Alfred-Wegener-Institut für Polar- und Meeresforschung Bremerhaven

Untersuchung zur Diversität und Funktion benthischer Mikroalgen und Protozoen im Nahrungsnetz mariner und limnischer Sedimente

Investigation on the diversity and function of microphytes and protists in the food web of marine and limnic sediments

DISSERTATION

zur
Erlangung des akademischen Grades
eines Doktors der Naturwissenschaften

(Dr. rer. nat.)

am Fachbereich Biologie/Chemie der
Universität Bremen

vorgelegt von

Karen Stumm

Bremen 2006

1. Gutachter: Prof. Dr. Victor Smetacek, Alfred-Wegener-Institut für Polar und Meeresforschung, Bremerhaven
2. Gutachter: Prof. Dr. Ulrich Bathmann, Alfred-Wegener-Institut für Polar und Meeresforschung, Bremerhaven

Den Sand, den Schlick, den nennt man Benthos,
darin ist ganz verblüffend viel los.
Der Phototroph' genießt das Licht,
derweil ein Krebschen drüberkriecht.
Es freut sich sehr der Flagellat
auf den Diatomeen-Salat.
Das Schneckchen schleicht auf weichen Sohlen,
um sich was Leck'res reinzuholen.
Der Wattwurm, allgemein beliebt
sich sehr viel Sand von vorn einschiebt.
Siebt sich die feinen Sachen aus
und drückt den Rest nach hinten raus.
Wie friedlich doch das Benthos ist,
auch wenn den And'ren man gern frisst.

Ein anonymer Wattpoet.

Table of contents

Zusammenfassung

Gegenstand dieser Doktorarbeit waren Untersuchungen zur Vertiefung des Verständnisses der Strukturen und der Entwicklungsdynamik mikrobieller Gemeinschaften, vor allem von Protisten. Die wichtigste Erkenntnis der letzten Jahren über Protisten war, dass sie gegenüber bisherigen Annahmen eine viel größere Rolle beispielsweise im Kohlenstoff- und Energiekreislauf in der Natur spielen.

Es wurden mehrere Studien zur saisonalen Dynamik und geographischen Verteilung von Mitgliedern der „kleinen benthischen Gemeinschaft“ in Süß- und Salzwasser-Systemen im gemäßigten und arktischen Klima durchgeführt. Eines der Ziele war die Entwicklung eines allgemeinen Modells der Wechselbeziehungen und der wirkenden Kontrollfaktoren in benthischen Gemeinschaften. Zur Durchführung der Analysen wurden Proben aus den oberen 3 mm von Oberflächensedimenten im Flachwasser von Seen und Meeresgebieten genommen. Die Abundanzen und Biomassen von Bakterien, Cyanobakterien, Diatomeen sowie von phototrophen und heterotrophen Nanoflagellaten wurden mit Hilfe der Epifluoreszens-mikroskopie ausgezählt. Ciliatenarten und Meiofauna wurden mit Hilfe der QPS (quantitative protagol staining) Färbetechnik lichtmikroskopisch bestimmt und gezählt. Wasser- und Lufttemperatur, Salzgehalt des Wassers, Wassergehalt des Sediments, Korngrößenverteilung, Gehalt von gesamtem und organischem Kohlenstoff und Stickstoff sowie Gehalt an Chlorophyll-*a* wurden gemessen, um den Einfluß dieser Umweltfaktoren auf die Organismengemeinschaft und auf deren gesamte Biomasse abzuschätzen.

Die Untersuchungen im feinsandigen Watt bei Dorum (Deutschland) ergaben, dass die bentische Gemeinschaft einem saisonalen Zyklus folgte. Im Winter und Frühling wurde sie im wesentlichen durch bottom-up Effekte kontrolliert. Ein zunehmender top-down Einfluss, vermutlich vor allem durch den Fraß von Meiofauna, wurde zum Sommer hin ermittelt. Im Herbst nahmen diese top-down Effekte wieder ab, begleitet von einer Erholung der Abundanzen microphytobenthischer Organismen. Untersuchungen von benthischen Gemeinschaften in küstennahen Sedimenten von Seen und marinen Regionen wurden in Deutschland, Nord Amerika (USA; Pennsylvania, New Jersey) und Grönland durchgeführt. Die Analysen ergaben beträchtliche Unterschiede zwischen den Biomassen der einzelnen Mitglieder der Gemeinschaften sowie der Zusammensetzung und Vielfalt der Ciliaten-Arten. Weiterhin wurde bei der Untersuchung der Ciliaten-Arten eine große Variabilität von Fraßtypen zwischen den verschiedenen Gemeinschaften ermittelt, herbivore Arten waren jedoch in allen Untersuchungsgebieten am häufigsten. Die ermittelten Unterschiede bei Biomassen und Abundanz der Organismen sowie bei der Artenvielfalt der Ciliaten an den

