in the *Idotea* treatments, but nevertheless eelgrass productivity increased with higher *Idotea* densities. The positive effect of epiphyte consumption compensated for the negative effect of direct grazing on eelgrass. Detrimental effects of *Idotea* on macrophytes have usually been observed, when the population reached very high abundances and other food sources were scarce (Duffy et al. 2003). During a two year monitoring period I noticed very few scars of *Idotea* grazing on eelgrass in the Kiel Fjord, implying that this mechanism plays no important role in this region. Grazing scars of *Rissoa* were observed not at all in the field, but occurred during cultivation of this species under extremely high densities in the laboratory. The deterioration of eelgrass found in southern Norway was also associated with very high *Rissoa* densities and found to be a single incident (4200 m^{-2}, Fredriksen et al. 2004).

In conclusion, the survival of the structuring species in this ecosystem – the eelgrass – is strongly connected with grazer identity and the effect of grazers can vary from mutual, to neutral, to antagonistic with changing density.

Recent studies have challenges the traditional view according to which the interaction of grazers and periphyton has been regarded as a unidirectional negative relationship (Kahlert & Baunsgaard 1999, Hay et al 2004, Hillebrand et al. 2004). This study supports the hypotheses that consumer can enhance the photosynthetic capacity of primary producers either directly by nutrient excretion or indirectly via reduced competition (McCormick & Stevenson 1991, Taylor & Rees 1998). High densities of both gastropod species increased nitrogen content and photosynthetic capacity of epiphytes, whereas *Idotea* only affected the productivity. Thus density-dependent mutual interactions existed not only between eelgrass and mesograzers but also between mesograzer and epiphytes.

Species-level characteristics of mesograzers had important effects in the epiphyte-eelgrass system and therefore, the functional group concept should only be used with cautiousness as proposed by Duffy et al. (2001). Grazer species composition and abundance are likely to be both essential factors in estimating the potential impact of mesograzers. The functional differences among generalist mesograzers varied considerably at the same abundance and with increasing grazing activity. This emphasises the importance of integrating the effect of the locally and temporally variability of grazer abundances in the assessment of grazing effects in macrophyte communities.

3. Effects of mesograzers on epiphyte diversity and composition

3.1. Introduction

Currently, there is much interest in the understanding how diversity at different trophic levels influences ecosystem processes. The growing rate of species extinction caused by human influences on ecosystems is superimposed on the natural factors that control species diversity. The local diversity of autotrophic organisms is regulate by local processes such as competition, grazing and abiotic conditions and by large-scale processes such as dispersal, speciation and connectivity (Hillebrand & Blenckner 2002, Hillebrand 2003).

Much research effort has been invested into studying the effects of local factors such as grazing and nutrient enrichment on algal communities in freshwater and marine environment (Steinmann 1996, Hillebrand et al. 2000). The impact of grazing on algal diversity is supposed to be dependant on the productivity of the system. Increasing nutrient supply and thus, productivity generally enhances the growth and dominance of few highly edible species (Proulx & Mazumder 1998). Under these conditions, the effect of grazers on diversity may be positive as far as they graze on the most common algal species (Worms et al. 1999). The effect may be reversed under low productivity, where grazing mainly reduced species richness. Furthermore, selectivity, spatial heterogeneity of grazing and the intensity of grazing pressure can influence the impact of herbivores on plant diversity (Lubchenco 1978, Sommer 1999a). In accordance with the 'intermediate disturbance hypothesis', a medium grazing pressure is assumed to be associated with the highest plant diversity (Abrahams 2001).

I tested this hypothesis with four mesograzer species in experimental eelgrass systems. The isopod *Idotea baltica*, the amphipod *Gammarus oceanicus* and the gastropods *Littorina littorea* and *Rissoa membranacea* were used to test the impact of growing mesograzer density on epiphyte diversity and composition.

3.2. Methods

Experimental design

I conducted mesocosm experiments (see Chapter 2.2) to test the impact of four common grazer species on epiphyte diversity and taxonomic composition.

Taxonomic composition and diversity of epiphytes

Two eelgrass shoots out of each mesocosm were carefully scraped using a special plastic scraper and a scalpel to transfer attached epiphytes into 250 ml of filtered seawater. The samples were fixed with 1% Lugol's iodine and counted under an inverted microscope in 3 ml Utermöhl-chambers. A minimum of 400 cells was counted for dominant species and the

whole chamber was counted to account for rare species. Biovolume was used as proxy for biomass following the method of Hillebrand et al. (1999).

Statistics

The Shannon-Wiener index (H') was used as measure of diversity. To test for significant treatment effects on diversity and evenness of epiphytes, I conducted linear and second order polynomial regressions. MANOVAs were used to test the significant impact of grazer abundance on the proportional contribution of algal growth forms to epiphyte composition. Data were arcsine square root transformed. The analysis was done with the Pillai`s trace statistic, recommended for interdependent response variables (Scheiner 1993).

3.3. Results

Diversity and evenness of epiphytes

Grazing of the four studied mesograzers had significant but varying effects on epiphyte diversity (H') (Fig. 3.1). The isopod *Idotea baltica* significantly reduced diversity (r^2 = 0.84, p = < 0.0001) and the amphipod *Gammarus oceanicus* had significant positive effects on epiphyte diversity (R^2 = 0.82, p = < 0.0001). The impact of the gastropods showed a weak unimodal pattern (*Littorina littorea*: R^2 = 0.68, p = < 0.0001 and *Rissoa membranacea* (R^2 = 0.78, p = < 0.0001). The species-specific effect on evenness followed exactly the same pattern as the impact on epiphyte diversity (Fig. 3.2). *Littorina* was the only species, which affected the number of epiphyte species (R^2 =0.85, p = < 0.0001). Epiphyte species were lost under high grazing pressure of this gastropod (Fig.3.3).

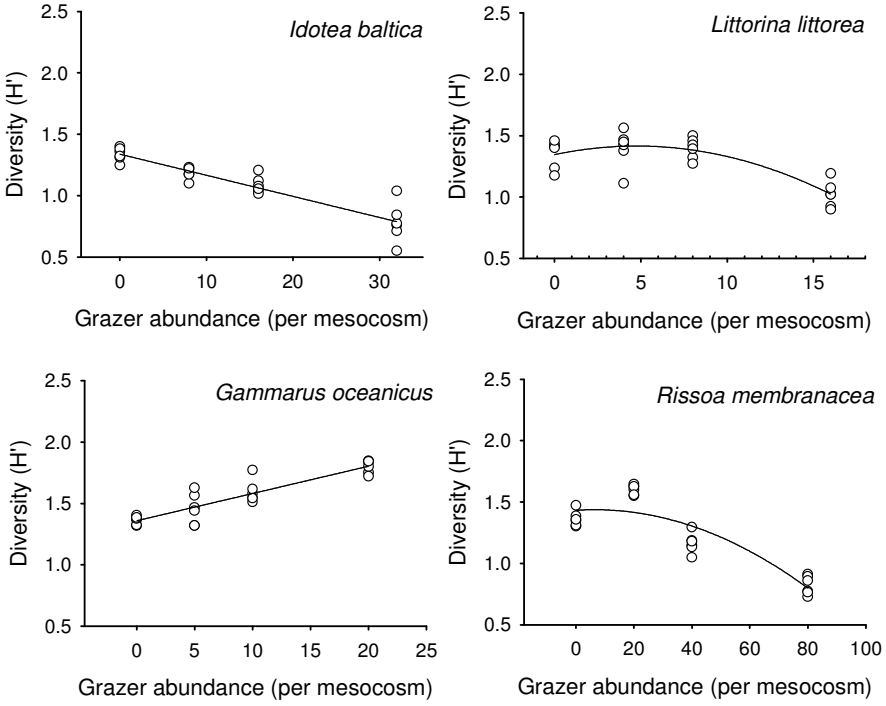

Figure 3.1. Diversity of epiphytes growing on eelgrass in experiments with natural abundances of four common mesograzers found in northern temperate macrophyte systems.

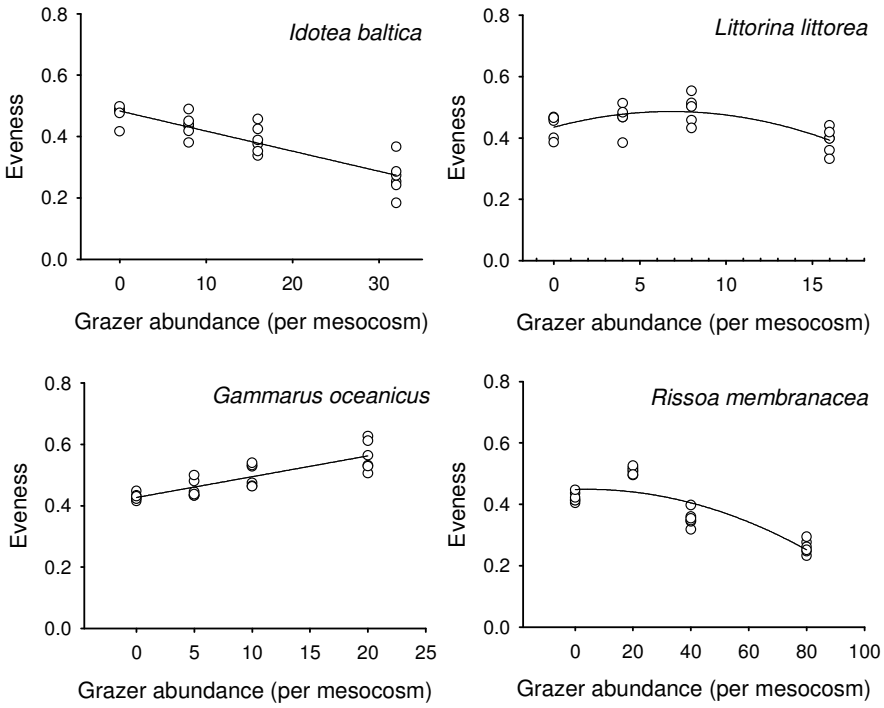

Figure 3.2. Evenness of epiphytes growing on eelgrass in experiments with natural abundances of four common mesograzers found in northern temperate macrophyte systems.

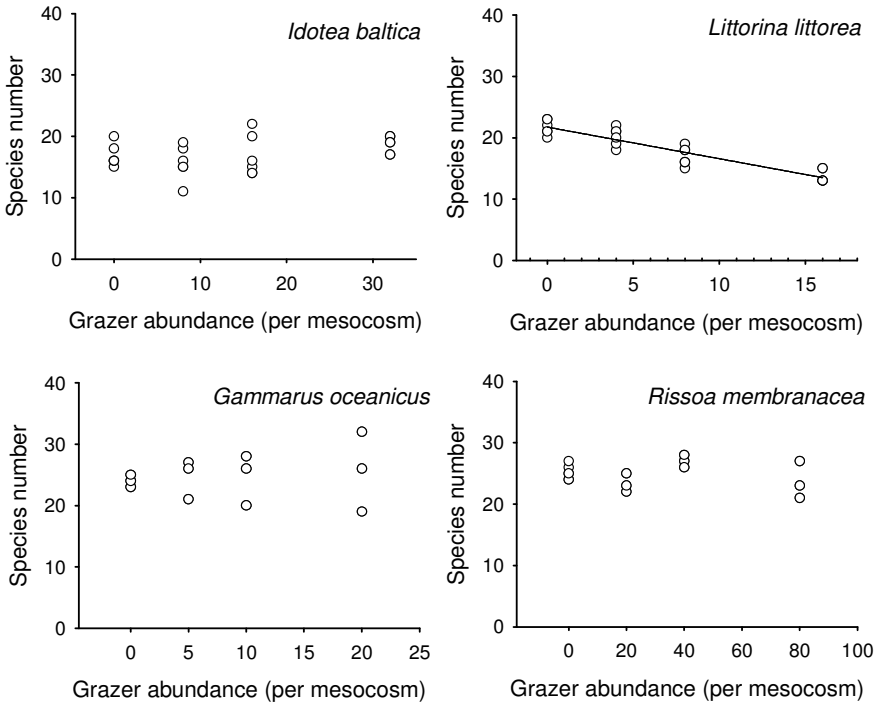

Figure 3.3. Number of epiphyte species growing on eelgrass in experiments with natural abundances of four common mesograzers found in northern temperate macrophyte systems.

Algal growth forms

Epiphyte composition was clearly dominated by diatoms, which constituted from 74 to 99% of epiphyte biovolume, but small filamentous algae were also present. Cyanobacteria were extremely rare in occurrence and were omitted from the analyses.

The diatoms showed a high differentiation in growth forms and cell sizes. The most important prostrate diatom species was the strongly adhering *Cocconeis scutellum* (Fig. 3.4A); mobile forms were represented by various *Amphora*, *Diploneis*, *Gyrosigma*, *Navicula* and *Pleurosigma* species. Stalked forms mainly consisted of *Licmophora debilis*, whereas *L. gracilis*, *L. communis*, *Achnanthes brevipes* and *A. minutissima* were of minor importance concerning epiphyte biovolume. The only tube-living diatom was *Berkeleya rutilans* (Fig. 3.5) and diatom chains were mainly represented by *Melosira nummuloides* (Fig. 3.4C).

Filamentous algae were mostly represented by the red alga *Acrochaetium secundatum* (Fig. 3.5) and the brown alga *Myrionema sp.*, other genera like *Ceramium*, *Pilayella* and *Polysiphonia* occurred only erratically.

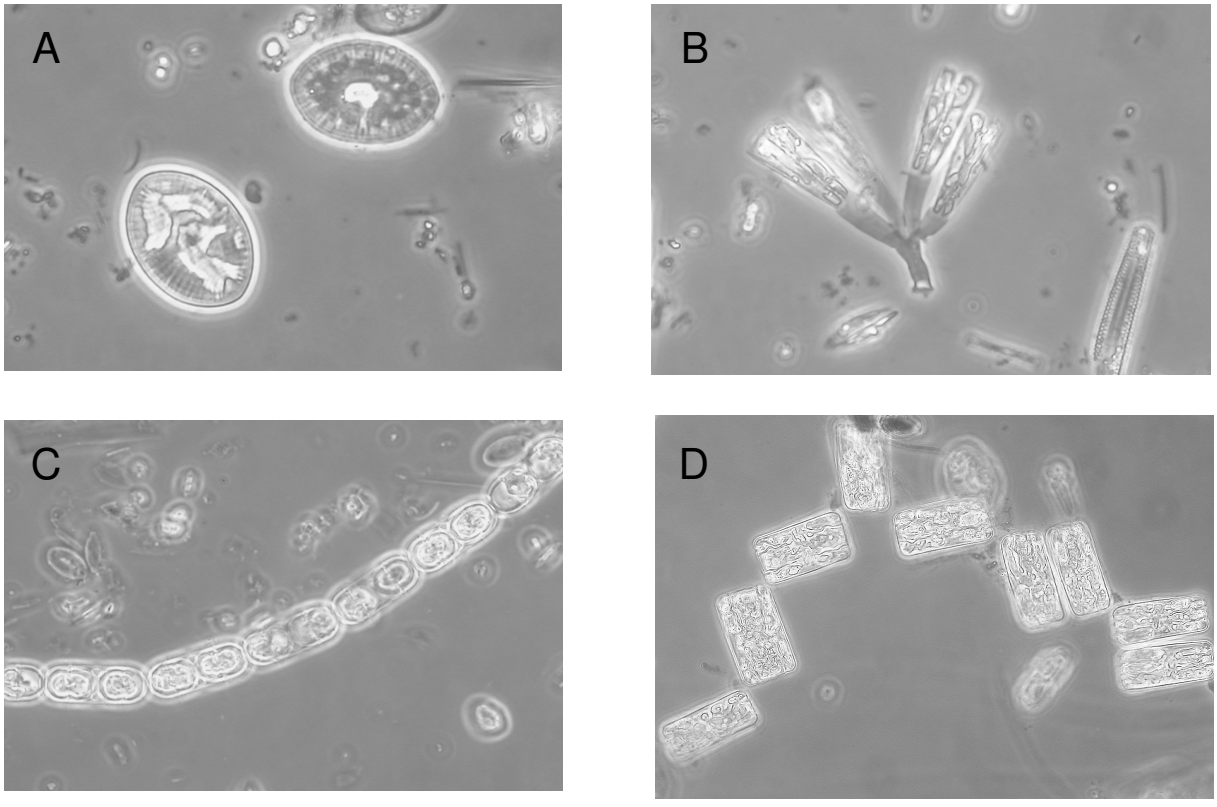

Figure 3.4. Common diatom species growing as epiphytes on eelgrass. (A) *Cocconeis scutellum,* (B) *Licmophora sp.*, (C) *Melosira nummuloides* and (D) *Grammatophora marina*.

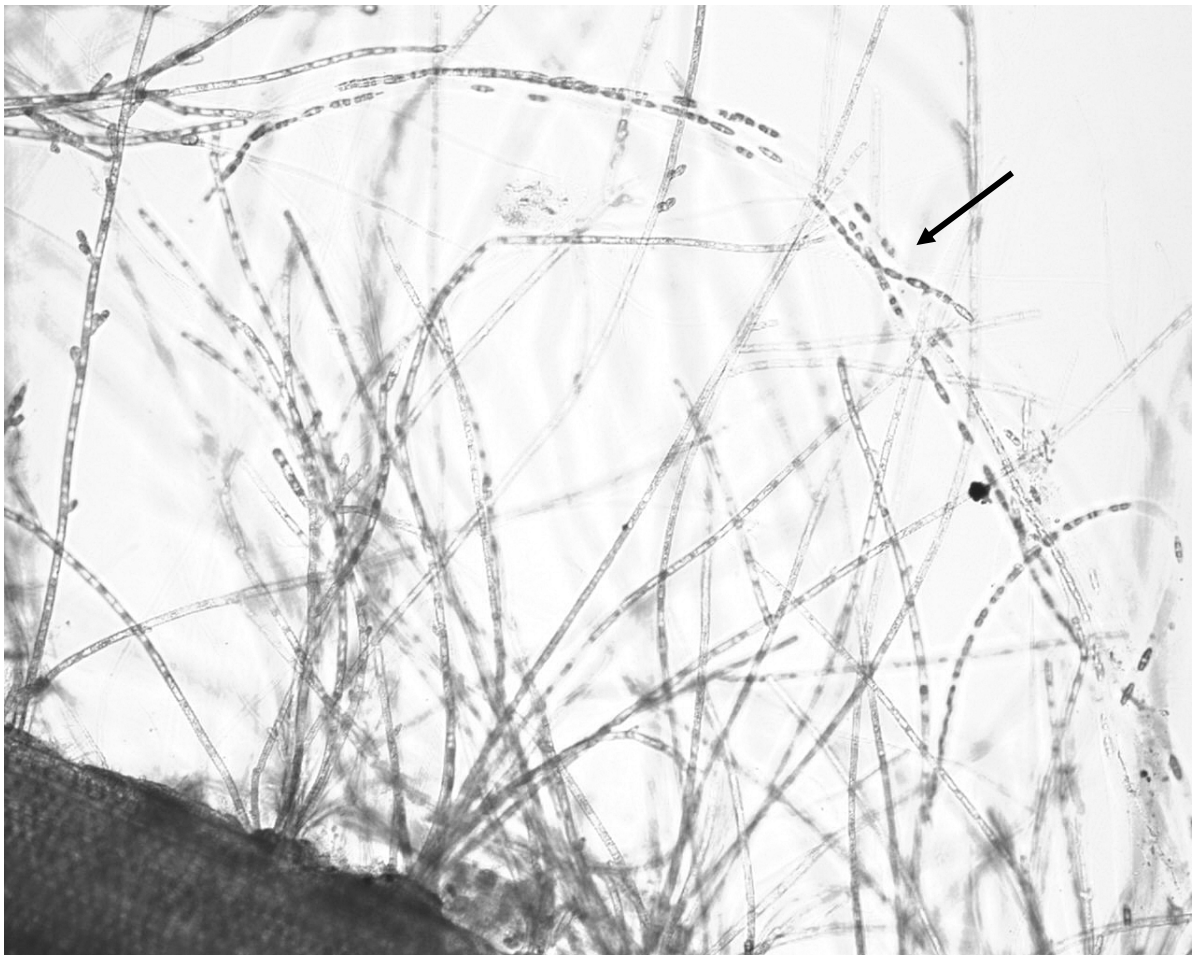

Figure 3.5. The red alga *Acrochaetium secundatum* and the tube-living diatom *Berkeleya rutilans* (arrow).

Table 3.1. Results of MANOVA of grazer impact on epiphyte composition.

species	Pillai's trace value	F	p
Idotea	2.34	13	<0.0001
Gammarus	1.63	4.3	<0.0001
Littorina	1.46	3.4	0.0004
Rissoa	2.32	12.4	<0.0001

All four studied mesograzers significantly influenced the composition of epiphytes (Table 3.1). The grazing of *Idotea* (Fig. 3.6A) had a strong negative effect on diatoms chains ($p = 0.003$) and filamentous algae ($p = 0.0002$). Tube-living ($p = 0.037$) and stalked diatoms ($p= 0.003$) were significantly reduced only in the treatment with the highest grazer abundance. Prostrate forms profited from the grazing impact on the other growth forms ($p = 0.018$).

Gammarus (Fig. 3.6C) had a likewise negative influence on diatom chains (p = 0.0003) and filamentous algae (p = 0.002), but stalked and prostrate forms increased their proportional contribution to total epiphyte biovolume in the presence of this mesograzer. This effect was only significant for prostrate forms (p = 0.0002). In contrast, *L. littorea* (Fig. 3.6B) grazed relative unselectively on all present algae, only under the highest grazing pressure, where the epiphyte biomass was reduced to very low values, prostrate forms (p = 0.003) increased their proportion and diatom chains were reduced (p = 0.0002). *Rissoa* (Fig. 3.6D) significantly effected all growth forms. Stalked (p = 0.0002), chain-forming (p = 0.0002), tube-living diatoms (p = 0.0002) and filamentous algae (p = 0.007) were reduced, whereas prostrate forms gained in importance (p = 0.0002).

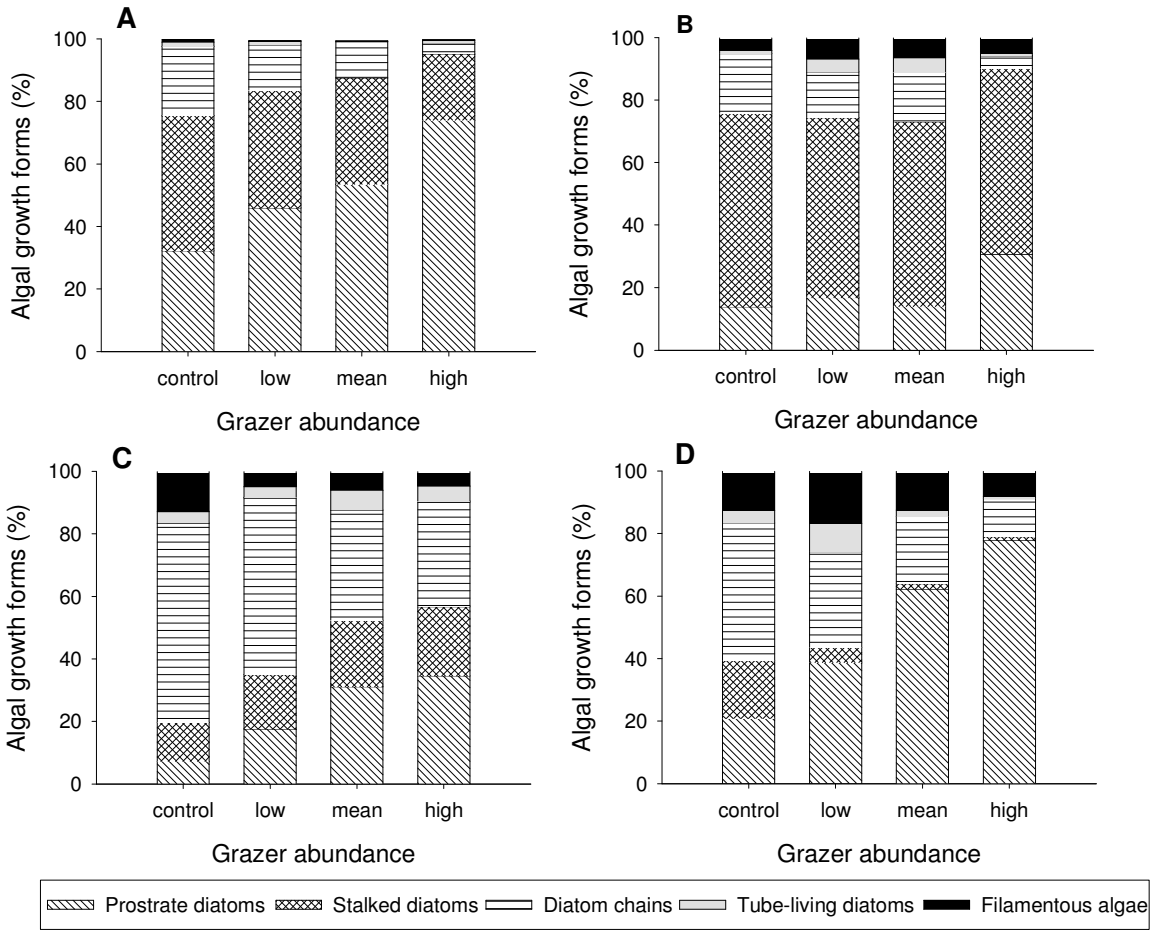

Figure 3.6. Algal growth forms of epiphytes presented as mean percent contribution to total epiphyte biovolume. (A) *Idotea baltica*, (B) *Littorina littorea*, (C) *Gammarus oceanicus* and (D) *Rissoa membranacea*.

Grazing impact

Linear regressions of epiphyte biomass (µg chl a cm^{-2} eelgrass, EB) on grazer numbers per mesocosm (N) and grazer biomass per mesocosm (AFDM, GB) showed the different quantitative impact of the four studies mesograzers.

Grazer number:

Idotea	EB = -0.0031 N + 0.18	R^2 = 0.67	n = 24
Gammarus	EB = -0.0031 N + 0.20	R^2 = 0.60	n = 24
Littorina	EB = -0.0128 N + 0.21	R^2 = 0.83	n = 24
Rissoa	EB = -0.0019 N + 0.25	R^2 = 0.85	n = 24

Grazer biomass:

Idotea	EB = -0.82 GB + 0.18	R^2 = 0.67	n = 24
Gammarus	EB = -1.03 GB + 0.20	R^2 = 0.60	n = 24
Littorina	EB = -0.85 GB + 0.21	R^2 = 0.83	n = 24
Rissoa	EB = -2.58 GB + 0.25	R^2 = 0.85	n = 24

The slope of regression was 4-fold steeper for individual *Littorina* than for both crustacean species and 7-fold steeper than for a single *Rissoa.* Considering grazer biomass, the regression for *Rissoa* showed the steepest slope, about 3-fold stronger than for the other species. Accordingly, the periwinkle had the strongest grazing impact per individual and *Rissoa* had the strongest grazing impact per biomass.

3.4. Discussion

Generally, grazing is supposed to reduce plant diversity, but plant diversity may increase compared to ungrazed controls, if the competitively dominant autotrophic species are consumed preferentially (Lubchenco 1978, Worm et al. 1999, Hillebrand et al. 2000). A meta-analysis on the effects of grazing in terrestrial and aquatic ecosystems proposes the hypothesis that the trend of the impact of grazers depends on the productivity of the studied system (Proulx & Mazumder 1998). Grazing decreased plant diversity in nutrient-poor habitats and this trend is reversed in nutrient-rich habitats ('grazer reversal hypotheses'). The intensity of the grazing pressure is yet another factor that may influence the direction of the effect of grazing on plant diversity. 'Intermediate' mortality of plant species is supposed to promote coexistence of competing species and thus, the highest plant diversity should occur under low to intermediate grazing pressure (Huston 1979, Sommer 1999a, Abrams 2001). Both hypotheses demand that grazers are able to select passively or actively for the dominant plant species.

The impact of natural densities of mesograzers varied considerably between the four species in our study. A clear positive effect of grazing was found for *Gammarus*, a species, which feeds preferentially on filamentous algae and diatom chains, the last being the dominant algae growth form in this experiment. Stalked and prostrate diatoms profited under these conditions and epiphyte diversity increased accordingly. This is well in line with the impact of

various grazers on plant diversity in terrestrial and aquatic ecosystems (Lubchenco 1978, Steinman 1996, Collins et al. 1998, Hillebrand et al. 2000). The effect on epiphyte biomass was not density-dependant for this species (Jaschinski & Sommer in prep.), but the taxonomic composition and thus, quality of the epiphyte assemblage was influenced by the strength of amphipod grazing pressure.

In contrast, *Idotea* had a negative effect on epiphyte diversity. This species also fed mainly on filamentous algae and diatom chains, but stalked forms were negatively affected, too, resulting in a growing dominance of the prostrate diatom *Cocconeis scutellum*. The difference in the trends of grazing impact is probably caused by the broader diet of *Idotea* compared to the amphipod and the varying epiphyte composition in both experiments. The chain-forming diatom *Melosira nummuloides* dominated the epiphyte assemblages in the amphipod experiment, whereas the stalked diatom *Licmophora debilis* showed the highest biovolume in the isopod experiment. The effect of *Idotea* on algal diversity in an epiphyte community under nutrient enrichment and dominated by diatom chains was equally positive as for *Gammarus* (see Chapter 5). Therefore, the degree of herbivore selectivity and algal composition can influence the impact of grazing on algal diversity.

The impact of both gastropod species on epiphyte diversity showed the expected unimodal pattern. The effect was weak for *Littorina* and more distinct for *Rissoa*.

The feeding mode of taenioglossan gastropods such as *Littorina* and *Rissoa* enables these species to ingest a wide variety of food types ranging from microalgae and small filamentous algae to tough, leathery macrophytes (Steneck & Watling 1982, Barker & Chapman 1990, Fredriksen et al. 2004). *Littorina* exerted an equally strong grazing pressure on all present algal growth forms even on the prostrate diatom *C. scutellum*, but rare species tended to be extinguished by this grazer at high abundance.

The selectivity of *Littorina* depends on the biomass level of the algal assemblage (Sommer 1999b). The biomass of epiphytes in my experiment was in a range (<1µg chl cm^{-2}), where the periwinkle is supposed to graze rather unselectively. This assumed low degree of selectivity is in good accordance with the effect of *Littorina* on epiphyte species composition. The low selectivity of the periwinkle may explain the weaker positive effect of intermediate grazing pressure in this study compared to earlier results (Sommer 1999a).

Unlike the periwinkle, the small gastropod *Rissoa* did not eliminate *C. scutellum*, which generated a mono-layer crust on the eelgrass leaves. This species exerted the strongest grazing pressure on stalked diatoms, but all other growth forms were also negatively affected, except the dominant prostrate species *C. scutellum*, which was more abundant in the grazed treatments. This feature may be the result of a passive selectivity caused by a smaller radula and less muscular force as *Littorina*. Then again, *Rissoa* is capable of feeding directly on eelgrass leaves, if other food sources are scarce. Maybe this gastropod selects

actively for the easily removable overstory of erect algal species as long as the food quantity is sufficient.

In this study, only the grazing impact of gastropods fit the hypotheses that moderate grazing pressure and thus, moderate plant mortality generate the highest diversity in plant communities via prevention of competitive exclusion (Huston 1979). The results confirm the importance of the selectivity of grazers and the composition of the algal assemblage in influencing the strength and direction of the impact of grazers on plant diversity.

4. Top-down and bottom-up control in an eelgrass-epiphyte system

4.1. Introduction

The relative importance of resource supply and higher order interactions in structuring ecosystems has been the sustained interest of ecologists in aquatic systems. The availability of nutrients and predation are assumed to play a fundamental role in regulating natural populations and communities. Bottom-up and top-down forces can simultaneously influence aquatic communities (Worm et al. 2000, Hillebrand 2002, Brett & Goldman 1997), but their relative fortitude varies between sites, season and life stages (Lotze et al. 2001, Hillebrand & Kahlert 2002). Environmental conditions, food-web architecture and composition furthermore influence the interactions of bottom-up and top-down control (Leibold et al. 1997). High diversity on lower trophic levels and the presence of omnivory may limit top-down effects.

Seagrass meadows, which experienced a dramatic and widespread decline in recent years (Hauxwell 2003), rank among the most productive and diverse coastal benthic ecosystems. In these systems the interactions between macrophytes, epiphytes and mesograzers complicate accurate predictions regarding consequences of environmental changes like eutrophication or diminished predation pressure from large fish (Jackson et al. 2001). Epiphytes (mostly diatoms and filamentous algae) colonize seagrass leaves and thereby attenuate light (Brush & Nixon 2002) and reduce the exchange of nutrients and gases at the leave surface (Sand-Jensen 1985). However, in some cases seagrass may profit from epiphytes: nitrogen supply through fixation by blue-greens and protection from desiccation and ultraviolet radiation in intertidal habitats can counteract the deleterious effects of epiphytes (McRoy et al. 1973, Penhale & Smith 1977). Nutrient enrichment is generally linked to enhanced epiphyte production and negative effects on seagrass growth, but positive reactions of seagrasses to higher nutrient supply were also found indicating a nutrient limitation (see Hughes et al. 2004 and references therein).

The direct and indirect impact of nutrients in seagrass systems is influenced by grazing of invertebrates, which play a key role in structuring marine benthic communities (Jernakoff & Nielsen 1997, Duffy & Hay 2000, Duffy et al. 2001). Small mobile herbivores (mesograzers, mainly crustacean and gastropod species) are nearly ubiquitous in macrophyte systems worldwide and can be present at very high densities. Mesograzers can mediate the potential detrimental effects of eutrophication by feeding preferentially on epiphytes (Neckles et al. 1993, Hauxwell et al. 1998, Hillebrand et al. 2000) and are a crucial link between primary producers and higher trophic levels (Edgar & Shaw 1995). Nevertheless, the effect of grazing on epiphytes is not altogether negative. Mesograzers are supposed to enhance the photosynthetic capacity of periphyton by removing the overstory of cells and by destroying the boundary layer, which impedes nutrient diffusion (McCormick & Stevenson 1991).

Furthermore, sloppy feeding and excretory products can promote nutrient availability (Hillebrand et al. 2000).

Several studies have individually manipulated nutrients or grazing in seagrass systems, but only one study has simultaneously dealt with bottom-up and top-down influences in controlling epiphyte and macrophyte dynamics (Neckles et al. 1993). Investigations in coastal and freshwater communities showed a high degree of interaction in these two factors. Nutrients effects were weaker in the presence of grazing and the effect of grazers was enhanced by nutrient enrichment (Hillebrand et al. 2000, see Hillebrand 2002 and references therein).

In this study, I experimentally tested the independent and interactive effects of nutrient enrichment and epiphyte grazing on the dynamics of an experimental eelgrass-epiphyte system. I analysed the direct and indirect consequences on epiphyte composition, epiphyte and eelgrass productivity and nitrogen limitation to test the following hypothesis:

(1) Epiphyte biomass and areal productivity are enhanced by nutrient enrichment and decreased by grazing.

(2) The consequences of grazing and nutrient supply are interactive for epiphytes. Grazing pressure will be enhanced by nutrient enrichment and nutrient effects will be dampened in the presence of grazers.

(3) Eelgrass growth is reduced by nutrient enrichment and increased by grazing.

(4) The nitrogen content of primary producers is enhanced by nutrient enrichment.

Additionally, a field study was conducted to investigate the seasonally varying impact of nutrient supply and grazing pressure on primary producer production.

4.2. Methods

Experimental design

I conducted mesocosm experiments to test the impact of nutrient enrichment and grazing on primary producers in an eelgrass-epiphyte system. The experiment took place in a constant temperature chamber in 125 l aquaria. Summer conditions were established concerning light and temperature. The aquaria were illuminated by HQI-lamps with a 16 h day and 8 h night cycle. The light intensity was 100 µmol m^{-2} s^{-1} at the water surface. The temperature in the constant temperature chamber was set to 17°C. However, due to a warming-effect of the lamps the water temperature in the aquaria was slightly higher (~18.5°C). Sand-filtered brackish deep water from the Kiel Fjord (salinity: 13.8 PSU ± 0.3) was used and additionally filtered with a 0.8 µm membrane filter to avoid contamination with plankton species. Continuous water circulation was created using pumps and the water was exchanged (up to 90% of the total volume) every day. Periphyton growing on the walls was removed every day before the water exchange.

The mesocosms were filled (5 cm) with 1 mm-sieved homogenized sediment which consisted mainly of fine sand with a low organic content. After 24 h 80 freshly harvested eelgrass shoots were planted in each mesocosm (320 shoots m^{-2}, average abundance in the Kiel Fjord in summer). Only shoots with at least four leaves were selected and the average length of shoots was 40 cm. On the following day the mesocosms were stocked with 64 *Idotea baltica* each (256 m^{-2}, average abundance in the Kiel Fjord in early summer). The isopod *Idotea baltica* is the most important mesograzer in vegetated areas in the Baltic Sea. All experimental material was collected at the Falkenstein Beach in the inner Kiel Fjord, Western Baltic Sea, Germany (54°21'/10°9'). The experiment was terminated after ten days. At this time, the eelgrass was harvested, placed in plastic bags and stored frozen until further processing.

The experiment was conducted with a factorial combination of three nutrient levels and grazing/no grazing activity. Nutrients (N + P) were applied at ambient, moderate and high concentrations. Ambient concentrations were characteristic of the Kiel Fjord in summer (4 µmol l^{-1} N and 0.25 µmol l^{-1} P), moderate concentrations were two-fold enriched and high concentrations were four-fold enriched. The highest nutrient level is characteristic for regions with a strong decline of eelgrass (Neckles et al. 1993). Silicate levels were high (16 µmol l^{-1}). Each of the six treatment combinations were replicated three times.

Nutrient concentrations (nitrate and nitrite, ammonium, phosphate and silicate) were measured in an autoanalyser (Skalar SANplus) on a daily basis.

Epiphyte biomass

Epiphyte biomass was measured using chlorophyll *a* as proxy. Eight eelgrass shoots were randomly selected from each mesocosm. Sample processing: see chapter 2.

Epiphyte composition

Two eelgrass shoots from each mesocosm were carefully scraped and attached epiphytes were transferred into a defined volume of filtered seawater. The samples were fixed with 1% Lugol's iodine and counted under an inverted microscope using 3 ml Utermöhl-chambers. A minimum of 400 cells was counted for dominant species and the whole chamber was counted to account for rare species. Biovolume was used as proxy for biomass, following the methods of Hillebrand et al. (1999), and the data were normalized to unit eelgrass surface area.

Eelgrass growth

At the end of the experiment eight shoots from each mesocosm were used to measure eelgrass growth. Sample processing: see chapter 2.

Eelgrass and epiphyte productivity

Procedure: see chapter 2

Elemental composition

Procedure: see chapter 2

Seasonal variations in epiphyte accumulation, C:N ratio and DIN

I collected eelgrass from our sampling site monthly from April 2001 to December 2002 and 40 eelgrass shoots were randomly selected. Thirty eelgrass shoots were processed for epiphyte and eelgrass biomass and ten eelgrass shoots were processed for nutrient content of epiphytes and macrophytes. Water nutrient concentrations were measured on a monthly basis.

Statistics

A two-factorial ANOVA was used to test for significant effects of the independent factors nutrient enrichment and grazing on epiphyte biomass, epiphyte and eelgrass productivity and C:N ratios. Tukey`s test was applied to distinguish significantly different treatments. A two-factorial MANOVA was used to test the significant impact of nutrients and grazing on the proportional contribution of algal growth forms to epiphyte composition. Data were arcsine square root transformed. The analysis was performed with the Pillai`s trace statistic, recommended for interdependent response variables (Scheiner 1993).

I calculated standardized mean difference (D) to compare the size of effects between the factors nutrients and grazing according to Hillebrand and Kahlert (2001). The response of epiphyte biomass and areal productivity as well as eelgrass growth to nutrient enrichment was computed between the treatments no grazer-enriched and no grazer-ambient (D_n). The response to grazing was calculated between the treatments no grazer-ambient and grazer-ambient (D_g).

4.3. Results

Epiphyte responses

Epiphyte biomass and areal productivity increased significantly with nutrient enrichment and decreased in the presence of grazers (Fig. 4.1A+B). Significant interactions were found between grazer and nutrient effects (Tab. 4.1). Nutrients effected epiphytes stronger than grazing. The highest supply of nutrients caused a 5-fold enhancement of epiphyte biomass and productivity, whereas grazers reduced epiphyte biomass and areal productivity to half of the ungrazed treatments at most. The absolute effect of grazers was positively influenced by nutrient enrichment. Epiphyte biomass and areal productivity were most strongly reduced

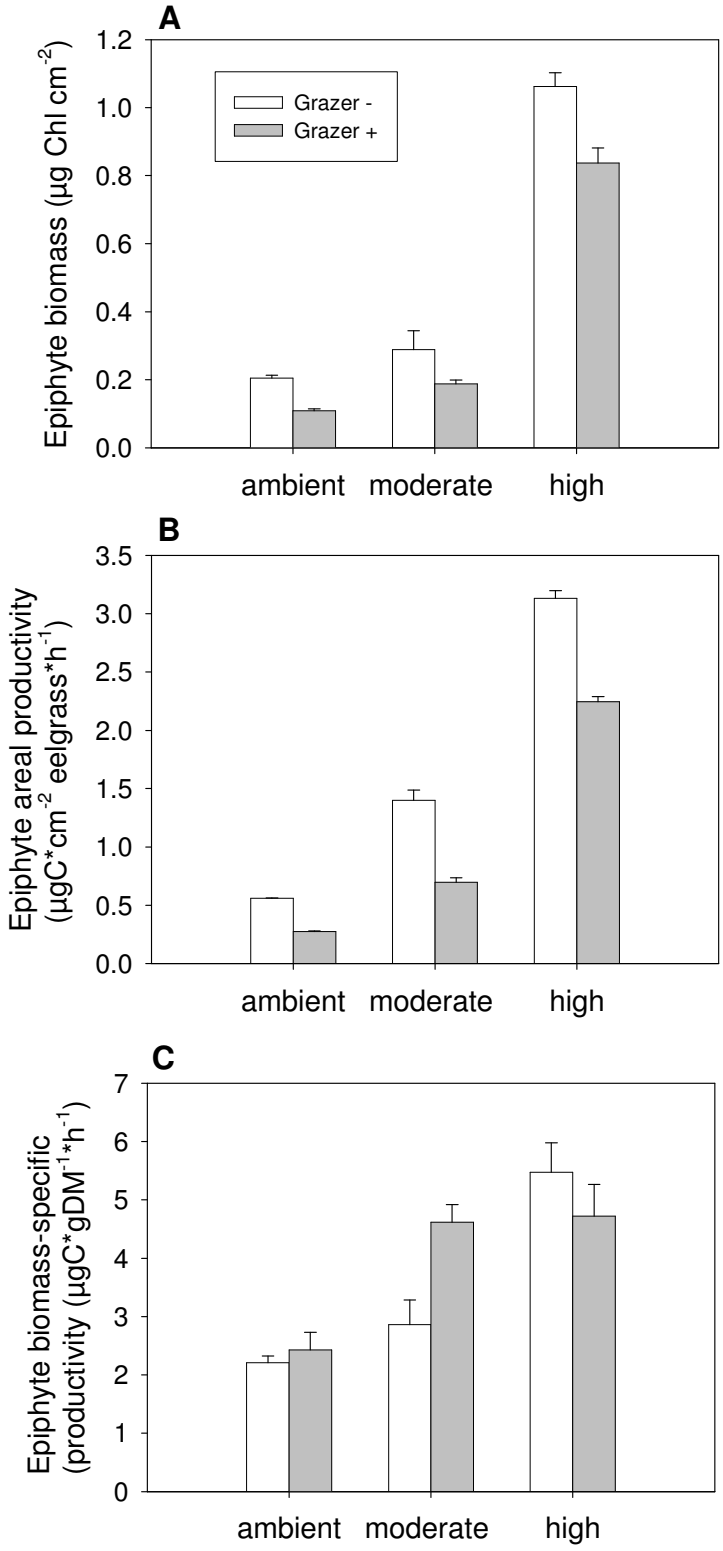

Figure 4.1. Epiphyte response (means ± SD) to nutrient enrichment and grazing. (A) Epiphyte biomass, (B) epiphyte areal productivity and (C) epiphyte biomass-specific productivity

Table 4.1. Results of univariate two-factorial ANOVAs on epiphyte and eelgrass responses to nutrient enrichment and grazing

Source of Variation	DF	MS	*F*-ratio	p-level
Epiphyte biomass				
Grazer	1	0.09	76.6	<0.001
Nutrient enrichment	2	1.14	986.9	<0.001
Grazer x nutrients	2	0.01	6.9	0.01
Epiphyte areal productivity				
Grazer	1	1.76	681.7	<0.001
Nutrient enrichment	2	8.25	3190.6	<0.001
Grazer x nutrients	2	0.14	54.9	<0.001
Epiphyte biomass-specific productivity				
Grazer	1	0.75	4.9	0.047
Nutrient enrichment	2	11.58	74.9	<0.001
Grazer x nutrients	2	2.39	15.5	<0.001
Eelgrass growth				
Grazer	1	0.80	165.9	<0.001
Nutrient enrichment	2	1.77	366.2	<0.001
Grazer x nutrients	2	0.01	2.2	0.157
Eelgrass biomass-specific productivity				
Grazer	1	0.05	5.0	0.045
Nutrient enrichment	2	0.12	12.5	0.001
Grazer x nutrients	2	0.00	0.0	0.979
Epiphyte C:N				
Grazer	1	0.00	0.0	0.959
Nutrient enrichment	2	13.34	117.8	<0.001
Grazer x nutrients	2	0.05	0.5	0.647
Eelgrass C:N				
Grazer	1	0.00	0.0	0.959
Nutrient enrichment	2	13.34	117.8	<0.001
Grazer x nutrients	2	0.05	0.5	0.647

under high nutrient concentrations. Vice versa, grazing reduced the positive effect of fertilisation on epiphyte biomass and areal productivity. Epiphyte biomass-specific productivity increased significantly with nutrient enrichment (Fig. 4.1C). The positive grazer impact was only significant at moderate nutrient concentrations ($p = 0.002$, Tukey's test) and at the highest nutrient supply the effect of gazers on epiphyte productivity was negative although not significantly.