verschiedenen untersuchten Standorten konnten nicht auf die Unterschiede der gemessenen Umweltfaktoren wie Salzgehalt des Wassers, Klima oder Kohlenstoffgehalt des Sediments zurückgeführt werden. In statistischen Analysen wurde der Gehalt von Chlorophyll-*a* als einziger Faktor ermittelt, der die Biomassen und Abundanz der Organismen der benthischen Gemeinschaft beeinflusst hatte. Die Lieferanten von Chlorophyll-*a* konnten jedoch nicht eindeutig ausgemacht werden. Es schien sich die Hypothese von Manini et al. (2003) zu bewahrheiten, nach der die Zusammensetzung und damit die Verfügbarkeit von Kohlenstoff für die Organismen in mikrobiellen Gemeinschafen im küstennahen Flachwasser von großer Bedeutung ist. In einer Reihe von Laborexperimenten wurde der Einfluss des Fehlens aller Makrograzer, sowie der Anwesenheit einzelner kleiner Makrograzer wie *Hydrobia ulvae*, *Corophium* sp., Chironomiden Larven oder Polychaeten auf die benthischen mikrobiellen Gemeinschaften an den verschiedenen Standorten analysiert. In einem Langzeit Feldexperiment auf Sylt wurde der Einfluss des Wattwurms *Arenicola marina* auf die strukturelle Zusammensetzung mikrobieller Gemeinschaften untersucht. Die Ergebnisse dieser Experimente zeigten, dass kleine Makrofauna-Organismen eher positiv stimulierende Effekte auf die mikrobielle Gemeinschaft ausüben als negative durch Fraß. Darüber hinaus legten die Ergebnisse des Langzeitausschlusses von *A. marina* nahe, dass auf lange Sicht die Rolle dieses Grazers von anderen Grazern übernommen wurde.

Quervergleiche zwischen den Ergebnissen aus dieser Doktorarbeit und Literaturdaten führen zu dem Schluss, dass die trophischen Ebenen in mikrobiellen Gemeinschaften im Feinsand mariner und limnischer Systeme fließend sind und die meisten Organismen eher opportunistisch das fressen, was am meisten vorhanden ist, als sich spezialisiert von bestimmten Arten zu ernähren. Die Dominanz des omnivoren Fraßtypus sowie der Fraß von Detritus, stimulierende Effekte durch Bioturbation und Rückkopplungsmechanismen zwischen verschiedenen Organismen erzeugen ein eng verwobenes Nahrungsnetz. Die grundlegenden Eigenschaften von Mikroben, wie große Populationen, sehr kurze Generationszeiten mit hoher Verbreitungskapazität, tragen zur Bildung von Systemen mit schnell wechselnden Artzusammensetzungen bei. Die Resultate und Schlussfolgerungen dieser Doktorarbeit bestätigen die Gültigkeit des allgemeinen Models von Fretwell (1977) auch für benthische mikrobielle Nahrungsnetze in Flachwasserbereichen. Danach formen top-down Effekte die trophische Struktur. Die fundamentalen Parameter einer Gemeinschaft wie beispielsweise deren gesamte Biomasse, Abundanz und Produktion werden jedoch von den grundlegenden Eigenschaften des Ökosystems, wie Nährstoff- und Kohlenstoffverfügbarkeit, Temperatur und Lichtintensität, also von den bottom-up Effekten bestimmt.

Summary

This thesis aimed at achieving a deeper understanding of the dynamics and structure of microbenthic communities with a focus on protists. The most important discovery concerning protozoa in recent years is the fact that they play a much more important role in for example carbon and energy flow in nature than previously believed. In this thesis several studies were presented which focus on the seasonal dynamics and the spatial distribution of components of the small benthic community in marine and freshwater environments in temperate and arctic climates and which aim at establishing a generalised model of relationships and controlling factors in small benthic food webs. Samples of the upper 3 mm of the surface of soft sediment were collected in coastal freshwater and marine sites in Germany, USA and North East Greenland. The abundance and biomass of bacteria, cyanobacteria, diatoms and nanoflagellates (phototrophic and heterotrophic) were enumerated by means of epifluorescence microscopy. Ciliate species and meiofauna were enumerated with the help of light microscopy after QPS (quantitative protagol staining). Environmental data, such as temperature, salinity, sediment water content, grain size distribution, total and organic carbon- and nitrogen content as well as chlorophyll-*a* values were determined in order to study their influence on the community structures and total biomass.