The epiphytes found on eelgrass consisted mostly of diatoms and a small portion of red and brown algae. The diatoms showed a high variety of growth forms and cell sizes. Prostrate forms were dominated by *Cocconeis scutellum*, which formed a strongly adhering monolayer on the eelgrass leaves. Diatoms on gelatinous stalks consisted mostly of *Licmorphora* species and diatom chains were mostly represented by *Melosira nummuloides*. Tube-living

diatoms like *Berkeleya rutilans* were of minor importance. The main filamentous algae were the red alga *Acrochaetium secundatum* and the brown alga *Myrionema sp.*, both very small species (<10mm). Cyanobacteria were rare in occurrence and omitted from the analyses.

There were highly significant effects of nutrients (Pillai's trace value = 1.86, F = 23.3, p<0.0001) and grazing (PT = 0.97, F = 59.0, p<0.0001) on the proportional contribution of the different algal growth forms (Fig. 4.2) to epiphyte composition. Significant interactions between these two factors were also found (PT = 1.61, F = 7.4, p = 0.0001).

Nutrient enrichment had negative effects on the proportions of prostrate (p = 0.0002, Tukey's test) and stalked forms (p = 0.008). Chain-forming diatoms (p = 0.0002) and filamentous algae profited from nutrient enrichment (p = 0.0169). The proportions of filamentous algae increased especially in the moderate enrichment treatment. The presence of *Idotea baltica* significantly increased the proportional contribution of prostrate diatoms (p = 0.0004). Tube-living diatoms were significantly enhanced by grazing only in the moderate enrichment treatment (p = 0.049). Grazing had a strong negative effect on chain-forming diatoms (p = 0.0004) and filamentous algae (p = 0.0015). The importance of grazers was diminished in the presence of high nutrient concentration.

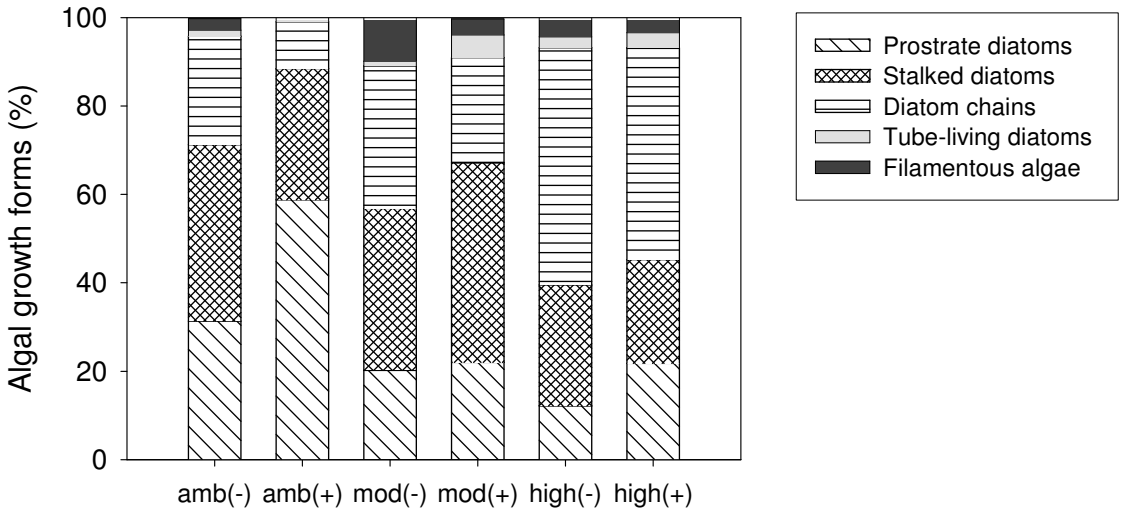

Figure 4.2. Proportional contribution of algal growth form to total epiphyte biovolume in response to nutrient enrichment and grazing.

Eelgrass responses

Eelgrass growth and biomass-specific productivity (Fig. 4.3) were significantly affected by the presence of grazers and nutrient enrichment (Tab. 4.1), but no interactions of these two factors concerning the measured parameters were found. Grazing had always a positive

effect on eelgrass productivity, which increased about 40%. Nutrient enrichment at moderate concentrations enhanced eelgrass growth, whereas high nutrient concentrations considerably reduced eelgrass growth.

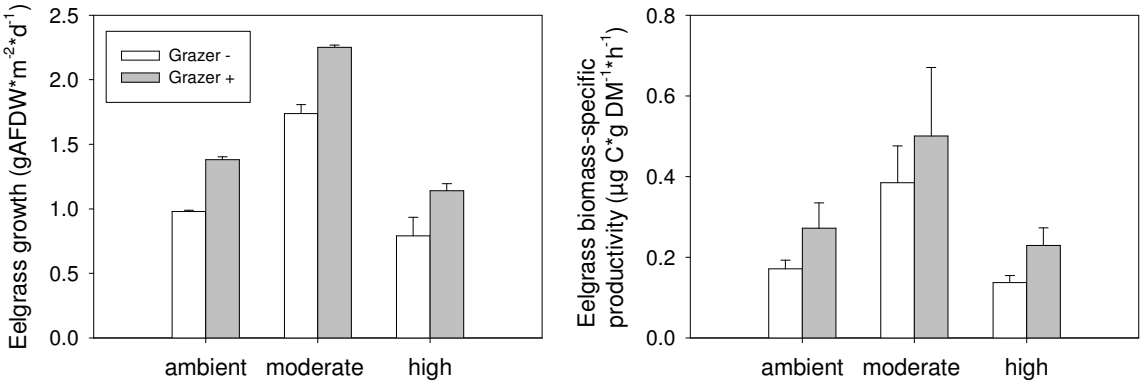

Figure 4.3. Eelgrass response (means ± SD) to nutrient enrichment and grazing. (A) Eelgrass areal productivity and (B) eelgrass biomass-specific productivity.

Comparison of effect sizes

Both nutrients and grazing had important effects on epiphyte biomass and production. The magnitude of effect sizes on epiphytes was higher for nutrient enrichment than for grazing (epiphyte biomass: D_n = 23.6, D_g = -10.7; epiphyte production: D_n = 45.3, D_g = -6.7). The impact of nutrients and grazing on eelgrass growth was smaller and the effect of grazing was more pronounced than the effect of nutrient enrichment (eelgrass growth: D_n = -1.5, D_g = 4.5).

Nitrogen content of epiphytes and eelgrass

The comparison of epiphyte and eelgrass C:N ratios with naturally occurring values during the course of the year (Fig. 4.6) indicated a nitrogen limitation (Fig. 4.4). Nutrient enrichment had significant positive affects on the nitrogen deficiency in both primary producer groups. The presence of grazers had no effects on the nitrogen content.

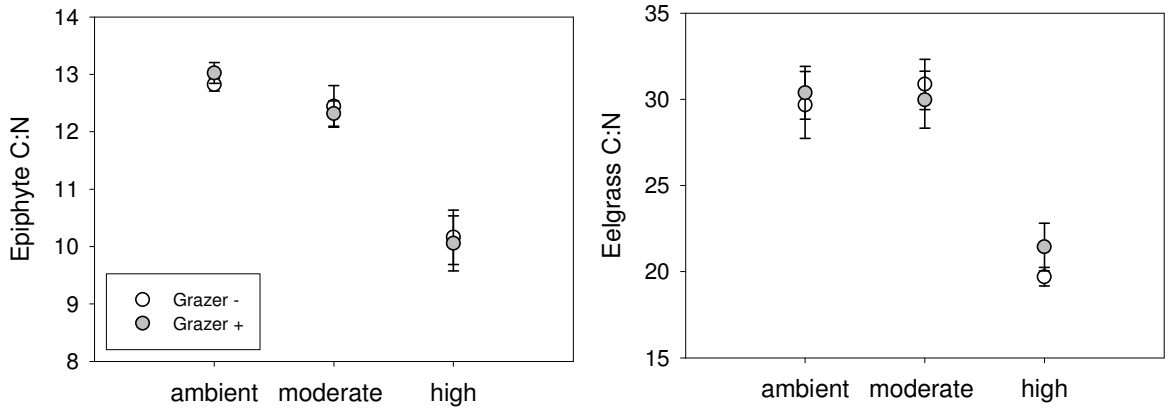

Figure 4.4. Effect of nutrient enrichment and grazing on molar C:N ratios in epiphytes and eelgrass (means ± SD).

Seasonal variations in epiphyte biomass, epiphyte and eelgrass nitrogen content and dissolved inorganic nitrogen (DIN)

From April 2001 to December 2002 epiphyte accumulation on eelgrass leaves varied by an order of magnitude (Fig. 4.5) with maxima occurring in June and October. A depression of epiphyte biomass was observed in late summer in both years.

The C:N ratio of epiphytes and eelgrass was directly opposed to dissolved nitrogen concentrations (Fig. 4.6). DIN concentrations were high in winter and low in summer, whereas the nitrogen content of the studied primary producers is high in winter and low in summer. This supports the assumption, that epiphytes and eelgrass are nitrogen limitated in summer. No correlation between epiphyte biomass and DIN concentrations or mesograzer abundance (Gohse-Reimann, unpublished data) could be found.

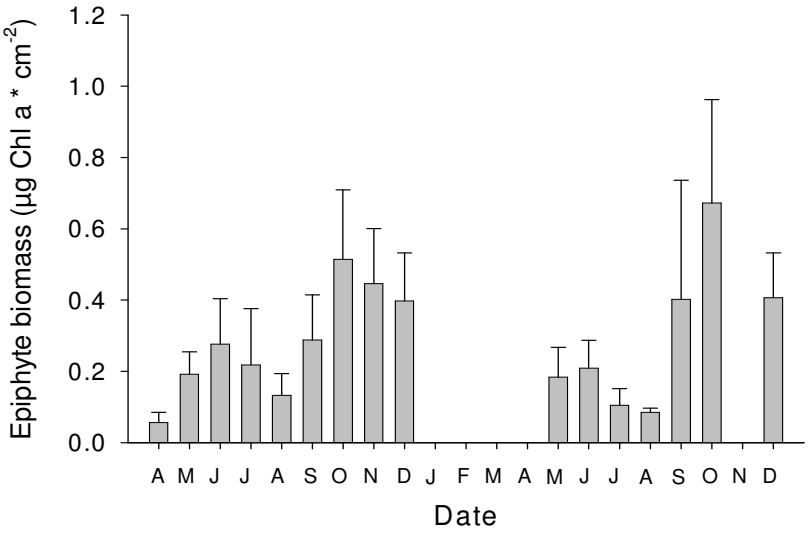

Figure 4.5. Seasonal epiphyte biomass (means ± SD) on eelgrass in the Kiel Fjord, Falkenstein Beach.

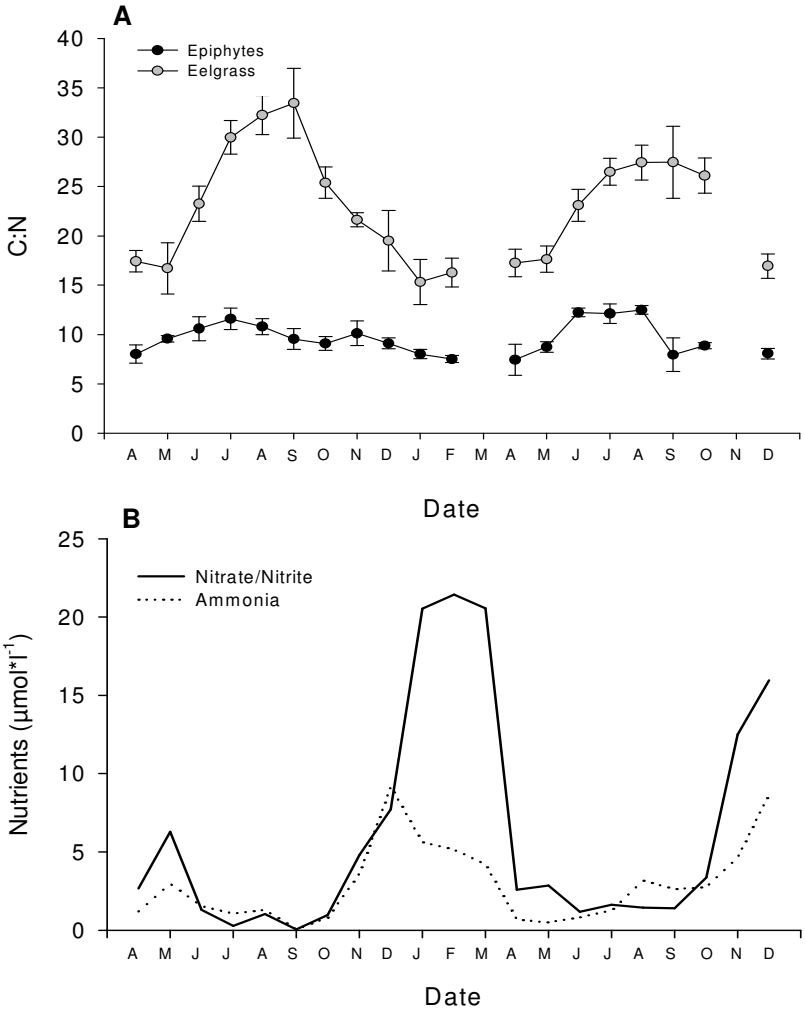

Figure 4.6. (A) Seasonal molar C:N ratio of epiphytes and eelgrass in the Kiel Fjord, Falkenstein Beach. (B) Inorganic nitrogen concentrations in the water column in the Kiel Fjord, Falkenstein Beach.

4.4. Discussion

I found strong impacts of nutrient enrichment and mesograzers on epiphyte and eelgrass dynamics in my experiment. Higher nutrient supply enhanced epiphyte biomass and areal productivity, whereas grazing reduced both parameters (supporting hypothesis 1). Within the range of experimental manipulations, the effect of nutrients was stronger than the effect of grazing and both factors were interactive (supporting hypothesis 2). The composition of epiphyte growth forms was antagonistically affected by the isopod *Idotea baltica* and by fertilisation. Eelgrass growth was enhanced at intermediate nutrient concentrations and suppressed under high nutrient supply (partially supporting hypothesis 3). Furthermore, the C:N ratio increased significantly in macrophytes and epiphytes with growing nutrient supply, indicating a nitrogen limitation of both primary producer groups (supporting hypothesis 4).

The accumulation of epiphytes and the growth of their macrophyte host are controlled by multiple factors including light, temperature, nutrient availability and the abundance of mesograzers. The individual effects of nutrients and grazing in marine benthic macrophyte systems have been shown in numerous studies (see Hughes et al. 2004, Bokn et al. 2003), whereas relatively few experimental studies have tackled both top-down and bottom-up control simultaneously (Neckles et al. 1993, Worm et al. 2000).

In this experiment - conducted under early summer conditions - I found antagonistic effects of nutrient enrichment and grazing. Higher nutrient supply increased epiphyte biomass and productivity, whereas the presence of grazers reduced epiphyte accumulation. The results are consistent with previous experimental studies in coastal systems (Hootsman & Vermaat 1983, Wear et al. 1999, Hillebrand et al. 2000). The effect of fertilisation on epiphytes was stronger than the effect of grazing and top-down and bottom-up forces were highly interactive. The impact of grazers on epiphyte biomass and areal productivity was stronger under enhanced nutrient supply and nutrient enrichment was more efficient in the absence of grazers. These results corroborated previous findings from coastal and freshwater systems (Hill et al. 1992, Neckles et al. 1993, Rosemond et al. 1993, Hillebrand et al. 2000). The stimulation of consumption under high nutrient supply is supposed to be a functional response in a food-limited situation and benthic herbivores are proposed to be highly prone to qualitative or quantitative food limitation (Lamberti 1996). Furthermore, nutrient enrichment favoured the growth of chain-forming diatoms and filamentous algae in our experiment. These algae were preferentially eaten by *I. baltica* (see Chapter 3).

Thus, a trade-off between grazing susceptibility and nutrient up-take facilitated the enhanced consumption under enriched conditions. Accordingly, epiphytes and grazers profited concurrently from bottom-up effects. A positive response concerning growth and reproduction of grazers could not be tested because of the short duration of our experiment.

However, extremely high growth rates and reproductive success was found for *I. baltica* in another longer study under nutrient conditions similar to our high nutrient treatment (Gohse-Reimann, unpublished data).

The presence of grazers may further interfere with nutrient effects via indirect mechanisms. Many grazer species not only diminish epiphyte biomass, but also enhance nutrient content and the biomass-specific productivity of epiphytes. A better supply of nutrients via excretion or sloppy feeding and the reduced competition for nutrients, space and light via destruction of the three-dimensional epiphytic layer by grazing may be considered as possible mechanisms (Hillebrand 2002). The importance of such mechanisms was corroborated by the significant higher biomass-specific productivity of epiphytes in the presences of *I. baltica* under moderate enrichment. However, this effect was reversed in the high nutrient treatments, where the selectivity of the isopod for the highly productive diatom chains reduced the epiphyte biomass-specific productivity.

The composition of epiphytes was stronger affected by grazing than by higher nutrient supply. Chain-forming diatoms (mostly *Melosira nummuloides*) and filamentous algae benefited from nutrient enrichment, whereas prostrate (mostly *Cocconeis scutellum*) and stalked forms (mostly *Licmophora* species) occurred in diminished proportions. *M. nummuloides* is a species with a strong response to nutrients, especially to nitrogen enrichment (Hillebrand & Sommer 1997, Hillebrand et al. 2000). This is supported by my experiment and the observed growing contribution of this species to epiphyte biomass on eelgrass in the Kiel Fjord in autumn, when nutrient concentrations increase after their summer depression.

Additionally, a functional change took place. The stalked diatoms were dominated by the small species *Licmophora debilis* (314 μm^3) under ambient enrichment. With increasing nutrient supply the proportions of larger species like *L. gracilis* (942 μm^3) and *Achnanthes brevipes* (3140μm^3) increased. Due to the higher surface to volume ratio small algae have a higher capability to compete for nutrients than large algae and should be competitively dominant under the low ambient nutrient regime. The same pattern was found for sediment microalgae under enrichment (Sundbäck & Snoeijs 1991). The group of prostrate diatoms underwent a change from the tightly adhering *C. scutellum* to mobile pennate forms like *Nitzschia sp*. and *Amphora sp*.. *C. scutellum* forms a mono-layer crust on the eelgrass leaves and thus, should be discriminated most by a growing three-dimensional overstory of cells promoted by nutrient enrichment. Mobile diatoms may even profit from these structures, because they can grow as secondary epiphytes on filamentous forms.

The species most favoured by nutrient enrichment, *M. nummuloides*, was also the species, which experienced the highest reduction in the presence of grazers. This pattern suggests a trade-off between grazing resistance and nutrient uptake efficiency as mentioned above.

Generally, chain-forming diatoms and filamentous algae were preferentially consumed by *I. baltica* and prostrate forms profited from this behaviour. The removal of upright, large growth forms is a consistent pattern in benthic microalgae communities under grazing pressure (Nicotri 1977, Hillebrand et al. 2000, Hillebrand & Kahlert 2001).

Generally, this experiment indicated a simultaneous top-down and bottom-up control of epiphyte composition and biomass. This assumption is furthermore corroborated by our field study. The seasonal correlation between epiphyte biomass and water nitrogen concentrations was very poor, as was the correlation between epiphyte biomass and mesograzer abundance. Thus, no general dominance of bottom-up or top-down control could be found at the study site. The strong decrease of epiphyte accumulation and the simultaneous increase in mesograzer biomass in late summer (Gohse-Reimann unpublished data) suggests a stronger effect of grazing pressure than of nutrients at this time of the year. This is corroborated by stable carbon isotope data implying a high proportion of epiphytes in the diet of mesograzers in early summer (see Chapter 6). Despite the constantly high grazing pressure, epiphyte biomass increased once again in autumn. Decreasing C:N ratios indicated a simultaneous increase in nitrogen supply, probably caused by the mixing of the water column in the first storm events in September. Thus, bottom-up effects may be stronger than grazing in controlling epiphyte biomass in autumn.

Seasonal variations in top-down and bottom-up control of primary producers were found in experimental studies for periphyton in different habitats, but there is no clear general trend presumably because of local differences in abiotic and biotic conditions (Neckles et al. 1993, Hillebrand 2002). Experimentally, I simulated early summer conditions, where epiphyte biomass seems to be regulated concurrently by low nutrient supply and moderate grazing pressure. The pronounced nutrient limitation that primary producers experienced in the field at this time of the year may explain the strong effects of nutrient enrichment in my experiment.

Eelgrass productivity increased under moderate nutrient enrichment and decreased under high nutrient supply. Thus, the results supported the assumption that seagrasses are more often controlled by nutrient limitation than by light as earlier studies suggested (see Hughes et al. 2004 and references therein). Seasonal C:N values corroborated a strong nitrogen limitation of eelgrass in summer. The negative impact of nutrient enrichment via a higher epiphyte load became apparent at a nutrient level, which is related to eelgrass decline in North American estuaries (Moore & Wetzel 2000) and occurred in the Kiel Fjord only in winter.

In addition to the strong but contrary bottom-up effects of nutrient enrichment, I found a clear positive effect of epiphyte grazers on eelgrass growth. This is consistent with previous studies, although the effect may be species-specific and density-dependent (William &

Ruckelshaus 1993, Philippart 1995, Duffy et al. 2001). Grazing may even mitigate the effects of moderate eutrophication in coastal systems.

The well-documented decrease in large predatory fish in coastal regions has brought forth the hypothesis that over-exploitation of top predators may diminish the predation pressure on smaller fish. Consequently, their prey, the mesograzers, should decline in numbers resulting in the same negative consequences for macrophytes as eutrophication (Williams & Heck 2001).

Despite the strong reduction of the top-predator cod (*Gadus morhua*) in the Kiel Bight by commercial fisheries (Bobsien 2006), the abundances of *I. baltica* in eelgrass beds seems to be relative constant compared with older data (Worthmann 1975). The juvenile cods found previously in eelgrass beds feed preferentially on crustacean mesograzers and fish eggs (Worthmann 1975). Thus, the release of grazing pressure on mesograzers could have been compensated by the simultaneous release of grazing pressure on other fish predators, which also favour amphipods and isopods as prey (Bobsien 2006). This hypothesis is corroborated by an increase in abundances of small-sized fishes, especially the sea stickleback *Spinachia spinachia* and the viviparous blenny *Zoarces viviparous*, which consumed about 40 % of the annual amphipod and isopod production at our study site (Bobsien 2006).

Thus, negative consequences of eutrophication and over-exploitation of top predators may have been mitigated by the highly structured and diverse eelgrass community in the study area so far.

In conclusion, both nutrient enrichment and grazing pressure had strong and antagonistic effects on epiphyte biomass, productivity and composition. Top-down and bottom-up control acted simultaneously and were highly interactive in the studied early summer situation. Seasonal variations in epiphytes, nutrients and mesograzers indicated varying strength of both effects in the course of the year. The effect of nutrients on eelgrass was ambiguous because of nutrient limitation and the competition between eelgrass and epiphytes. The effect of mesograzers on eelgrass growth was always strongly positive, which supported the relevance of higher order interactions in maintaining the health of the economically and ecologically important coastal macrophyte systems.

5. Grazer diversity effects in an eelgrass-epiphyte-microphytobenthos-system

5.1. Introduction

Intensive studies in terrestrial food webs have shown that the diversity of primary producers can strongly influence ecosystem functioning (see Hooper et al. 2005 for overview). However, the consequences of the loss in consumer diversity have been studied only recently (Emmerson et al. 2001, O'Connor & Crowe 2005, Duffy et al. 2005, Gamfeld et al. 2005). Since all natural ecosystems include more than one trophic level, and consumer species can exert strong impacts on ecosystem processes and community structure (Jackson et al. 2001, Duffy 2002), it is important to consider the effects of diversity in multitrophic systems. Furthermore, the fact that species at higher trophic levels seem to be more often subject to extinction than primary producers (Jackson et al. 2001, Petchey et al. 2004) underpins the necessity of exploring the consequences of losses in consumer diversity.

Conceptual models predict that varying consumer diversity and composition can generate a wider range of effects on ecosystem processes than changes in primary producer diversity alone (Thébault & Loreau 2003, Petchey et al. 2004, Fox 2004). Resource availability, food web structure, functional traits of lost species and bidirectional effects can create complex responses of ecosystem processes to varying diversity in a multitrophic system (Duffy 2002, Worm & Duffy 2003, Hillebrand & Cardinale 2004).

In this study I focus on three questions:

First, how does consumer diversity affect total prey biomass? Consumer grazing impact can increase with higher consumer diversity via two mechanisms: the selection and the complimentarity effect (Loreau & Hector 2001, Hooper et al. 2005). The selection effect hypothesis postulates that species with a large impact on prey biomass are more likely to be present with increasing diversity and thus, dominate the mixtures. The complementarity effect enhances resource use via niche partitioning and facilitation. Experimental studies addressing the impact of consumer diversity on primary producer biomass are rare in marine systems and the results are ambiguous. Gamfeld et al. (2005) reported a reduction of microalgae biomass with growing ciliate diversity. No evidence for mesograzer diversity effects were found on algae biomass in rock-pools (Matthiesen et al. 2006), whereas Duffy et al. (2005) documented that mesograzer diversity enhanced epiphyte grazing only in the presence of predators.

The second question, I addressed in my experiment was, whether consumer diversity influences prey diversity in the experimental system. Consumer pressure shows a unimodal

relationship with prey diversity (Worm et al. 2002), but the relationship of diversity effects on different trophic levels remains unclear (Hunter & Price 1992, Terborgh 1992). Dyers and Letourneau (2003) reported a positive effect of consumer diversity on prey diversity in an endophytic system as postulated by conceptual models (Dunne et al. 2002, Thébault & Loreau 2003, Petchey et al. 2004), but increasing mesograzer species richness decreased total benthic community diversity in a seagrass system (Duffy et al. 2003).

Third, do these effects persist under high nutrient availability? The diversity/productivity relationship on the primary producer level has been topic of much debate in terrestrial ecology for more than 50 years (see Tilman 1999 for review). More recent studies focus on the influence of nutrient availability and accordingly productivity on the relationship between consumers and prey diversity (Proulx & Mazumder 1998, Hillebrand 2003). Multivariate models and empirical studies show that these factors have interactive effects on prey diversity (Kondoh 2001, Worms et al. 2002). Consumer diversity effects as a special sort of consumer effects may vary accordingly under different nutrient regime.

I present the results of a mesocosm experiment testing the effect of grazer diversity on epiphyte and microphytobenthos assemblages within a multi-trophic eelgrass system. The eelgrass *Zostera marina* is one of the most abundant marine macrophytes in northern temperate regions and it is a structuring species of ecologically and economically important ecosystems. Some of the organisms associated with eelgrass, the so-called mesograzers (mainly small crustaceans and gastropods), play an important role in this system as they remove the epiphytes; and thus, enhance eelgrass growth and survival (see Hughes et al. 2004 for overview). Furthermore, they are a crucial link between primary producers and higher trophic levels (Edgar & Shaw 1995).

In my experiment, I focused on the microalgae assemblages in the experimental eelgrass-system. Microalgae can be successfully used as model systems to explore the consequences of diversity loss at the consumer level (Gamfeldt et al. 2005, Matthiesen et al. 2006). Results can be obtained over a short period because of the short generation time of the microalgae. The mesocosm design had the additional advantage of providing a more natural environment than the usual small-scale experiments.

Material and Methods

Experimental design

I manipulated grazer species richness in 54 indoor mesocosm units (diameter: 30 cm, height: 60 cm), equally distributed in nine tanks (117 x 93 x 60 cm). Each mesocosm was filled with 2 mm-sieved sediment from the field (height: 10 cm). Each experimental unit was planted with 20 freshly harvested eelgrass shoots (average abundance in the Kiel Fjord in summer, ~350 shoots * m^{-2}) and left undisturbed for four days (Fig. 5.1). The grazer abundance in the Kiel Fjord was comparable to the grazer abundance in my experiment. Three common mesograzers, the isopod *Idotea baltica* (*Idotea*, I, hereafter), the amphipod *Gammarus salinus* (*Gammarus*, G) and the periwinkle *Littorina littorea* (*Littorina*, L), were used as consumers. In addition to the start and the control (no grazer) treatments, three richness levels were used (1, 2, 3, all combinations). Each treatment was replicated in six independent mesocosms in a randomised design. Grazer abundances introduced into the grazer treatments were related to the average natural abundances in summer (Gohse-Reimann, unpublished data). The initial grazer biomass was 50 mg AFDM (ash-free dry mass) corresponding to 18 *Idotea*, 24 *Gammarus* or 6 *Littorina* in the single grazer treatments. Mixed-grazer treatments were stocked using a substitutive design whereby the biomass of all grazers was kept constant.

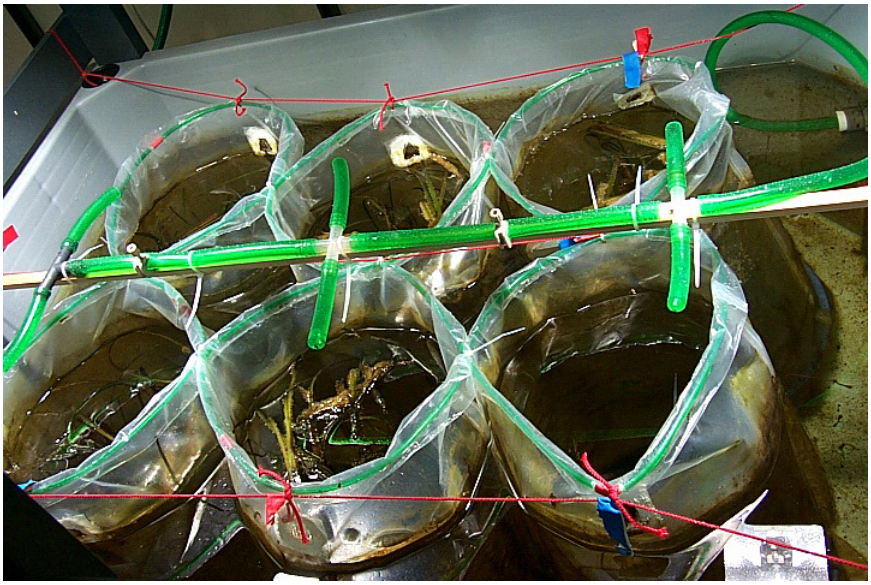

Figure 5.1. Experimental eelgrass units

Samples were taken at the beginning (time 0, three control mesocosms), after 7 days (three mesocosms of each treatment) and after 21 days of the incubation (three mesocosms of each treatment). Generally grazing effects work more quickly than nutrient effect. Grazers remove prey biomass instantly, whereas nutrient enrichment acts more slowly via prey reproduction. Earlier experiment under similar conditions showed significant grazing effects after 10 days. Considering the higher nutrient concentrations in this study, I decided to take the first samples after 7 days. Nutrient enrichment in the field, caused by storm event, produced significant increase in epiphyte biomass three weeks later.

The mesocosms were supplied independently with a constant flow of sand-filtered brackish deep water from the Kiel Fjord (salinity: 14.7 PSU ± 0.7). Water flowed out of each tank continuously through a hole, 2 cm in diameter, that was covered with a 1-mm plastic mesh. Nutrients from the inflow to the experimental units were determined on a daily basis by using an auto-sampler following the methods of Grasshoff et al. (1983). Nutrient concentrations of the inflowing water were as follows: nitrate 9.1 µmol l^{-1} ± 2.7, ammonium 3.7 µmol l^{-1} ± 1.2, phosphate 0.8 µmol l^{-1} ± 0.3 and silicate 18.4 µmol l^{-1} ± 1.2. The nutrient concentrations in the Kiel Fjord were as follows: nitrate 1.6 µmol l^{-1}, ammonium 1.3 µmol l^{-1}, phosphate 0.2 µmol l^{-1} and silicate 5.1 µmol l^{-1}. Thus, the experimental nutrient concentrations were about four times enriched compared to the field data. The light and temperature regime was adapted to summer conditions with a 16 h day and 8 h night cycle (100 µmol s^{-1} m^{-2}, 18.5 ºC).

Sampling and sample processing

Samples were taken after the introduction of consumers (t0), after 7 d and after 21 d. Microphytobenthos on the sediment surface was sampled according to Aberle and Wiltshire (2006). Subsequently the sediment samples were preserved with liquid nitrogen by using the cryolander-technique (Wiltshire et al. 1997). The micro-slicing of the sediment surface was carried out according to Wiltshire (2000) and the sediments layers were fixed with Lugol's solution. For the determination of algal cell number, biovolume, and taxonomic composition, the samples were transferred to a Sedgewick Rafter chamber. After settlement the sampled cells were counted under an inverted microscope and converted to biovolume following the methods of Hillebrand et al. (1999).

After the sediment samples were taken, all eelgrass shoots were uprooted and transferred to a container with filtered seawater to collect attached grazers. Subsequently, the eelgrass was placed in plastic bags and stored frozen until further processing. Two eelgrass shoots out of each mesocosm were carefully scraped using a special plastic scraper and a scalpel to transfer attached epiphytes into a defined volume of filtered seawater. The samples were fixed with 1% Lugol's iodine and counted under an inverted microscope in 3 ml Utermöhl-

chambers. A minimum of 400 cells was counted for dominant species and the whole chamber was counted to account for rare species. Biovolume was used as proxy for biomass.

The eelgrass shoots were dried to a constant weight for 48 h at 60° C and subsequently combusted for 8 h at 540° C to determine AFDM. The eelgrass surface area was calculated using the formula surface (mm^2) = AFDM (g) x 588.88 ($R^2 = 0.97$, $P < 0.001$), determined by measuring and weighing 100 eelgrass shoots. Eelgrass leaf production was measured by a variation of the leaf-marking technique: at the beginning of the experiment all the eelgrass shoots were marked with a needle hole 1 cm above the first node with roots. Six shoots out of each mesocosm were cut at the marking place and the length and the width of new leaves (without hole) and the growth of old leaves were measured. The production of biomass was calculated as AFDM per day using the formula mentioned above.

Statistics

To test for significant differences between grazer treatments first one-way ANOVAs were implemented using the factor grazer composition and the response variables microalgal biovolume and diversity, and eelgrass and secondary production, followed by Newman-Keuls post hoc-tests (composition effect). To detect significant grazer species richness effects, planned contrasts comparing the three-grazer treatment against all single-grazer treatments were applied (richness effect).

Net biodiversity effects (ΔY) were calculated according to Loreau and Hector (2001) as an additional estimate of diversity effects. ΔY was tested against zero with a two-sided t-test. A significant net biodiversity effect shows that the effect in the combinations is higher than expected from the single-grazer treatments. To calculate the expected share of each species in the combinations (IG, IL, GL, IGL), I used the means of the single-grazer treatments (n=3). The increase of net biodiversity effects from two to three grazer species was tested with a linear regression.

Differences in taxonomic composition were tested with non-metric multi dimensional scaling (MDS) using PRIMER 5.2 (© 2001 Primer-E Ltd.). MANOVAs were used to test the significant impact of grazer treatments on the proportional contribution of algal growth forms to epiphyte and microphytobenthos composition. Data were arcsine square root transformed. The analysis was performed with the Pillai`s trace statistic, recommended for interdependent response variables (Scheiner 1993).

5.2. Results

Consumer diversity effects on ecosystem processes

After the first seven days of the experiment, epiphyte biomass detected as biovolume was highest in the control treatment and decreased with consumer species richness (Fig. 5.2A). Grazer species richness (Table 1) and species identity showed significant effects; *Idotea* and *Gammarus* reduced epiphyte biomass significantly more effectively than *Littorina* ($p \leq 0.001$). Neither grazer species richness nor species identity had significant effects on microphytobenthos biomass (Fig. 5.2D). *Littorina* had the strongest impact on microphytobenthos biomass followed by *Gammarus* and *Idotea*. The total algal biomass at the sediment surface was generally one order of magnitude lower than the epiphyte biomass. Epiphyte species richness and diversity (H', based on the Shannon-Wiener function) were lowest in the control treatment and increased with grazer species richness (Fig. 5.2B + C). I found significant effects of grazer species richness on epiphyte species richness and diversity (Table 5.1). The impact of *Littorina* differed significantly from *Idotea* and *Gammarus* as the periwinkle had a less positive effect on epiphyte diversity than the two crustacean species ($p \leq 0.001$), but there was no significant effect of grazer species identity on epiphyte species richness. Epiphyte evenness showed the same trend and was significantly affected by grazer species richness (Table 5.1) and grazer species identity ($p \leq 0.001$). Microphytobenthos taxon richness and diversity provided similar values for the control- and the grazer-treatments after seven days (Fig. 5.2E + F). The diversity increased slightly with increasing grazer diversity and *Littorina* had a more negative impact on microphytobenthos diversity than *Gammarus* and *Idotea*, but these differences were not significant ($p > 0.05$). I found no significant effects on microphytobenthos evenness.

After 21 days the control treatment had again the highest epiphyte biomass values, but no significant effect of grazer species richness on epiphyte biomass was found (Fig. 5.3A, Table 1). Species identity affected epiphyte biomass furthermore and *Littorina* continued to show the weakest impact on epiphyte biomass ($p \leq 0.001$). Neither grazer species richness nor species combination significantly affected microphytobenthos biomass (Fig. 5.3D, Table 1). Epiphyte and microphytobenthos biomass increased in all treatments and as much as 2 to 20 times higher biovolume was found compared to sampling after 7 days. However, this effect was only significant for the two-grazer treatments (t-test, $p \leq 0.012$ for epiphytes, $p \leq 0.005$ for microphytobenthos).

After 21 days control treatments continued to show the lowest epiphyte species richness, but grazer diversity no longer had a significant impact on epiphyte species richness (Fig 5.3B, Table 5.1). The significant difference between the impact of the periwinkles and the crustaceans remained constant ($p \leq 0.001$). In contrast, the diversity and evenness of epiphytes was highest in the single grazer treatments (Fig. 5.3C, Table 5.1), whereas two-

and three-grazer treatments were similar to the control treatment. Grazer species richness and combination did not significantly affect microphytobenthos taxon richness (Fig. 5.3E, Table 1). However, I found a similar trend for diversity and evenness as in the epiphyte assemblages (Fig. 5.3F). Overall, epiphyte and microphytobenthos diversity declined in all treatments after 21 days compared to the sampling after 7 days. The decrease in diversity with time was significant in all treatments for epiphytes (t-test, $p \leq 0.011$) and in the combined treatments for microphytobenthos (t-test, $p \leq 0.05$).

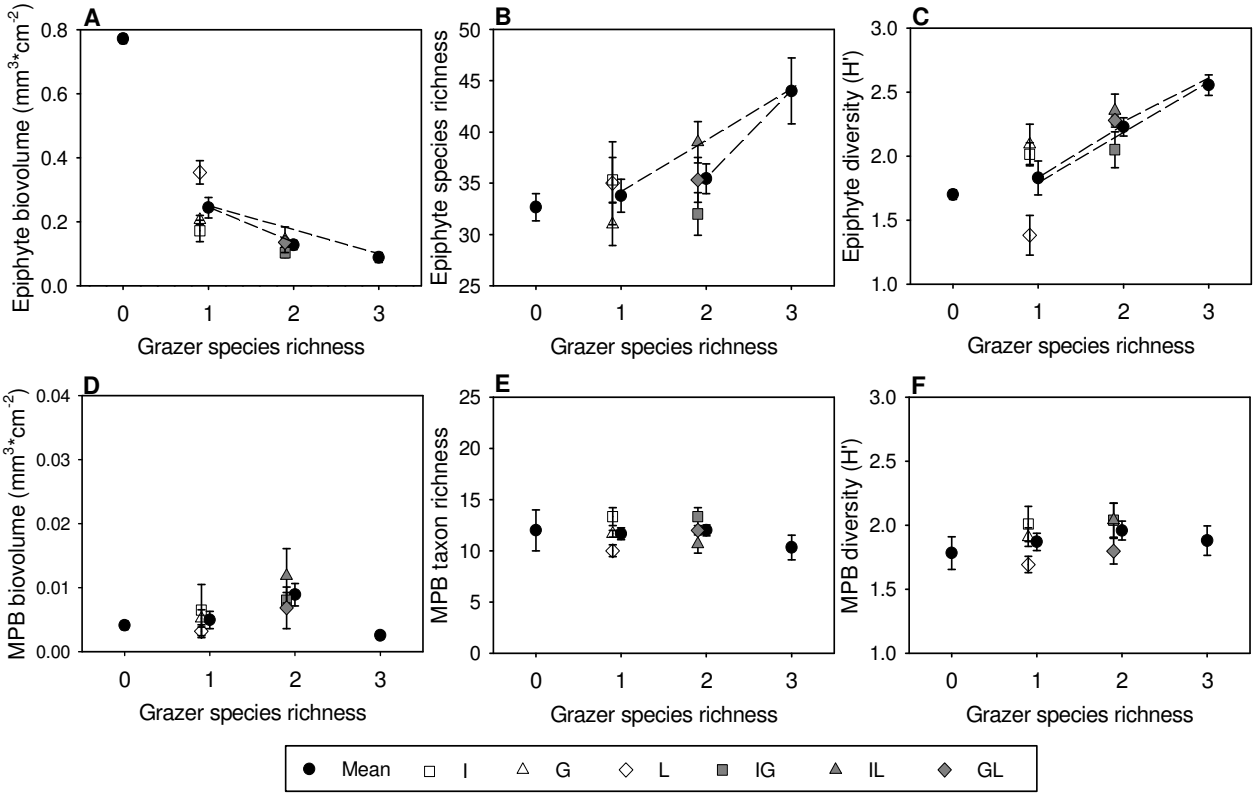

Figure 5.2. Effects of grazer diversity on ecosystem properties after 7 days of incubation. Filled circles present means with SE. Dashed lines show significant responses to grazer species richness. (A) Epiphyte biovolume, (B) epiphyte species richness, (C) epiphyte diversity, (D) microphytobenthos (MPB) biovolume, (E) MPB taxon richness and (F) MPB diversity.

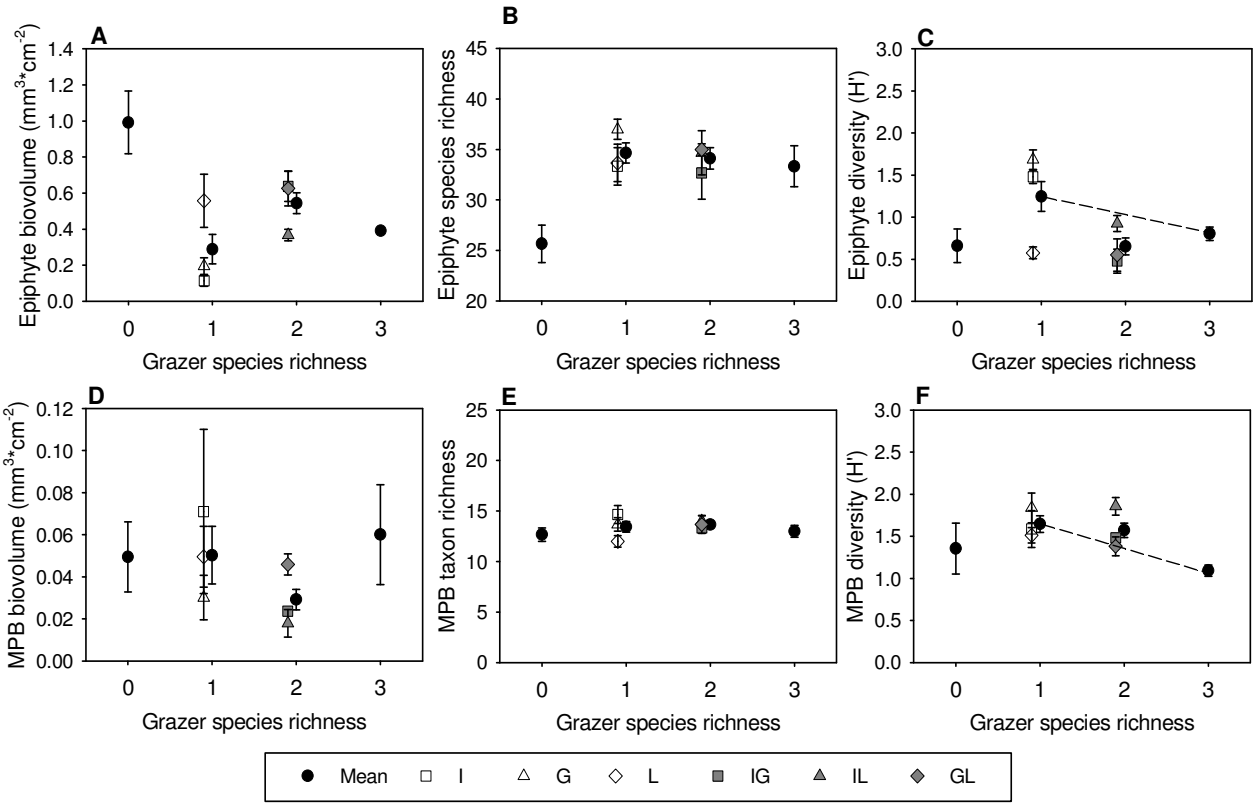

Figure 5.3. Effects of grazer diversity on ecosystem properties after 21 days of incubation. Filled circles present means with SE. Dashed lines show significant responses to grazer species richness. (A) Epiphyte biovolume, (B) epiphyte species richness, (C) epiphyte diversity, (D) microphytobenthos (MPB) biovolume, (E) MPB taxon richness and (F) MPB diversity.