The investigation of a habitat in a mudflat in Dorum (Germany) revealed that the benthic microbial food web varied with the season with a primacy of bottom-up control during winter and spring, an increasing influence of top-down forces mainly by meiofauna grazing towards summer, decreasing again towards autumn, accompanied by a recovery of microphytobenthos.

Investigations of soft sediment communities in different climate and geographic positions of freshwater and marine sites in Germany, North America (USA; Pennsylvania, New Jersey) and in North East Greenland were carried out. The results revealed considerable differences in the biomass of the small benthic components as well as in ciliate species composition and richness. The study of the ciliate species composition also exhibited a high variability of feeding types between the investigated sites, but the herbivorous feeding type seemed to be dominant in all sediments. Differences in total microbenthic biomass, abundance and ciliate species richness could not be attributed to the differences measured in carbon content, salinity or climatic parameters. The amount of chlorophyll-*a* was the only factor influencing the small benthic biomass. The sources of chlorophyll-*a* remained unclear. The hypothesis of Manini et al. (2003) stating that the composition of available carbon has a strong influence on the

structure and biomass of microbial communities in shallow sediments seemed to hold true for the investigated sites.

In a series of laboratory experiments the influence of the absence of all macrograzers as well as the presence of a single common abundant small macrograzer such as *Hydrobia ulvae*, *Corophium* sp. as well as Chironomid larvae and polychaets on the components of the microbenthic communities in sediments from all study sites were investigated. The structuring influence of *Arenicola marina* on components of the small food web was investigated in a long term field experiment on the island of Sylt. The results of the laboratory experiments and those of the field study revealed that the small macrofauna tended to have positive stimulating effects on the microbial community by bioengineering, rather than negative ones by grazing. The exclusion of *Arenicola marina* in a long term experiment revealed the fact that the role of this grazer was adopted by other grazers.

The results of this thesis and literature data led to the conclusion that in small communities of soft sediments the trophic levels are blurred and most species rather feed opportunistically on what is most available. The dominance of the omnivourous feeding type, detritus feeding, stimulating bioengineering effects and feed back mechanisms compose a highly networked food web. The fundamental attributes of microbes such as large absolute population sizes, short generation times and high dispersal capabilities, form a system with rapid changes in species composition.

The outcome of this thesis verified the model of Fretwell (1977) for benthic microbial food webs in coastal regions, predicting that top-down forces form the trophic structure, but the bottom-up attributes of the ecosystems, such as nutrients availability, temperature and light, determine the fundament of the community as total biomass, abundance and production.

General introduction

Protists are unicellular organisms of several unrelated eukaryotic linkages (Fenchel 1990). Cell sizes span from 2 µm to 20 mm covering a range of 1 : 10.000, compared to the much narrower range of body sizes of mammals of 1 : 750 (from shrew to blue whale, Fenchel 1990). Protists are found in nearly all systems where liquid water is available: terrestrial systems, marine and freshwater systems and even in brine channels of sea ice. The generation times of protists are short (3 h to 50 d) which enables them to make rapid use of resources. However, populations also tend to collapse as rapidly as they develop (Hausmann & Hülsmann 1996; Findlay & Watling 1998). Many protist species living in habitats with a short presence of water are able to escape temporarily by encystment (cysts, spores and sclerotia; Sleigh 1973). For many protists salinity seems to be a matter of acclimation and many species can be found in different water types from brackish to marine and in freshwater (Fenchel 1969; Sleigh 1973; Fenchel 1987; Patterson & Larsen 1991). The tolerable temperature for the life of protozoa seems also to be a matter of acclimation. The same species can be found in cold water at below 5°C and in hot springs up to 40-50°C. However, the lowest temperature for active life is defined by the freezing point of the surrounding water. The upper limit of even extreme thermophilic life is presumed to be at 150°C, when the thermal break down of chemical compounds (in proteins and DNA) can no longer be inhibited. Protists exhibit multifarious feeding strategies comprising photoautotrophy, phototrophy, mixotrophy and heterotrophy (herbivorous, carnivorous, detrivorous and omnivorous) and their abundance can reach sizes of several orders of magnitude (e.g. in sediment up to 10^6 cells ml^{-1}). The most important discovery concerning protozoa in recent years is the fact that they play a much more important role e.g. in carbon and energy flow in nature, than previously believed. This study aimed at investigating protists in benthic shallow soft sediments and their role within the small food web of marine and freshwater systems.