Table 5.1. Results of planned contrasts with the fixed factor grazer species richness. Significant results are shown in bold.

GRAZER RICHNESS EFFECTS	**7 days**		**21 days**	
	F	P	F	P
MPB biovolume	0.62	0.4446	0.2	0.6572
Epiphyte biovolume	**26.04**	**0.0002**	1.32	0.2695
MPB taxon richness	2.21	0.1592	0.33	0.5729
Epiphyte species richness	**11.22**	**0.0048**	0.39	0.5420
MPB diversity	0.01	0.9435	**13.89**	**0.0023**
Epiphyte diversity	**27.29**	**0.0001**	**10.59**	**0.0058**
MPB eveness	0.85	0.3728	**14.65**	**0.0018**
Epiphyte eveness	**18.17**	**0.0008**	**11.26**	**0.0047**
Eelgrass growth	0.94	0.3478	0.76	0.398
Secondary production	0.37	0.552	0.003	0.9576

Net biodiversity effects

I performed analyses of net biodiversity effects (ΔY). Significant net diversity effects of grazer richness were found for epiphyte biovolume and epiphyte diversity after 7 days (Fig. 5.4). Thus, epiphyte biomass was significantly lower and epiphyte diversity was significantly higher in the combinations (two species and three species) than the expected values from the single-grazer treatments. I found no significant effect of grazer species richness on net diversity effects.

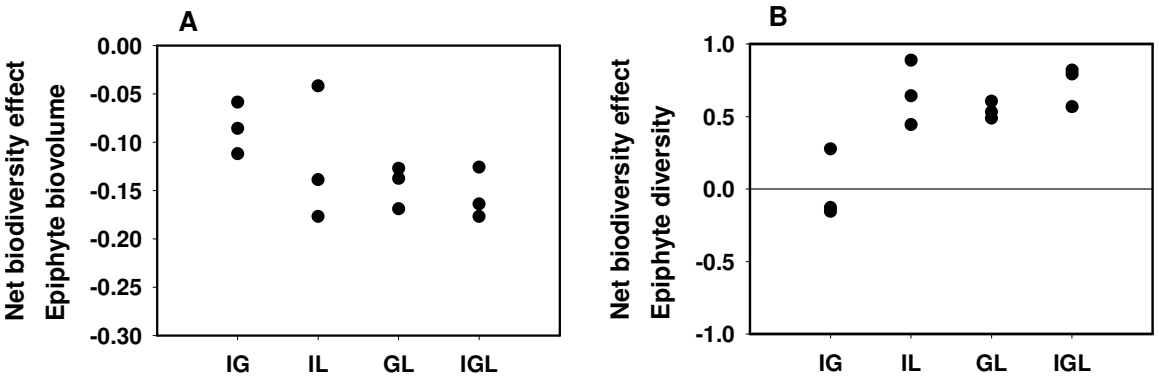

Figure 5.4. Net biodiversity effects for the different grazer combinations after 7 days of incubation. (A) Epiphyte biovolume, (B) epiphyte diversity.

Algal growth forms and taxonomic composition

Both microalgal assemblages were dominated by diatoms at the beginning of the experiment (microphytobenthos: 99%; epiphytes: 80% with 20% small brown algae mostly *Acrochaetium secundatum*). The diatoms in the epiphyte community mostly consisted of stalked forms (37%), with prostrate diatoms and chains contributed roughly equal shares (20 and 22%, respectively). Tube-living forms represented only 1% of total algal biovolume (Fig. 5.5A). In contrast, the microphytobenthos community was dominated by prostrate forms (over 90%) with only 7% comprised of chain forming and 0.4% of stalked diatom genera (Fig. 5.5B). After 7 days the dominant stalked forms (mostly *Licmophora sp.*) increased in the epiphyte assemblages in the control and the *Idotea* treatment but decreased in most other treatments. Prostrate diatoms (mostly *Cocconeis scutellum*) remained relatively constant and diatom chains (mainly *Melosira nummuloides* and *Fragilaria sp.*) tended to increase, most strongly in the *Gammarus* and *Littorina* treatments. Likewise, the tube-living forms (*Berkeleya sp.*) increased, especially in the two- and three-grazer treatments. The share of macroalgae

(mostly *A. secundatum*) decreased in all treatments. In the microphytobenthos assemblages, stalked forms (*Synedra sp.*) increased in the *Idotea* single-grazer and all two-grazer treatments and consequently prostrate growth forms (mostly *Pinnularia sp.*, *Stauroneis sp.*, *Nitzschia sp.*, *Navicula sp.* and *Amphora sp.*) decreased in these treatments to 40-70% of microphytobenthos biomass. Diatom chains (*M. nummuloides* and *Fragilaria sp.*) were reduced in almost all treatments.

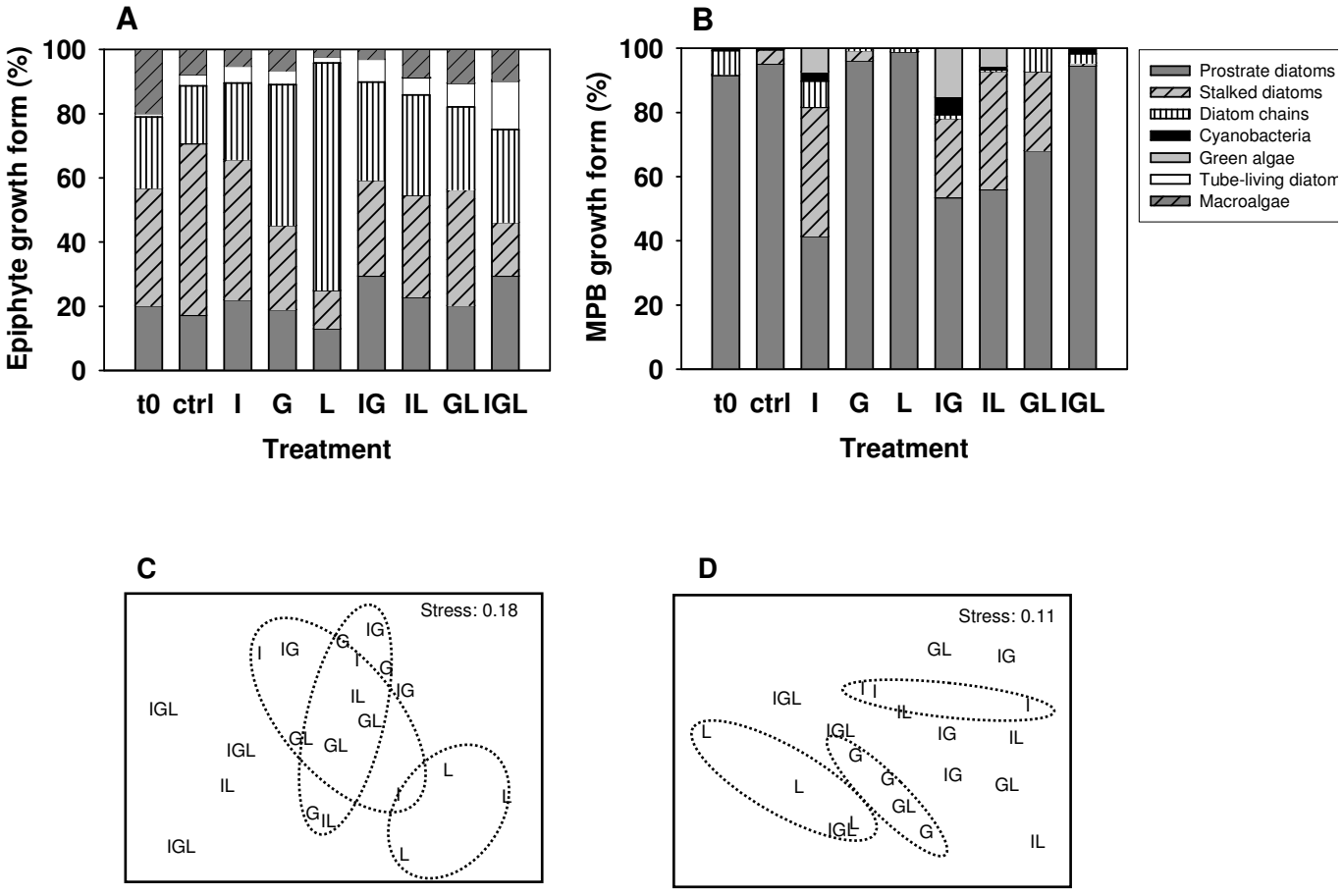

Figure 5.5. Algal growth forms and taxonomic composition after 7 days of incubation: (A) epiphyte growth forms, (B) microphytobenthos growth forms, (C). MDS-plots for the taxonomic composition of epiphytes and (D) MDS-plots for the taxonomic composition of microphytobenthos.

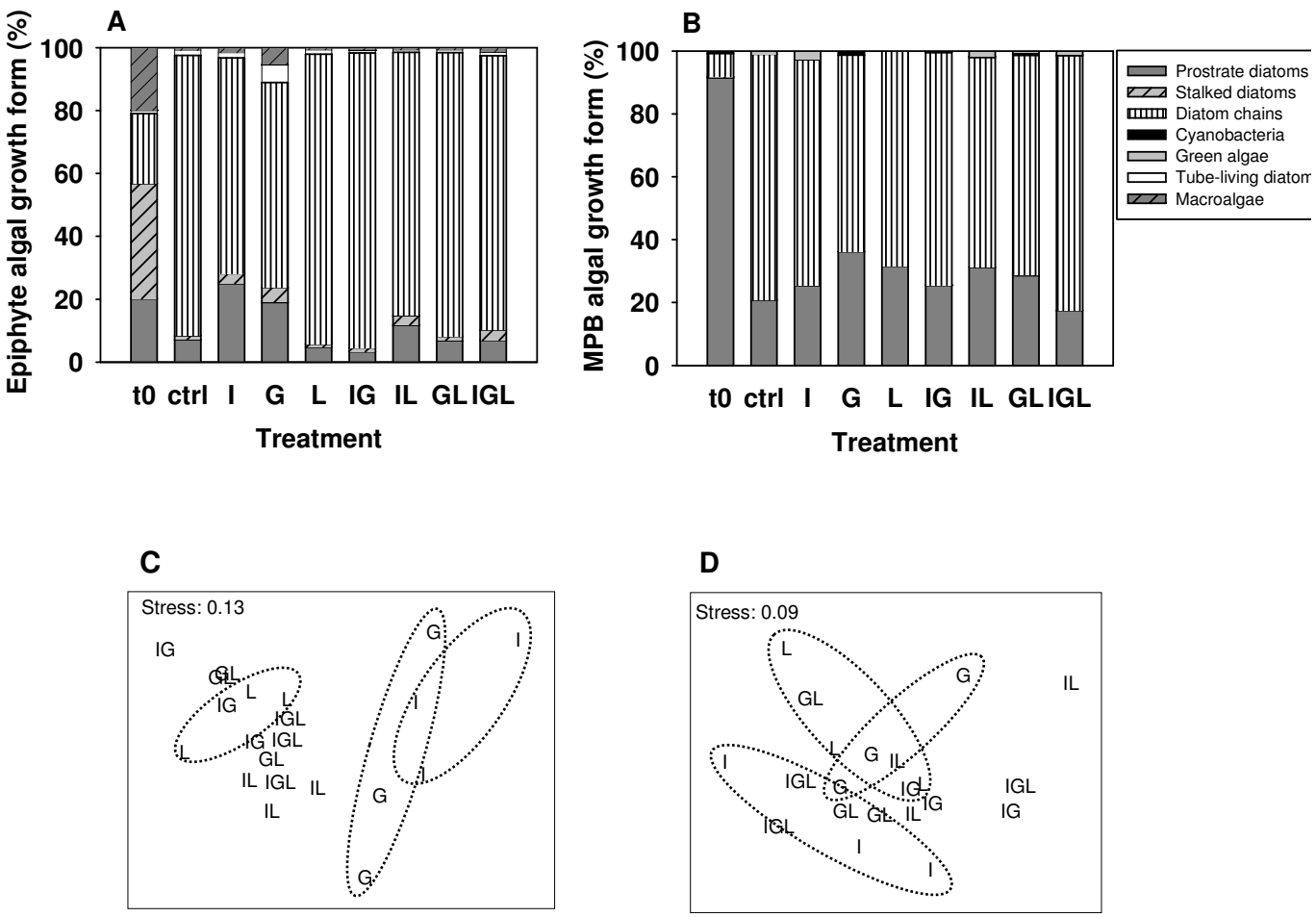

Figure 5.6. Algal growth forms and taxonomic composition after 21 days of incubation: (A) epiphytes growth forms, (B) microphytobenthos growth forms, (C). MDS-plots for the taxonomic composition of epiphytes and (D) MDS-plots for the taxonomic composition of microphytobenthos.

I found a significant impact of the different grazer treatments on epiphyte composition (Pillai's trace value = 2.27, F = 1.94, p = 0.012). The effect on microphytobenthos composition was not significant (PT = 1.82, F = 1.33, p = 0.16). Significantly different impacts on algal growth forms between all single-grazer treatments were found in the epiphyte assemblage for stalked forms ($p \leq 0.04$) and diatom chains ($p \leq 0.05$). In the microphytobenthos assemblages, *Idotea* had a significant different impact on prostrate ($p \leq 0.02$) and *Littorina* on stalked diatoms ($p \leq 0.04$). The effect on green algae differed significantly between all three grazer species (p = 0.0003). *Idotea* and *Gammarus* exerted relatively similar effects on the taxonomic composition of epiphytes, whereas the taxonomic composition of the microphytobenthos was more similar in the *Littorina* and *Gammarus* single-grazer treatment compared to the impact of *Idotea* (Fig. 5.5C, 5.5D).

After 21 days, clear composition changes were detected in all treatments and an overall dominance of chain-forming diatoms appeared in both microalgal assemblages (Fig. 5.6A, 5.6B). *Melosira*, initially only present in small amounts in the epiphyte assemblages, dominated both communities comprising between 60% and 92% (epiphytes) and between 45% and 77% (microphytobenthos) of the total algal community. Macroalgae were almost eliminated in most treatments. I found a significant impact of the different grazer treatments on epiphyte composition (PT = 2.23, F = 1.9, p = 0.016). The effect on microphytobenthos composition was not significant (PT = 1.45, F = 0.95, p = 0.55). Now *Littorina* had a different impact on epiphytes than *Idotea* and *Gammarus*, as significant effects on prostrate ($p \leq 0.015$) and stalked ($p = 0.025$) and chain-forming diatoms ($p \leq 0.005$) were observed. *Gammarus* had a significantly different impact on tube-living diatoms ($p \leq 0.0004$). For the microphytobenthos assemblages, all significant differences between the three grazers disappeared. Another obvious feature was the overall similarity in taxonomic composition of the microphytobenthos (Fig. 5D), whereas the single-grazer treatments with *Idotea* and *Gammarus* showed marked differences in the epiphytic community (Fig. 5C)

Discussion

I found varying impacts of grazer diversity on microalgal biomass, diversity and taxonomic composition within the experimental seagrass communities. The studied consumers, the isopod *Idotea*, the amphipod *Gammarus* and the periwinkle *Littorina*, showed only a significant impact on biomass and diversity with regard to epiphytic assemblages, whereas strong effects on algal growth forms and taxonomic composition occurred in both microalgal assemblages. The consumer diversity effects on epiphyte biomass and species richness were not consistent with time under high nutrient regime.

Initially, the results clearly showed that even low level consumer diversity can affect primary production in an eelgrass community. Epiphyte biomass was significantly reduced with increasing consumer species richness after seven days. Thus, the results corroborate the findings of recent studies in microbial foodwebs (Naeem & Li 1998; Gamfeldt et al. 2005). Theoretical framework in the literature has proposed that biodiversity effects on ecosystem processes are caused by two mechanisms: the selection and the complimentarity effects (Loreau & Hector 2001). The selection effect operates on the higher probability of dominance of species with strong effects, while the complementarity effect includes resource partitioning via niche differentiation and facilitation. I found a net biodiversity effect on epiphyte biomass as the effect of the combinations was significantly stronger than expected from the single-grazer treatments. This pattern stresses the importance of complimentarity effects in this experiment. The diverse impact of the studied consumers on the taxonomic composition of

the microalgal assemblages supported the fact that niche differentiation played a major role in our experiment.

The different qualitative grazing behaviour of co-occurring consumer species (specialists) seems to be fundamentally important to the relationship between consumer diversity and ecosystem function (Chapin et al. 1997, Gamfeld et al. 2005). Consumers with identical feeding behaviour were not found to have a positive diversity–production relationship (Fox 2004). My findings here of strong species effects on the composition of microalgal assemblages are in good correspondence with recent models (Thébault & Loreau 2003, Fox 2004). In these, it is predicted that a high degree of specialisation of consumers is necessary to cause significant effects of consumer diversity on prey biomass.

The biomass of the microphytobenthos community was not affected by grazer diversity in this study. Such an insusceptibility of microphytobenthos biomass to grazing impacts by macrofauna organisms is in good correspondence with studies conducted by Hillebrand and Kahlert (2002). These authors found that in contrast to epilithic algae, the effect of grazing on the microphytobenthos was negligible. Although grazers like *Idotea*, *Gammarus* and *Littorina* are known to graze on microphytobenthos, their effect is considered less strong than the impact of very effective microphytobenthos grazers such as hydrobiid snails and *Corophium sp*. (Gerdol & Hughes 1994). Additionally, the epiphyte biomass was 10 to 20 times higher than the microphytobenthos biomass and thus, greater availability of epiphytes could have partially neutralized the negative impact of macrofauna grazing on microphytobenthos biomass in my study.

In this experiment, high consumer diversity caused increasing epiphyte species richness at first. This positive effect of consumer diversity on prey diversity is in good agreement with theoretical predictions (Dunne et al. 2002, Thébault & Loreau 2003, Petchey et al. 2004) and results from a field study in an eelgrass bed, where macroalgae diversity was positively related to animal diversity (Parker 2001). A plausible explanation for such top-down diversity effects is the capability of consumers to mediate coexistence of their prey by feeding on the competitive dominant prey species and thus, confining competitive exclusion at the prey level (Paine 1966, Hillebrand 2003, Petchey et al. 2004). Consumer effects show a unimodal relationship with prey diversity, with the highest prey diversity related to “intermediate” mortality (Huston 1979). In this study, the grazing efficiency increased with growing mesograzer diversity and this effect had the adequate strength and was directed towards the dominant algae species, such that it positively affected epiphyte diversity. In contrast, Duffy et al. (2003) reported a negative effect of growing mesograzer diversity on benthic diversity. The mesograzer abundance in this study was about twofold enhanced compared to my experiment. The strong grazing pressure may have prevented a positive effect. Positive top-down effects of diversity are also reported in a terrestrial endophytic community, but not in a

detrital food web (Dyers & Letourneau 2003). Some authors have argued that the likelihood of top-down effects decline from aquatic to terrestrial and decomposer food webs (Polis & Strong 1996, Shurin et al. 2002). More tests of cascading effects of consumer diversity in different ecosystems and under different consumer pressure and nutrient supply are necessary to obtain more general conclusions.

After three weeks of incubation, a drastic change appeared in our experimental units: the consumer diversity effects on epiphyte biomass and species richness disappeared, although the effect of consumer species identity remained constant. A plausible explanation for this varying impact of consumer diversity with time is the high nutrient availability. The counteracting processes of herbivore grazing and nutrient enrichment on autotrophic biomass and diversity have received a lot of attention recently (Hillebrand & Kahlert 2002, Hillebrand 2003, Hughes et al. 2004). These studies reported that grazing pressure and nutrient availability can have strong antagonistic effects on prey biomass and diversity. Some studies focus on the influence of nutrient availability and accordingly productivity on the relationship between consumers and prey diversity (Proulx & Mazumder 1998, Hillebrand 2003). Multivariate models and empirical studies show that these factors have interactive effects on prey diversity (Kondoh 2001, Worms et al. 2002). Consumer diversity effects as a special sort of consumer effects may vary accordingly under different nutrient regime.

The nutrient concentrations in our experiment were in general in the range of moderate enrichment reported for estuaries in the case of anthropogenic eutrophication (Valiela 1992). However, the nitrogen and phosphorus concentrations were four times higher than the usual summer concentrations in the Kiel Fjord. During the course of the experiment, I found an overall increase in epiphyte and microphytobenthos biomass and a decrease in diversity in both microalgal assemblages. Such phenomena are usually found in communities under nutrient enrichment (Sundbäck & Snoeijs 1991, Hillebrand 2003, Hughes et al. 2004). Furthermore, effects on taxonomic composition were drastic in all treatments: both microalgal assemblages changed into monoculture-like communities consisting mainly of the highly productive filamentous diatom *Melosira nummuloides*. This species and its congener, *M. moniliformis*, are known for their ability to respond rapidly to nutrient enrichment, especially at high silicate concentrations like in this experiment (Hillebrand et al. 2000). The results support the presumption that nutrient effects – resulting in a high productivity – can neutralize consumer diversity effects.

In general, the data supported the hypothesis that in a prey-consumer-system higher consumer diversity can lead to a more efficient resource utilisation and consequently, to a stronger control of prey biomass. The importance of species identity and functional traits was emphasized. I showed that diversity on the prey level can be affected by diversity changes

on the consumer level. The inconsistency of consumer diversity effects with time revealed the overall importance of collateral factors e.g. nutrient conditions in a multitrophic system.

6. Carbon sources and trophic structure in an eelgrass (*Zostera marina* L.) bed based on stable isotope and fatty acid analyses

6.1. Introduction

Seagrass beds are widespread in shallow coastal waters and are considered as highly productive and diverse communities (Heck 1995, Lee et al. 2001). The eelgrass *Zostera marina* is a common species in subtidal habitats from the Arctic to the Mediterranean Sea. However, the high productivity of these systems is not due to the angiosperm production alone, since epiphytic and sediment-associated microalgae are known to contribute significantly to total system production (Daehnick et al. 1992, Nelson & Waaland 1997). Recent studies in seagrass ecosystems imply strong food web linkages between epiphytic and edaphic algae and consumers whereas fresh seagrass leaves are assumed to be of minor importance (Lepoint et al. 2000, Moncreiff & Sullivan 2001, Kang et al. 2003). Similar results have been found in saltmarsh and mangrove systems (Newell et al. 1995, Créach et al. 1997, Loneragan et al. 1997). The importance of algal material in comparison to vascular marine plants was confirmed in all studies, although saltmarsh grasses can contribute up to 50% to animal nutrition (Currin et al. 1995) and the contribution of detrital material is known to vary between seasons (Connolly et al. 2005).

The analysis of stable isotope ratios is a useful tool in determining the trophic pathways within marine food webs. Nevertheless, two complications can arise in the complex seagrass system. The relatively large number of potential carbon sources in coastal areas often complicates the detection of the most important carbon sources. Furthermore, this method relies on distinct differences in stable isotope values of primary producer groups. The similarity of sources frequently prevents a clear distinction between several sources, especially in seagrasses and their associated epiphytes (Loneragan et al. 1997, Connolly et al. 2005). Recent studies have tried to solve these problems by applying complementary methods. Fatty acid analysis has been found to be a reliable method to trace food sources in aquatic food webs, since the conservative transfer of specific fatty acids has been proven in laboratory experiments (Lee et al. 1971). A number of “indicator” fatty acids specific for algal groups like diatoms, dinoflagellates or red algae can be used as biomarkers (Kayama et al. 1989, Viso et al. 1993) and the quantitative pattern of all fatty acids, the fatty acid signature, can provide additional information, especially at higher trophic levels (Iverson et al. 2002). Kharlamenko et al. (2001) combined stable isotope and fatty acid analyses to study an eelgrass food web in a semi-enclosed bay in Siberia. They concluded that eelgrass carbon, via the detritus pathway, played an important role in the studied eelgrass community. Similar conclusions were reached in a stable isotope study of an eelgrass bed in Alaska (McConnaughey & McRoy 1979). In contrast, the view that eelgrass carbon plays a relative

minor part in trophic pathways of seagrass communities was confirmed in two other studies, using the same technique (Stephenson et al. 1986, McClelland 1998).
I used a combination of stable isotope and fatty acid analyses in this study in order to determine the relevance of epiphytes and sediment-associated microalgae in an eelgrass community in the Baltic Sea.

6.2. Methods

Study area

The research site was an eelgrass meadow adjacent to Falkenstein Beach in the inner Kiel Fjord, Germany (54°21'/10°9'). The Kiel Fjord is located in the Kiel Bight, a part of the Western Baltic Sea. The eelgrass meadow extended over an area of 23 ha and was interrupted by small, unvegetated patches (Bobsien 2006). Due to the special hydrological situation in the Baltic Sea, salinity ranges between 10 and 20 PSU, depending on discharge rates, prevailing winds and season. The astronomical tide range is negligible, but storm events can cause changes in water level. The studied eelgrass meadow extends from approximately 1.5 to 6 m depth. In June eelgrass constituted 91% of macrophyte biomass. The red algae grow attached to hard structures in the sediment. The sediment was sandy (grain size: 0.5-1 mm = 42%, >1 mm = 51%). The content of organic matter was low (< 1%). Grain size and sediment organic content were analysed using standard methods. The sand microflora biomass in surface sediments (0-0.5 cm) was 82.5 mg chl a m^{-2} (unpublished data).

Sample collection

Samples of phytoplankton, eelgrass, attached epiphytes, red algae and the most common macrozoobenthic organisms and fish species were analysed in this study. Samples were collected at 3 m water depth on 24 June 2002. All samples of macrophytes and consumers were collected by dredging, placed in plastic containers with water from the collection site and transported to the laboratory for sorting and further processing. The phytoplankton sample was collected with a plankton net (mesh size 20 µm) by towing it 10 times from bottom to surface and by combining the individual tows to one sample. Sand microflora, which consisted mostly of small prostrate diatoms in our study, is very difficult to sample directly. Some authors use composite muscle samples or the stomach content of a species known to feed exclusively on the assemblage of diatoms and bacteria in the sand as proxy (Newell & al. 1995, Moncreiff & Sullivan 2001). Unfortunately such a consumer does not exist in the studied eelgrass community. Instead, I measured sand microflora indirectly as detritus-free sediment. Scuba divers took 15 sediment cores (1cm inner diameter) within the eelgrass bed.

Sample processing

In the laboratory, the plant material (algae and eelgrass with epiphytes) was cleansed in 0.2 µm filtered sea water in order to remove detrital fragments and attached animals. Epiphytes were carefully scraped from the eelgrass blades and transferred to small amounts of filtered sea water using a special plastic scraper and a scalpel. The phytoplankton sample was filtered by a 64 µm sieve to remove zooplankton, faecal pellets and detritus. The cleaned epiphyte and phytoplankton samples were filtered on precombusted (450°C, 24 h) Whatmann GF/F filters. The sediment cores were deep-frozen, the top 0.5 cm was cut off, and 5 at a time were pooled to yield a single sample. Visible detritus was manually removed and the sediment samples were carefully rinsed with 0.2 µm filtered sea water. Observations with a dissecting microscope before and after the cleaning procedure of epiphytes, phytoplankton and sediment showed the successful removal of unwanted material. The composition of primary producers was not affected. All samples for stable isotope analysis were dried to constant weight (60°C, 24 h) and stored in a dessicator. All samples for fatty acid analysis were deep-frozen at -80°C.

All invertebrate species were kept alive overnight in filtered sea water to clear their guts. Muscle tissue was analysed for all fish species, *Carcinus maenas* and *Mytilus edulis*, the other invertebrate species were processed as whole organisms. Consumer and macrophyte samples for stable isotope analysis were dried to constant weight (60°C, 48 h). All samples were ground with an agate mortar and pestle as fine as possible and then stored in airtight plastic vials. The shells of the gastropods were discarded as far as feasible before this procedure. All fatty acid samples were deep-frozen at -80°C until further processing.

Stable isotope analysis

Eelgrass and algae subsamples were transferred into tin cups. The mesograzer subsamples were transferred into silver cups, treated with 0.2µl 10% HCl to remove carbonates and then dried again. The use of HCl to remove nondietary carbon in tissue used for stable isotope analysis has been questioned, because the $\delta^{15}N$ values can be influenced too, but the elimination of carbonates is absolutely necessary for some organisms, especially small gastropods and crustaceans that could only be sampled by crushing their shell or carapace. Preliminary analyses showed no statistically significant differences of $\delta^{15}N$ values in acid or no-acid treatments in the samples.

All consumer species were measured as individuals, except the small gastropod *Rissoa membranacea*, where 10 individuals were pooled in order to obtain sufficient material for analysis. All samples were combusted in a CN-analyser (Fisons, 1500N) connected to a Finnigan Delta plus mass spectrometer. $\delta^{15}N$ and $\delta^{13}C$ values were calculated as

$$\delta X\ (‰) = [(R_{sample}/R_{standard})-1] \times 1000$$

were X = ^{15}N or ^{13}C and R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Pure N_2 and CO_2 gas were used as primary standards and calibrated against IAEA reference standards (N1, N2, N3, NBS22 and USGS24). Acetanilide was used as internal standard after every sixth sample. The overall analytical precision was ± 0.1‰ for $\delta^{15}N$ and $\delta^{13}C$.

The model of Phillips and Gregg (2003), that provides a range of feasible source mixtures, was used to determine the carbon sources:

$$\delta_M = f_A\delta_A + f_B\delta_B + f_C\delta_C$$
$$1 = f_A + f_B + f_C$$

f_A, f_B and f_C are the proportion of source isotopic signatures (δ_A, δ_B and δ_C) which coincide with the observed signature for the mixture (δ_M). All possible combinations of primary producer contributions were analysed with an increment of 1%. These predicted mixture signatures were compared with the measured values. If they were within a tolerance of 0.01%, they were considered feasible solutions. I used only $\delta^{13}C$ values in the modelling because of the sensitivity of the model to fractionation corrections (Connolly et al. 2005). The fractionation is much larger for ^{15}N than for ^{13}C and can vary considerably between different species. I chose 0.5‰ as average fractionation increase of ^{13}C for estuarine ecosystems (France & Peters 1997).Calculations were carried out with IsoSource, a Visual Basic program, which is available for public use at http://www.epa.gov/wed/pages/ models.htm. Epiphytes, the red alga *Delesseria sanguinea* and sand microflora were used as possible carbon sources for most consumers. I ran the model using phytoplankton, epiphytes and sand microflora as main autotroph carbon sources for the blue mussel *Mytilus edulis*, the common starfish *Asterias rubens* and the small sandeel *Ammodytes tobianus*. These species are known to depend on phytoplankton carbon (Castilla 1972, Wiedemeyer & Schwamborn 1996, Muus & Nielsen 1999).

Trophic levels were calculated according to the model of Hobson and Welch (1992):

$$TL = 1 + (N_m - N_b) / TE$$

Where TL is the trophic level of the consumer, N_m is the $\delta^{15}N$ value of the consumer, N_b is the average basis $\delta^{15}N$ value and TE the trophic enrichment factor in this system. A TL close to 2

is consistent with herbivorous nutrition, whereas a TL ≥ 3 suggests a carnivorous diet. Averaging the $\delta^{15}N$ values for all primary producers, excluding phytoplankton, resulted in a mean of 7.0‰ as a baseline value to establish where TL 1 lies. The $\delta^{15}N$ values with the exception of carnivores ranged from 8.2‰ to 10.5‰, indicating for some consumers a trophic fractionation essentially lower than the mean value of 3‰ generally employed in aquatic systems. In general, trophic $\delta^{15}N$ enrichment of herbivores is highly variable (Vander Zanden & Rasmussen 2001). Therefore, I averaged the $\delta^{15}N$ values of herbivores (based on literature information) and calculated an average enrichment value (1.5±0.1‰). This fractionation was used to calculate trophic levels for potential herbivorous and omnivorous species. The trophic enrichment of carnivores is less variable. Provided that the average $\delta^{15}N$ value of primary consumers is used as baseline, the resulting error is generally minor (Vander Zanden & Rasmussen 2001). The trophic level of carnivores was determined using mean $\delta^{15}N$ value of the same herbivore species as a baseline (8.5±0.1‰) and 3‰ as the trophic enrichment factor.

Fatty acid analysis

The macrophyte and mesograzer samples were freeze-dried for 48 h, ground with an agate mortar and pestle and weighted. Macrophytes were processed as individuals, while mesograzers were pooled into three replicate samples containing three individuals, with the exception of *Rissoa membranacea* where 10 individuals were pooled to obtain sufficient material for analysis. Fatty acids were extracted, esterified and analysed on a gas chromatograph (Hewlett Packard 5890 Series II.) following the method of Wiltshire et al. (2000) using the GC temperature settings of von Elert (2002). To quantify the fatty acid content an internal standard of heptadecanoic (17:0) and tricosanoic fatty (23:0) acid methyl esters was used.

Statistics

Differences between potential primary food sources (eelgrass, epiphytes, sand microflora, *D. sanguinea* and phytoplankton) concerning stable isotopes were analysed by a 1-way ANOVA followed by a Student-Newman-Keul`s test.

Calculations for fatty acid signatures were only performed for fatty acids represented with at least one value above 1%. Fatty acid data were log-transformed and subjected to non-metric multi-dimensional scaling using the program package PRIMER 5.0.

6.3. Results

Stable isotope signature of primary producers

The stable carbon isotope ratios of the studied primary producers in the eelgrass bed of Falkenstein showed a wide range of mean values from -9.6 to -34.9‰ (Table 6.1). The quantitatively relevant primary producers eelgrass, epiphytes, sediment microflora, the red alga *Delesseria sanguinea* and phytoplankton showed significantly different values ($p < 0.001$). Eelgrass had a mean $\delta^{13}C$ value of -9.6‰, while that for its associated epiphytes (mainly prostate, stalked and tube-living diatoms) was -11.3‰. Thus, the epiphytes were only slightly depleted compared to the mean $\delta^{13}C$ value of eelgrass. The mean value for the sand microflora (mainly small, prostate diatoms) was -20.0‰. Phytoplankton was isotopically lighter showing a mean $\delta^{13}C$ value of -22.6‰. The dominant phytoplankton primary producer was the chain-forming diatom *Dactyliosolen fragilissimus*, a typical species of summer phytoplankton in the Kiel Bight. The red alga *D. sanguinea* had a substantially lighter mean $\delta^{13}C$ value than all other primary producers (-34.9‰). All other red algae had mean $\delta^{13}C$ values ranging from -16.9‰ to 24.6‰, a range, which included the mean $\delta^{13}C$ values of phytoplankton and sand microflora. However, these species were rather rare and their contribution to system primary production was therefore considered negligible.

Stable nitrogen isotope ratios were similar (7.2 to 8.1‰) for eelgrass and all red algae with the exception of *Ahnfeltia plicata* (10.3‰). The diatom-dominated samples (epiphytes, phytoplankton and sand microflora) had significantly lower mean $\delta^{15}N$ values than the macrophyte (eelgrass and *D. sanguinea*) samples ($p<0.001$).

Table 6.1. Mean $\delta^{13}C$ and $\delta^{15}N$ values of primary producers in an eelgrass bed in the Kiel Bight, June 2002. SD = standard deviation. Superscript letters shows significant differences.

Food sources	n	Average $\delta^{13}C$ ‰	SD	n	Average $\delta^{15}N$ ‰	SD
Rhodophyceae						
Ahnfeltia plicata (Hudson) Fries	3	-16.87	1.03	3	10.30	0.93
Ceramium rubrum (Hudson) C. Argardh	3	-17.43	0.15	3	8.00	0.13
Delesseria sanguinea (Hudson) Lamouroux	10	-34.85[a]	1.02	10	8.05[a]	0.85
Polysiphonia fibrillosa (Dillwyn) Sprengel	3	-24.61	0.17	3	7.23	0.17
Zostera marina Linnaeus	10	-9.64[b]	0.65	10	8.04[a]	0.32
Epiphytes on *Z. marina*	10	-11.31[c]	0.81	10	6.99[b]	0.28
Phytoplankton, 20µm	3	-22.56[d]	0.06	3	7.55[b]	0.06
Sediment microflora	3	-20.04[e]	0.23	3	6.00[c]	0.42

Table 6.2. Mean $\delta^{13}C$ and $\delta^{15}N$ values of consumers in an eelgrass bed in the Kiel Bight, June 2002. SD = standard deviation.

Consumer	n	Mean $\delta^{13}C$ ‰	SD	n	Mean $\delta^{15}N$ ‰	SD
Annelida						
Harmothoe imbricata (Linnaeus)	5	-18.81	0.51	5	11.88	0.14
Nereis diversicolor O.F. Müller	1	-17.83		1	11.75	
Bivalves and gastropods						
Lacuna vincta Montagu	3	-12.25	0.51	3	9.23	0.25
Littorina littorea (Linnaeus)	10	-17.27	0.98	10	9.50	0.20
Mytilus edulis (Linnaeus)	10	-21.81	0.41	10	8.40	0.19
Rissoa membranacea (J. Adams)	3	-14.37	0.17	3	8.21	0.06
Crustacea						
Amphitoe rubricata Montagu	10	-23.88	0.72	10	8.46	0.11
Carcinus maenas (Linnaeus)	5	-17.54	0.43	5	12.66	0.58
Corophium insidiosum Crawford	5	-18.05	0.06	5	8.78	0.06
Crangon crangon (Linnaeus)	10	-17.37	0.54	10	11.63	0.23
Erichthonius difformis Milne-Edwards	7	-18.61	0.89	7	8.24	0.23
Gammarus oceanicus (Segerstråle), 10 mm	10	-19.01	0.38	10	8.66	0.27
Gammarus oceanicus (Segerstråle), 16-20 mm	10	-23.80	0.74	10	9.43	0.14
Idotea baltica (Pallas), 8 mm	10	-17.75	0.35	10	8.92	0.25
Idotea baltica (Pallas), 15 mm	10	-17.08	0.48	10	9.36	0.36
Microdeutopus gryllotalpa A. Costa	3	-18.13	0.69	3	8.22	0.37
Mysis mixta Lilljeborg	5	-18.99	0.40	5	11.67	0.28
Palaemon adspersus Rathke	10	-19.43	0.66	10	10.49	0.18
Praunus flexuosus (O.F. Müller)	10	-19.47	0.22	10	11.37	0.48
Echinodermata						
Asterias rubens Linnaeus	5	-17.15	0.81	5	11.61	0.77
Fish						
Ammodytes tobianus Linnaeus	1	-20.36		1	12.29	
Gadus morhus Linnaeus	1	-19.03		1	14.41	
Gasterosteus aculeatus Linnaeus	5	-19.79	0.70	5	13.92	0.19
Gobiusculus flavescens (Fabricius)	5	-20.06	0.18	5	13.47	0.17
Nerophis ophidion (Linnaeus)	5	-18.21	0.77	5	12.35	0.61
Pholis gunnelus (Linnaeus)	3	-19.90	0.05	3	13.86	0.21
Pomatoschistus minutus (Pallas)	5	-17.29	0.81	5	13.89	0.28
Spinachia spinachia (Linnaeus)	5	-17.96	0.21	5	13.36	0.28
Syngnathus typhle Linnaeus	5	-18.29	0.89	5	13.00	0.27
Zoarces viviparus (Linnaeus)	1	-18.52		1	13.09	

Table 6.3. Results of the IsoSource model for consumers. Mean contributions of primary producers to consumer nutrition and the 1 to 99 percentile ranges are given.

Consumer	Plankton (%)	Epiphytes (%)	Sand microflora (%)
M. edulis	94 (90-97)	1 (0-2)	6 (1-10)
A. rubens	32 (1-60)	30 (22-39)	38 (1-77)
A. tobianus	68 (52-87)	5 (0-10)	27 (3-48)
	Epiphytes (%)	Sand microflora (%)	*D. sanguinea* (%)
H. imbricata	35 (6-62)	46 (3-92)	19 (2-35)
N. diversicolor	41 (19-63)	43 (8-78)	16 (3-29)
L. vincta	90 (87-92)	7 (3-11)	3 (2-5)
L. littoraea	49 (26-70)	37 (4-74)	14 (0-26)
R. membranacea	70 (61-83)	23 (3-38)	7 (1-14)
A. rubricata	26 (8-42)	30 (4-58)	44 (34-54)
C. maenas	47 (26-70)	37 (0-70)	16 (4-30)
C. insidiosum	43 (17-68)	42 (2-83)	15 (0-30)
C. crangon	47 (26-70)	37 (0-70)	16 (4-30)
E. difforme	40 (14-65)	43 (3-84)	17 (2-32)
G. oceanicus, 10 mm	35 (6-62)	47 (4-93)	18 (1-36)
G. oceanicus, 16-20 mm	24 (10-37)	30 (9-52)	46 (38-54)
I. baltica, 8 mm	45 (20-69)	40 (1-79)	15 (1-30)
I. baltica, 15 mm	45 (27-66)	43 10-72)	12 (1-24)
M. gryllotalpa	44 (23-67)	40 (3-73)	16 (4-30)
M. mixta	31 (4-60)	51 (5-94)	18 (2-35)
P. adspersus	30 (4-60)	49 (2-91)	21 (5-38)
P. flexuosus	29 (7-51)	51 (16-86)	20 (7-33)
G. morhua	33 (5-61)	44 (0-89)	23 (6-39)
G. aculeatus	27 (1-57)	48 (1-90)	25 (9-42)
G. flavescens	26 (3-47)	49 (16-86)	25 (11-37)
N. ophidion	37 (9-65)	45 (1-90)	18 (1-34)
P. gunnelus	25 (2-46)	51 (18-88)	24 (10-36)
P. minutus	39 (15-64)	46 (7-85)	15 (0-29)
S. spinachia	39 (10-66)	43 (0-89)	18 (1-34)
S. typhle	34 (12-56)	49 (14-84)	17 (4-30)
Z. viviparus	34 (6-62)	47 (3-92)	19 (2-35)

Stable isotope signature of consumers

Mean stable carbon isotope ratios of individual consumer species ranged from -12.2‰ for the gastropod *L. vincta* to -23.9‰ for the amphipod *Amphitoe rubricata* (Table 6.2). The mean $\delta^{13}C$ value for all consumers was -18.6‰. The bivalve *Mytilus edulis* (the only important filter feeder in this eelgrass bed) had a $\delta^{13}C$ value of -21.8‰, which was only slightly enriched compared to the $\delta^{13}C$ value for phytoplankton (-22.6‰). The IsoSource

model based on phytoplankton, epiphytes and sand microflora suggested that 94% of the mussels' carbon is phytoplankton-derived (Table 6.3). The common starfish *Asterias rubens* is considered to prey preferentially on *M. edulis*. However, its $\delta^{13}C$ value (-17.2‰) indicated additional food sources. The contributions of phytoplankton, epiphytes and sand microflora were all about the same (32%, 30% and 38%, respectively). Phytoplankton had the most likelihood of contributing to the sand eel *Ammodytes tobianus* (68%) followed by sand microflora (27%) and epiphytes (5%).

The $\delta^{13}C$ values for the three gastropods, *Lacuna vincta* (-12.2‰), *Rissoa membranacea* (-14.4‰) and *Littorina littorea* (-17.3‰), suggested a decreasing dependence on epiphyte carbon (90%, 70% and 49%, respectively) according to the IsoSource calculations (Table 6.3). The crustaceans exhibited a wide range of $\delta^{13}C$ values ranging from -17.1‰ for the isopod *Idotea baltica* to -23.9‰ for the amphipod *A. rubricata* indicating a mixed diet including epiphytes, sediment microflora and red algae or species, which feed on these items (mean 36% epiphyte-derived carbon, 44% sand microflora-derived carbon and 20% *D. sanguinea*-derived carbon). The carnivorous annelids *Harmothoe imbricata* and *Nereis diversicolor* had $\delta^{13}C$ values, -18.8‰ and -17.8‰ respectively, which suggested that they depend mainly on sand microflora for their ultimate carbon source (48% and 52%, respectively). A narrow range of interspecific differences was found in the $\delta^{13}C$ values for the 10 sampled fish species (maximum difference = 3‰), ranging from -17.3‰ for the sand goby Potamoschistus *minutes* to -20.1‰ for the tree-spined stickleback *Gasterosteus aculeatus*. These values for fish taken as a group suggest a diet consisting on average of 33% epiphyte-, 47% sand microflora- and 20% *D. sanguinea*-derived carbon.

In Fig. 6.1, $\delta^{13}C$ values for all consumers and all primary carbon sources (eelgrass, epiphytes, phytoplankton, sediment microflora and the red alga *D. sanguinea*) are plotted together. Animals were assigned to herbivore, omnivore and carnivore groups based on literature information. Herbivores had the greatest span in their $\delta^{13}C$ values, whereas omnivores and carnivores exhibited a more narrow range. No significant difference in mean $\delta^{13}C$ values was found between herbivores, omnivores and carnivores as grouped in Fig. 6.1 ($p = 0.444$).

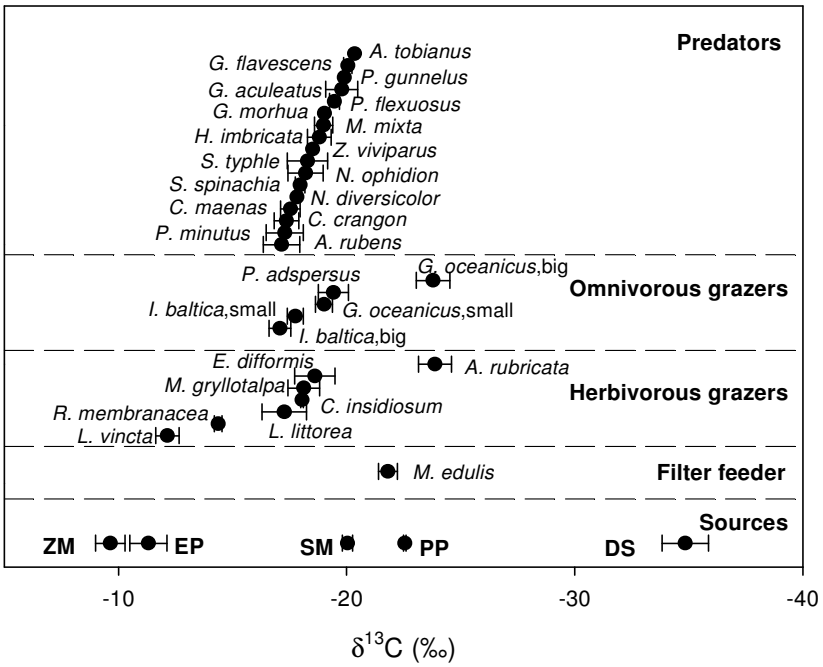

Figure 6.1. δ ^{13}C values (‰) for consumers and the different, potential primary carbon sources, collected from an eelgrass bed in the Kiel Fjord in June 2002. Mean ± SD (ZM = *Z. marina*, EP = epiphytes, SM = sediment microflora, PP = phytoplankton, DS = *D. sanguinea*).

Stable nitrogen isotope ratios for consumer species ranged from 8.2‰ for the gastropod *R. membranacea* to 14.4‰ for the cod *Gadus morhua* (Table 6.2), and where indicative of the trophic level. The filter-feeding blue mussel *M. edulis* (δ^{15}N = 8.4‰) occupied a very low trophic position, suggesting a largely herbivorous diet. The starfish A*. rubens* had a higher δ^{15}N value of 11.6‰ in accordance with its known carnivorous diet. The δ^{15}N values for the gastropods ranged from 8.2‰ to 9.5‰, corresponding to their herbivorous diet. The crustaceans showed a wide ranger of δ^{15}N values (8.2‰ for the amphipod *Microdeutopus gryllotalpa* to 12.7‰ for the predator *Carcinus maenas*), in concordance with their trophic positions from herbivory to carnivory. The crustaceans can be divided into three groups: herbivorous amphipods mostly small (≤1cm) and sessile like *Corophium insidiosum*, the omnivorous *Gammarus oceanicus*, *I. baltica* and *Palaemon adspersus* and the carnivorous shrimps *Praunus flexuosus* and *Crangon crangon*, the mysidacaean *Mysis mixta* and the green crab *C. maenas*. All fish had δ^{15}N values corresponding to higher trophic positions (12.3‰ – 14.4‰). The top predator was juvenile cod *G. morhua*. In Fig. 6.2 δ^{15}N values of all consumers are shown. No clear distinctions between trophic levels were apparent.

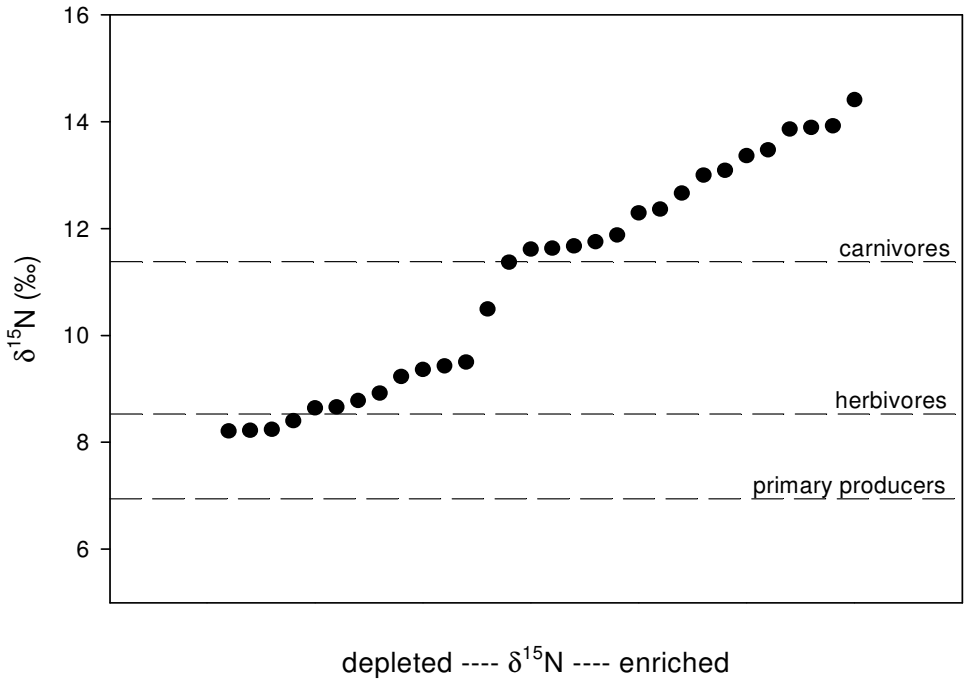

Figure 6.2. $\delta^{15}N$ values (‰) for all consumer samples collected from an eelgrass bed in the Kiel Fjord in June 2002. Dashed lines indicate a mean $\delta^{15}N$ value for all primary producers, excluding phytoplankton, and the ideal herbivore and carnivore $\delta^{15}N$ values assuming a trophic fractionation of 1.5‰ for herbivores and 3% for carnivores.

Fatty acid composition of potential food sources and consumers

Of the 38 fatty acids (FA) identified, the saturated fatty acids 14:0, 16:0 and 18:0 made up 72-79% of total fatty acid content in the diatom-dominated phytoplankton, epiphyte and sand microflora samples. The eelgrass and red algae samples showed considerably lower proportions of saturated fatty acids (28% and 46%, respectively). Characteristic fatty acids of eelgrass were 18:2(n-6), 18:3(n-4), 18:3(n-3) and 18:4(n-3). The epiphytic assemblage consisted mainly of pennate diatoms; red and brown algae contributed only a small proportion to the total biomass. The high concentrations of 16:0 (57%) are typical for the fatty acid composition of diatoms. Specific biomarkers for diatoms are 16:1(n-7) and 20:5(n-3) and these were present in small quantities. The phytoplankton fatty acid composition differed only in one aspect from the epiphyte fatty acids: the amount of the fatty acid 14:0 was much higher (55%) and correspondingly the amount of the fatty acid 16:0 much lower (18%). The sand microflora, in contrast, showed a total absence of unsaturated C_{20}-fatty acids including 20:5(n-3) and a higher quantity of 15:0, a fatty acid characteristic for anaerobic bacteria. Another biomarker fatty acid for aerobic heterotrophic bacteria 18:1(n-7) occurred in small amounts in all potential primary food sources.

The red alga *D. sanguinea* contained high quantities of 20:4(n-6) in accordance with data reported by Khotimchenko and Vaskovsky (1990) for *Delesseria violacea*.

Table 6.4. Biomarker fatty acids that were used in this study to identify primary food sources in an eelgrass system in the Kiel Bight.

Fatty acid	Biomarker for	References
16:1(n-7)	Diatoms	Desvilettes et al. (1997), Viso & Marty (1993)
20:5(n-3)	Diatoms	Desvilettes et al. (1997), Viso & Marty (1993)
18:3(n-3)	Zostera marina	Nichols et al. (1982), Khotimchenko (1990), Kharlamenko et al.(2001), this study
18:4(n-3)	Zostera marina	Nichols et al. (1982), Khotimchenko (1990), Kharlamenko et al. (2001), this study
20:4(n-6)	Red algae	Kayama et al. (1989), Khotimchenko & Vaskovsky (1990), this study
15:0	Anaerobic bacteria	Findlay et al. (1990), Desvilette et al. (1993)
18:1(n-7)	Aerobic bacteria	Findlay et al. (1990)

The dominant fatty acid in all consumers was 16:0. Other saturated fatty acids were of no importance. The major monoenic fatty acids were in decreasing order of significance: 18:1(n-9), 16:1(n-7) and 18:1(n-7). Oleic acid 18:1(n-9) is a major fatty acid of most marine animal lipids (Dahl et al. 2003). The only relevant polyunsaturated fatty acid in all consumers was 20:5(n-3), which is characteristic for diatoms. The fatty acid signatures of all consumers alone and together with potential food sources were subjected to non-metric multi-dimensional scaling (MDS) to evaluate similarities. The first MDS plot (Fig. 6.3A) demonstrated that the primary food sources epiphytes, sand microflora, *D. sanguinea* and phytoplankton were more similar to consumers than eelgrass concerning fatty acid composition. Eelgrass showed little similarity to other primary consumers and the consumers. In a second MDS plot (Fig. 6.3B) consumers were grouped into herbivorous gastropods (*R. membranacea*, *L. vincta*, *L. littorea*) and omnivorous crustaceans (*G. oceanicus* and *I. baltica*), whereas the carnivorous green crab *C. maenas* and polychaete *H. imbricata* showed little similarity to the other consumer species and each other.

Biomarker fatty acids in dominant consumers

The biomarker fatty acids used to identify food sources in this study are listed in Table 6.4. Biomarker fatty acids for eelgrass (Fig. 6.4A) were present in all consumer species, but only in insignificant amounts (≤ 1.2%). In contrast, all consumers had high levels of the fatty acids 16:1(n-7) and 20:5(n-3) characteristic for diatoms (Fig. 6.4B). The relatively low values of 16:1(n-7) in *C. maenas* might be due to elongation of this fatty acid to 18:1(n-7), which occurs in some marine animals (Dahl et al. 2003).

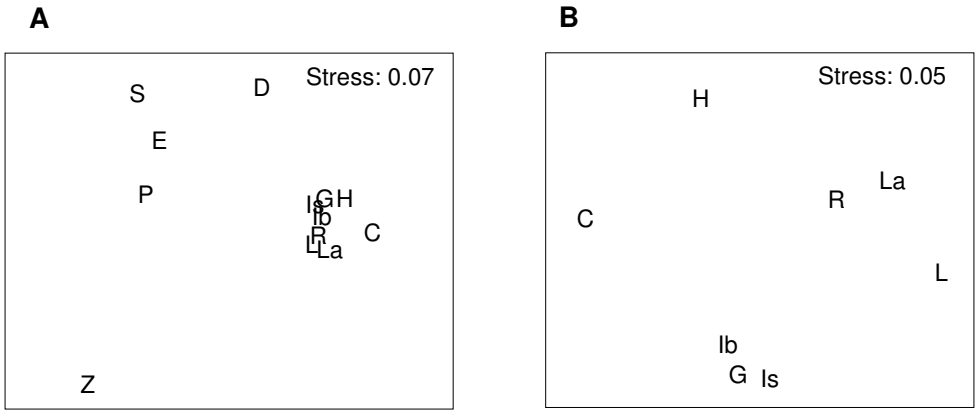

Figure 6.3. Multi-dimensional scaling (MDS) plots of potential primary food sources and consumers. (A) Primary producers and consumers and (B) consumers alone. Stress <0.05 gives an excellent representation in a MDA analysis while stress <0.1 gives a good representation. (Z = *Z. marina*, E = epiphytes, S = sand microflora, D = *D. sanguinea*, P = phytoplankton, G = *G. oceanicus*, Is = *I. baltica* 8 mm, Ib =*I. baltica* 15 mm, R = *R. membranacea,* L = *L. littorea,* La = *L. vincta*, C = *C. maenas* and H = *Harmothoe imbricata*)

All consumers contained small amounts of 15:0, the biomarker for anaerobic bacteria (≤0.7% of total FA, fig. 4C). However, significant amounts of 18:1(n-7), a biomarker for aerobic heterotrophic bacteria, were found in all animal species with the exception of *L. vincta*. The high values of 18:1(n-7) in *C. maenas* could be caused by the elongation of 16:1(n-7) mentioned above. The fatty acid 20:4(n-6), characteristic for red algae, occurred in low amounts in all consumers (Fig. 6.4D); the highest amount was present in *G. oceanicus* (4% of total FA).

The primary fatty acid of phytoplankton (14:0) was merely found in low amounts in the studied consumer species (Fig. 6.4E). The unsaturated fatty acid 16:0, the dominant fatty acid in diatoms, was found in high amounts in all species with the exception of *C. maenas*. Epiphytes and sand microflora contained relevant amount of this fatty acid, however, the concentration in phytoplankton was significantly lower. The high content of 14:0 may be caused by the flagellates *Emilia huxleyi*, found in the phytoplankton sample. Prymnesiophyceae exhibit a high content of this fatty acid (Viso & Marty 1993).

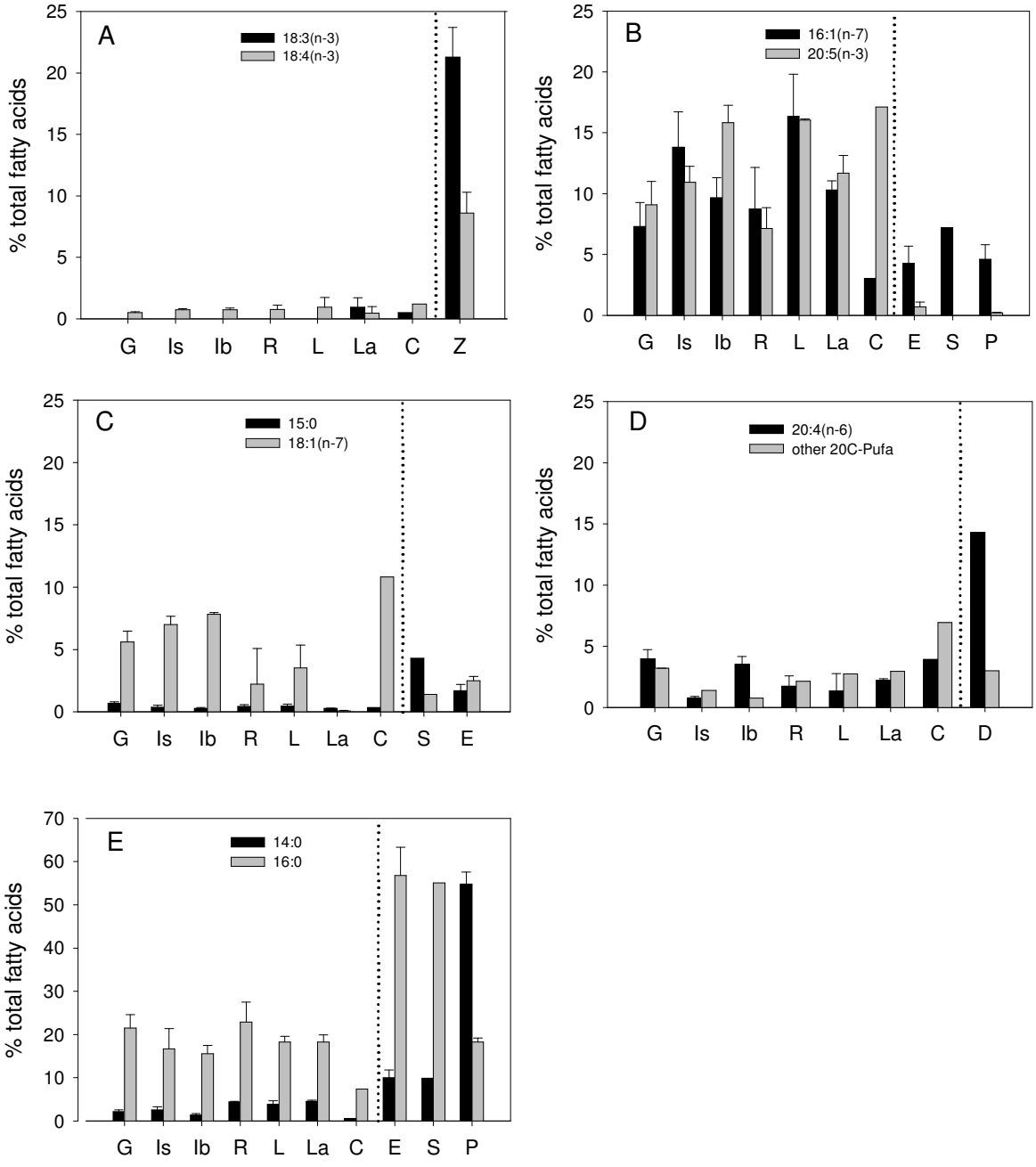

Figure 6.4. Biomarker fatty acids in dominant animals and primary food sources: (A) for eelgrass, (B) for diatoms, (C) for bacteria, (D) for red algae and (E) phytoplankton, mean ± SD. The dotted lines separate primary producers and consumer species. (Z = *Z. marina*, E = epiphytes, S = sand microflora, D = *D. sanguinea*, P = phytoplankton, G = *G. oceanicus*, Is = *I. baltica* 8mm, Ib =*I. baltica* 15mm, R = *R. membranacea,* L = *L. littorea,* La = *L. vincta*, C = *C. maenas*)

6.4. Discussion

The importance of epiphytes as carbon sources

Stable carbon isotopic values and fatty acid composition of primary producers and consumers in the studied eelgrass bed strongly supported the assumption of a food web mainly based on epiphytes and sand microflora. Red algae and phytoplankton appear to be of minor importance in this system. The contribution of eelgrass seemed to be negligible.

Stable isotope studies are increasingly being used to determine the relative contributions of different sources of primary production to higher trophic levels in a multitude of aquatic ecosystems. The importance of seagrass, saltmarsh plants or mangroves versus epiphytic or edaphic microalgae has been the subject of a long-standing debate in shallow estuarine benthic ecosystems (Currin et al.1995, Moncreiff & Sullivan 2001, Connolly et al. 2005) and stable isotope studies can be a useful tool to answer this question. Nevertheless, the frequently occurring similarity of stable carbon isotope values of seagrasses and epiphytes can obscure the distinction between these two carbon sources (Loneragan et al. 1997, Connolly et al. 2005) as found in this study. To determine the contribution of these primary producers to higher trophic levels a combined approach is necessary.

The conservative transfer of fatty acids from primary producers to higher trophic levels was first demonstrated in laboratory experiments (Lee et al. 1971) and later in many food web studies in natural assemblages (Falk-Petersen et al. 2002, Dahl et al. 2003). A comparison of the fatty acid signature of primary producers and consumer species sampled in this study strongly suggests that fresh eelgrass leaves are of no relevance for the carbon flow in this food web. The negligible amounts of the main biomarker fatty acid for eelgrass 18:3(n-3) (Nichols et al. 1982, Kharlamenko et al. 2001) found in consumers support this assumption. The contribution of phytoplankton carbon to higher trophic levels was also assumed to be of minor importance in this eelgrass bed.

In accordance with the results of the fatty acid analyses the contribution of primary producer carbon to higher trophic levels was calculated for epiphytes, sand microflora and red algae. According to the model of Phillips and Gregg (2003) epiphytes and sand microflora were the major carbon sources, whereas red algae were of minor importance. No significant difference in $\delta^{13}C$ values was found between herbivores, omnivores and carnivores as grouped in Fig. 6.1, although the contribution of epiphyte carbon to herbivores (mean 52%) was higher than for the other two groups (34% and 35%, respectively).

The most important herbivorous grazers in terms of consumer biomass were the gastropods *Rissoa membranacea* and *Littorina littorea* (Gohse-Reimann, unpublished data). The gastropod *Lacuna vincta* was of no quantitative importance. The small gastropod *R. membranacea* is mainly found on eelgrass leaves and had $\delta^{13}C$ values closest to those of

epiphytes (70% epiphyte-derived carbon). *Littorina littorea* appears to have a diet based more strongly on sand microflora (49% epiphyte-derived carbon). High levels of 16:1(n-7) and 20:5(n-3) in both species confirmed the importance of diatoms in their diet (Viso & Marty 1993, Desvilettes et al. 1997).

The dominant omnivorous crustacean in this eelgrass bed was *Idotea baltica* (Gohse-Reimann, unpublished data), the most important benthic mesograzer in the Baltic Sea (Orav-Kotta & Kotta 2004). This isopod is known for its wide range of food sources including edaphic and epiphytic microalgae, filamentous algae, macroalgae, eelgrass and small invertebrate species (Franke & Janke 1998, Orav-Kotta & Kotta 2004). The importance of epiphytes and benthic diatoms for *I. baltica* was supported by the presumed high contribution of epiphyte and sand microflora carbon (mean 45% and 42%, respectively) to their diet and the high amounts of the fatty acids 16:1(n-7) and 20:5(n-3), characteristic for diatoms. The biomarker fatty acids for eelgrass were present in negligible concentrations. The same held true for *Gammarus oceanicus*, but this amphipod contained lower amounts of 16:1(n-7) and 20:5(n-3) compared to *I. baltica*. Stable carbon isotope values also indicated a lesser importance of epiphyte-derived carbon for this species (mean contribution 30%) and an increase in red algae-derived carbon (mean contribution 32%). This result is in good accordance with a previously reported diminished grazing impact of amphipods on epiphytes compared to isopod mesograzers by Duffy et al. (2001). Both mesograzers are purported to be crucial links between primary production and higher trophic levels in seagrass systems (Edgar & Shaw 1995).

The mean contribution of epiphyte carbon to the diet of the studied fish species was 33%, whereas sediment microflora and *D. sanguinea* contributed 47% and 20%, respectively. This is in good accordance with gut analyses, which revealed a diet consisting mostly of isopods, amphipods and copepods in slightly varying amounts, planktonic organisms being of minor importance (Bobsien 2006).

Primary producer biomass was dominated by eelgrass in June (54 g AFDW m^{-2}), followed by in descending order red algae (4.9 g AFDW m^{-2}), sand microflora (1.3 g AFDW m^{-2}), epiphytes (0.5 g AFDW m^{-2}) and phytoplankton (0.3 g AFDW m^{-2}) (unpublished data). In contrast, productivity rates measured in laboratory experiments under summer conditions showed that epiphytic algae (89.9 mg C m^{-2} d^{-1}) had a higher primary production rates than eelgrass (57.5 mg C m^{-2} d^{-1}). Previous studies have reported equal contributions of eelgrass and epiphytes to annual system carbon production (Borum & Wium-Andersen 1980, Thom 1990). Primary production of sand microflora ranged from 108 to 3312 mg C m^{-2} d^{-1} in the sediments of *Halodule wrightii* beds in Mississippi Sound, where irradiance at the sediment surface was 80 to 900 µmol m^{-2} s^{-1} over a yearly cycle (Daehnick et al. 1992). If I assume their lowest production values characterise the summer rates of the sand microflora in this

study, when irradiance reaching sediment is 100 µmol m^{-2} s^{-1}, benthic algal production (i.e. epiphyte and sand microflora) would be approximately three times greater than that of eelgrass.

Nutritional quality, digestibility and chemical defence of primary producers can additionally influence selective grazing. Benthic diatoms, dominating the epiphyton and sand microflora in this system, are considered as highly nutritious food sources (Klumpp et al. 1992, Créach et al. 1997), whereas eelgrass leaves were deficient in nitrogen compared to epiphytes (C:N = 23 and 12, respectively). Furthermore eelgrass contains lignin, which promotes structural rigidity to the leaves but increases the proportion of indigestible material. In addition, deterrent phenolic compounds present in eelgrass are known to impede herbivory (Harrison 1982). Therefore, it can be assumed that eelgrass in the Kiel Bight primarily provides habitat and shelter for consumers, whereas food is mainly supplied indirectly by providing space for attached epiphytes or partially via the detritus pathway.

The change of $\delta^{13}C$ values during early decomposition is marginal, but the content of characteristic fatty acid is strongly decreased (Kharlamenko et al. 2000). The small amounts of 18:3(n-3) and 18:4(n-3), found in consumers in this study, may have originated from eelgrass detritus. The minor importance of eelgrass as carbon source for sediment bacteria, found in four eelgrass beds in temperate regions (Boschker et al. 2000), supports the assumption that the role eelgrass as carbon source is negligible in the studied community. Kharlamenko et al. (2000), using the same techniques as in our study, concluded that eelgrass detritus contributed relevant amounts to consumer nutrition. Their study took place in a shallow, semi-enclosed bay with little water exchange. Eelgrass detritus may be more relevant under such hydrological conditions than at the more exposed study site.

Recent stable isotopic studies confirmed the importance of microscopic algae in seagrass food webs (Lepoint et al. 2000, Moncreiff & Sullivan 2001, Connolly et al. 2005). A strong dependence on seagrass carbon was only found in a tropical food web for herbivorous fish species (Marguillier et al. 1997). The results of this study corroborate not only the importance of epiphytic algae in eelgrass systems, but also the significance of the frequently neglected sand microflora as found in other coastal ecosystems like tidal flats, saltmarsh and mangrove communities (Newell et al. 1995, Créach et al. 1997, Kang et al. 2003).

Food web structure

The suspension-feeding blue mussel *Mytilus edulis* occupies a low trophic position in the studied food web and its main carbon source was phytoplankton. Wiedemeyer and Schwamborn (1996) reported likewise the predominance of phytoplankton as a carbon source for this mussel in the Kiel Fjord. Mussels are preyed upon by the starfish *Asterias rubens* (TL = 3.1) and the green crab *Carcinus maenas* (TL = 3.5). The starfish is mainly

specialised as a predator on blue mussels; however, $\delta^{13}C$ values suggested that starfish carbon was partially epiphyte-derived possibly through preying on periwinkles. The green crab has a wider utilisation of food items including small crustacean species and benthic annelids, which explains the higher trophic level of this predator.

Herbivores had the largest range of $\delta^{13}C$ values, varying from the small gastropod *Lacuna vincta* (-12.2‰) to the amphipod *Amphitoe rubricata* (-23.9‰). *L. vincta* contained small amounts of eelgrass biomarker fatty acids and its $\delta^{13}C$ value was close to that of epiphytes, indicating that its diet consisted mostly of epiphytes and to a smaller degree of eelgrass. This small gastropod is known to graze directly on the living tissue of macrophytes (Fredriksen 2003), and it can regulate epiphyte biomass in eelgrass communities (Nelson & Waaland 1997). However, this species was not abundant at our study side and therefore of minor importance in this eelgrass system. *A. rubricata* was found living in tubes on the red algae *D. sanguinea*. The mean $\delta^{13}C$ value of this amphipod was closest to the value for *D. sanguinea*, suggesting that this red alga provided not only shelter, but also supplied a relevant part of its diet. *A. rubricata* is found only rarely at our study site. This species is more common in the red algae zone below the eelgrass meadow. Another small gastropod, *R. membranacea* (-14.4‰), had a lower $\delta^{13}C$ value than *L. vincta.* This species may be regarded as an important herbivorous epiphyte grazer in this system. In late summer *R. membranacea* can be found in huge numbers on the eelgrass leaves. All other herbivores showed intermediate $\delta^{13}C$ values indicating a more general diet.

The observed continuous distribution of trophic positions supports the assumption that omnivory is a critical feature of this ecosystem. Therefore, my data agree with the hypothesis that consumer species in aquatic ecosystems make use of every possible trophic position in the food web (France et al. 1998). The lower $\delta^{15}N$ values of juvenile *I. baltica* and *G. oceanicus* compared to adults (Table 6.2) may suggest an ontogenetic change in feeding behaviour. Such feeding plasticity was also found in fish species in this eelgrass system (Bobsien 2006).

All sampled fish species are considered carnivores. The two-spotted goby (*Gobiusculus flavescens*), the sea stickleback (*Spinachia spinachia*), the straightnose pipefish (*Nerophis ophidion*) and the broad-nosed pipefish (*Syngnathus typhle*) are the most common fish species in this eelgrass system (Bobsien 2006). The relatively small range in $\delta^{13}C$ values supported the assumption that the studied fish species are generalists feeding on essentially the same crustacean prey species (Bobsien 2006). The dominant crustacean carnivore in this study was the green crab *C. maenas*, another generalist species (S. Gohse-Reimann, unpublished data). The other carnivorous crustacean species occurred only sporadically during the course of the year. The top predator in this study, juvenile cod (*Gadus morhua*), which preyed in large schools in eelgrass beds in the Kiel Bight 30 years ago (Worthmann

1975), are now greatly reduced in abundance. Only two individuals were caught in the course of the year.

Conclusions

This study emphasises the major importance of benthic microalgae (epiphytic and sediment-associated) for the carbon flux in eelgrass systems. The trophic contribution of the structuring macrophyte of this system (*Zostera marina*) appeared to be minimal and red algae and phytoplankton seemed to be of minor importance. The studied food web was characterised by a large proportion of generalist feeders in every group of consumers and by a high degree of omnivory. Overall, the combination of multiple stable isotope analyses and fatty acid analysis has been proven to be a useful tool in investigating two major approaches in the research of marine coastal ecosystems: the flux of carbon and food web structure.

7. General conclusions

Overall, this study revealed the complex interactions of grazing pressure, nutrient supply and diversity in seagrass ecosystems. The structure of plant assemblages is generally assumed to be controlled by consumers, resource supply and abiotic factors. Top-down and bottom-up forces can simultaneously regulate plant biomass, composition and diversity in marine communities (Worm et al. 2000, Hillebrand 2002, Brett & Goldman 1997), but the environmental and biological context influence their relative fortitude (Leibold et al. 1997, Lotze et al. 2001, Hillebrand & Kahlert 2002). Additionally, the proposed connection between consumer diversity and ecosystem processes in multi-trophic food webs has attracted the attention of ecologists recently (Duffy 2002, Fox 2004, Petchey et al. 2004, Gamfeldt et al. 2005).

This thesis showed that the functional differentiation of mesograzers plays an important role in structuring the relationship between eelgrass and epiphytic algae. The magnitude and direction of their effects can vary even between superficially similar mesograzer species. Therefore, the functional group concept should be used with caution as suggested by Duffy et al. (2001), because the assigning of species to a functional group according to size and diet does not necessarily imply functional redundancy. I found that mesograzer abundances can not be neglected in the assessment of mesograzer impact in eelgrass systems. Natural densities of the four studied common mesograzers had different relationships with epiphyte and eelgrass productivity. The impact of both gastropods was linear; the effect of *Idotea* exponential and *Gammarus* density had no significant relationship with primary producer productivity at all.

The fact of seasonally and spatially varying mesograzer abundances (Thom et al. 1995) emphasises the importance of density-dependent effects.

I found, that the effects of mesograzers on epiphytes were not altogether negative. Gastropods enhanced the nutrient content of epiphytes and the photosynthetic capacity of epiphytes increased with growing densities of *Idotea*, *Littorina* and *Rissoa*. This is the first experimental evidence in marine benthic systems that the impact of grazers may promote algal biomass-specific productivity as proposed by McCormick and Stevenson (1991).

Positive effects of grazers on epiphyte diversity are postulated under the framework of the “intermediate disturbance hypothesis” (Connell 1978). In this study, only the two gastropod species complied with the prediction that intermediate algal mortality results in the highest diversity, caused by the prevention of competitive exclusion (Huston 1979). The results strengthen the relevance of grazer selectivity and algal composition in controlling the impact of mesograzers on epiphyte diversity.

Furthermore the relevance of both top-down and bottom-up effects in controlling epiphyte biomass and productivity could be demonstrated. Nutrient supply and grazing pressure

simultaneously influenced the primary producers in eelgrass beds as found in other aquatic communities (Worm et al. 2000, Hillebrand 2002, Brett & Goldman 1997) and the effects were highly interactive for epiphytes. The results of the two-year field survey suggest that the relative fortitude of both top-down and bottom-up forces on epiphytes varied seasonally. In spring and early summer low nutrient supply and grazers at low densities appear to regulate epiphyte biomass equally; in summer strong top-down effects reduced epiphyte accumulation efficiently, and in autumn high nutrient concentrations significantly increased epiphyte biomass despite constant high grazer densities.

The effect of species richness has been the focus of much ecological research (see Loreau et al. 2001 for overview). With few exceptions, however, studies have concentrated on grassland plants and aquatic microbial systems. It is not until recently that diversity effects in multitrophic systems have been analysed (Duffy et al. 2003, 2005, Petchey et al. 2004, Gamfeldt et al 2005). I showed that increasing mesograzer diversity resulted in an enhanced grazing efficiency and a reduction in epiphyte biomass. Similar patterns were found in plankton and pond ecosystems (Gamfeldt et al 2005, Wojdak 2005). Furthermore a cascading diversity effect from the consumer to the prey level was demonstrated. Both effects were only found in the epiphyte assemblage, no significant impact of mesograzer diversity was found for the microphytobenthos. Consumer diversity effects were the result of differential resource use (the complementarity effect). These findings perfectly match predictions from a recent model, which postulates that consumers had to be different in their food intake to cause positive diversity effects (Thébault & Loureau 2003).

However, the effects of mesograzer diversity disappeared under high nutrient supply in the course of three weeks. My experiment suggests that the number of consumer species in a system can have significant effects on primary producer assemblages, and that these effects can depend on the ecological context.

In summary, my thesis provides a detailed overview on the complicated interrelationship in mesograzer-epiphyte-host systems.

8. Appendix

Effects of acidification in multiple stable isotope analyses

Abstract

The effect of in situ acidification on the stable isotope ratios of carbon and nitrogen was tested in several mesograzers living in an eelgrass system. Dried and ground samples of individuals were weighted in silver cups and treated in situ with 10% HCl. Control samples were measured without acidification. This treatment to remove inorganic carbon significantly decreased the $\delta^{13}C$ values up to 1.77‰. The $\delta^{15}N$ values were not affected by this method of acidification. In contrast to the acid washing method the tested procedure seems suitable to remove inorganic carbon in small invertebrate species.

Introduction

Stable isotope analysis has been proven to be an important tool to understand food web dynamics in aquatic ecosystems. Stable carbon isotopic signatures are commonly used to recognize and quantify potential food sources (Stephenson et al. 1986, Moncreiff & Sullivan 2001, Fredriksen 2003). However, samples, which contain nondietary carbon with deviating $\delta^{13}C$ values, can cause problems in this methodological approach. Especially, sediment samples, carbonate encrusted algae and molluscs and crustacean, which are too small to dissect muscle material, can comprise relevant amounts of inorganic carbon. The shell of molluscs is formed by calcium carbonate and the basically chitinous body wall of crustaceans is usually reinforced with calcium carbonate to generate a rigid exoskeleton. Earlier stable isotope studies used different variations of the acid washing method to remove nondietary carbon (Fry et al. 1982, Peterson & Howard 1987, Sullivan & Moncreiff 1990). Bunn (1995) found, that this method significantly influences the $\delta^{15}N$ values of shrimp samples. Nevertheless this method is used in many recent benthic studies with several variations concerning acid concentration and duration of the bathing in acid (Marguillier et al. 1997, Kharlamenko et al. 2001, Moncreiff & Sullivan 2001, Fredriksen 2003, Kang et al. 2003). Some studies totally dispense with acidification (Fourqurean et al. 1997, Jennings et al. 1997, Loneragan et al. 1997, Connolly et al. 1995) and a few studies used an *in situ* acidification procedure (Deegan & Garritt 1997, Herman et al. 2000). Here, the pulverized samples were acidified with a small amount of relative high concentrated HCL. To investigate the effect of this method on stable isotope ratios I analysed small grazing organisms collected in an eelgrass bed in the Kiel Fjord, Western Baltic Sea.

Method

The study included eight species: The isopod *Idotea baltica*, analysed were adult (15mm) and juvenile specimen (3-5mm), the amphipods *Gammarus oceanicus* (10mm) and *Amphithoe rubricata* (13mm), the shrimp *Praunus flexuosus* (25mm) and *Crangon crangon* (30-35mm) and gastropods *Rissoa membranacea* (5mm) and *Lacuna vincta* (5mm). All animals were kept alive overnight in filtered sea water to clear their guts (Hobson & Welch 1992), rinsed with distilled water and dried to constant weight (60 °C, 48 h). Ten individuals respectively were ground with an agate mortar and pestle as fine as possible and then stored in clean airtight plastic vials till further processing. In the case of the small gastropods, ten individuals were pooled in order to obtain sufficient material for analysis. Two splits of each sample (0.4-0.8 mg depending on the species) were weighted into silver cups. One sample was acidified with 0.2 µl 10% HCL, the other served as control. The samples were dried for one hour at 60 °C and then another 0.2 µl 10% HCl was added to confirm the complete removal of inorganic carbon. No effervescence indicates that the procedure is accomplished (Nieuvenhuize et al. 1994). The samples are dried again for 12 hours at 60 °C to remove hydrochloric acids to avoid contamination of the CN-analyser. Directly afterwards, the cups are closed, compacted and analysed.

All samples were combusted in a CN-analyser (Fisons, 1500N) connected to a Finnigan Delta plus mass spectrometer. $\delta\,^{15}N$ and $\delta\,^{13}C$ ratios were calculated as

$$\delta X\ (‰) = [(R_{sample}/R_{standard})-1] \times 1000$$

were X = ^{15}N or ^{13}C and R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Pure N_2 and CO_2 gas were used as primary standard and calibrated against IAEA reference standards (N1, N2, N3, NBS22 and USGS24). Acetanilide was used as internal standard after every sixth sample. The overall analytical precision was ±0.1‰ for $\delta\,^{15}N$ and $\delta\,^{13}C$.

The differences between no acid and acid treated samples were tested with paired t-tests for each species.

Results and discussion

All species showed a decrease in δ ^{13}C values (Fig. 1) ranging from 0.12‰ in *Praunus flexuosus* to 1.77‰ in *Amphitoe rubricata*. The difference between no acid and acid treatment was not significant for the two larger species *Praunus flexuosus* and *Crangon crangon* (Table 1). The acid treatment had no significant effect on the δ ^{15}N values (Fig. 1). No significant difference in variation between individuals in the acid treated samples was found for δ ^{13}C values. The variation in δ ^{15}N values increased from mean 4.0% to 5.7%, but the change was not significant (Table 1).

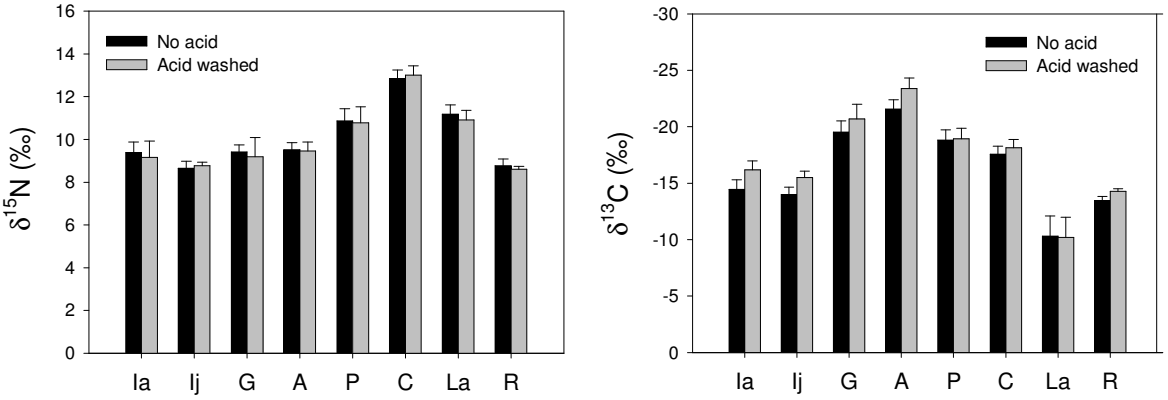

Fig. 1. Mean stable isotope composition (±SD) of δ ^{15}N and δ ^{13}C ratios in no acid and acid treatments (Ia = adult *I .baltica*, Ij = juvenile *I. baltica*, G = *G. oceanicus*, A = *A. rubricata*, P = *P. flexuosus*, C = *C. crangon*, L = *L. vincta* and R = *R. membranacea*)

Bunn's study (1995) on the effects of acid washing resulted in statistically and ecologically important changes in δ ^{15}N values. Additionally the variation among individuals broadened in δ ^{13}C values and δ ^{15}N values leading to a decrease in statistical power for testing differences between samples. Goering et al. (1990) also found changes in HCL treated samples and suggested that the different loss of compounds containing nitrogen caused these results. These results implicated that different organic nitrogen compounds had varying δ ^{15}N values. The presumed leaching of small molecules while rinsing the samples is supported by the fact that grounding increased the effect of acid washing (Bunn 1995).

The in *situ* acidification method evades these problems and can be efficiently applied to samples were removal of nondietary carbon is absolutely necessary. In larger

Table 1. Mean stable isotope ratios (±SD) of crustacean and the results of paired t-test between no acid and acid washed samples

	no acid	acid washed		
species	$\delta^{15}N$	$\delta^{15}N$	t	P
I. baltica	9.38±0.48	9.15±0.77	0.815	0.426
I. baltica, juv.	8.65±0.33	8.78±0.15	-1.081	0.294
G. oceanicus	9.41±0.33	9.19±0.9	0.730	0.475
A. rubricata	9.52±0.31	9.46±0.42	0.406	0.690
P. flexuosus	10.86±0.57	10.78±0.74	0.270	0.790
C. crangon	12.84±0.41	13.01±0.43	-0.861	0.401
L. vincta	11.18±0.44	10.91±0.45	0.730	0.506
R. membranacea	8.77±0.30	8.61±0.14	0.875	0.431
	no acid	acid washed		
species	$\delta^{13}C$	$\delta^{13}C$	t	P
I.baltica	-14.44±0.87	-16.17±0.80	4.63	<0.001
I.baltica, juv.	-13.98±0.66	-15.48±0.60	5.31	<0.001
G.oceanicus	-19.52±1.00	-20.70±1.30	2.263	0.036
A.rubricata	-21.57±0.84	-23.34±0.92	4.635	<0.001
P.flexuosus	-18.82±0.90	-18.94±0.91	0.287	0.777
C.crangon	-17.56±0.70	-18.14±0.73	1.827	0.084
L. vincta	-10.32±1.77	-10.19±1.78	-0.089	0.933
R. membranacea	-13.46±0.36	-14.27±0.24	3.227	0.032

animals it is possible to discard parts containing calcium carbonate like shells in molluscs and exoskeletons in crustacean, but small species are usually crushed in
total. These samples can contain significant amount of nondietary carbon that can influence the results on potential food sources. Furthermore, the nitrogen in the exoskeleton originates from the diet and may be relevant in determining consumer food sources.

In contrast to the acid washing method our results suggests, that the *in situ* acidification method can be used to eliminate nondietary carbon without significantly influencing the chemical composition of the studied samples.

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Acknowledgement

I would like to thank Prof. Dr. Ulrich Sommer, who supervised this thesis and gave me the opportunity to resume my research after quite a long break.

For most valuable criticism and comments on this thesis, I want to thank Nicole Abele-Malzahn, Sabine Flöder, Rolf Karez and Ilka Peeken.

Nutrient, C/N and stable isotope analyses were performed by Thomas Hansen.

Nicole and Dani provided the data on microphytobenthos (Chapter 5) and fatty acids (Chapter 6),

I'm grateful to the crew of the "Polarfuchs", who supported me during my field survey.

Furthermore, I want to thank numerous scuba divers, who provided large amounts of eelgrass.

Especially, I want to thank Sandra Gohse-Reimann, who worked in the same project and Cordula Stielau, who provided much useful information and was always a amiable room mate.

The experiment in Chapter 5 was planned and carried out in cooperation with Nicole and Sandra. Special thanks to Nicole, who always supported me in the somewhat tedious preparation of the manuscript.

I would like to thank Sabine and Dani for their support during the sampling for the stable isotope and fatty acid analyses.

Financial support was provided by the DFG (SO 145/20).

Als letztes möchte ich meinen Eltern danken, die mich immer unterstützt haben.

Erklärung

Hiermit erkläre ich, daß ich die vorliegende Dissertation, abgesehen von der Beratung meines Betreuers, selbstständig angefertigt habe und daß sie nach Inhalt und Form meine eigene Arbeit ist. Sie wurde an keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Die Promotion soll im Fach Biologische Meereskunde erfolgen. Hiermit erkläre ich, daß ich Zuhörer bei meiner Promotionsprüfung zu lasse.

Sybill Jaschinski