Characteristics of benthic habitats

Benthos, from the Greek word “to benthos” means “depth and thicket”, describes all organisms on underwater grounds including hard and soft bottoms. Oceanic benthic habitats cover around 361 x 10^6 km^2 of the earth´s surface. If the widespread aquatic areas of groundwater, rivers and lakes are included, 72 % of the earth´s surface is covered by benthic habitats. Large parts of the underwater grounds are covered by sediments and nearly all are inhabited by organisms.

Shallow littoral regions of freshwater and marine systems, the subject of this investigation, with water depths of less than 1 km, cover around 27 x 10^6 km^2 and exhibit one of the most productive ecosystems on earth. Their productivity by far exceeds that of the open oceans (Meyer-Reil & Köster 1993; Barnes & Hughes 1999).

In general, sediments consist of three primary components: (1) particulated mineral matter, including clay, carbonates, and nonclay silicates, (2) inorganic components of biogenic origin (silicon oxide as from of diatom frustules, calcium carbonate from bones, shells), (3) allochthonous and autochtonous organic matter in various stages of decomposition. The mineral matter and inorganic components originate primarily from eroded terrestrial material and biomineralized matter. The biotic environment determines the quality and quantity of the allochthonous organic matter. In marine habitats the majority of the organic material originates from labile phytoplankton whereas in freshwater habitats the organic supply arises mainly from plant material (Capone & Kiene 1988). In near coastal regions where the transition between land and sea is fluent (marshland, saltmarshes) as well as in areas where freshwater flows into the ocean, the input of organic matter originates from a mixture of plant and planktonic material, whereby the proportion of plankton increases with increasing distance to the coast (Capone & Kiene 1988).

The composition of light, temperature, wind and water current form a diverse variety of coastal shallow soft sediment habitats. The exposure of the coast to wind and wave action as well as the slope of the ground determine the wave shock, the water current and the therewith connected particle size distribution (Knox 2000). The distribution of particle sizes is responsible for the characteristic steepness of chemical gradients in sediments, for example the thickness of the oxic layer. In shallow coastal soft sediments the oxic layer reaches thicknesses from just a few millimetres to centimetres (Gray 1981). These sediment characteristics (grain size distribution, thickness of the oxic layer, penetration depth of light) are known to play a prominent role in structuring the composition of soft sediment communities (Fenchel 1969; Higgins & Thiel 1988; Jansson 1967). The organisms can also influence their environment e.g. by physical activity (bioturbaion, absorbtion, excretion) and so for instance derange the stacked chemical layer. A prominent example of a near shore bioengineer is the lugworm *Arenicola marina*. By burrowing down into the anoxic region and flushing their tubes with fresh water the worms form an oxic environment within the mainly anoxic surrounding which in turn is colonized by oxic meioorganisms and microorganisms (Reise 1985).

Intertidal mudflat

The soft sediment of intertidal mudflats constitutes a highly unstable environment, due to the continuously changing water level, currents and temporary exposure of the sediment to the air. Sand grains move permanently and the temperature and salinity change due to the tides and weather. The temperature in remaining water puddles and of sediment exposed to the air approaches the air temperature, depending on exposure time. The temperature of the North Sea for instance ranges from 0°C during winter to around 20°C during summer. Sediment temperature of exposed sediment can reach 35°C and even more by direct insolation (Reineck 1983). Salinity fluctuation can reach 25 PSU (Reineck 1983). A reduction of salinity may be caused by dilution by freshwater input from rain and rivers as well as by wind induced displacements of the water tongue of a nearby river (Wolff 1983). An increase of salinity may be caused by evaporation due to insolation or freezing when the salt remains in solution. Salinity fluctuations can create serious stress to organisms due to osmotic pressure variances. Organisms living in such fluctuating environments have been forced to evolve special adaptation strategies.

The small benthic community

The small benthic community of a soft sediment is composed of organisms belonging to various taxonomic groups (as viruses, bacteria, algae, fungi, flagellates, protozoa, arthropod, nematode, molluscs, kinorynchia, gastrotrichia) of different size classes (nano- and microbenthos <42µm; meiobenthos 42 µm - 1 mm; macrobenthos >1 mm). Depending on body size and morphological equipment the organisms of soft sediments live attached to the sand grains, in between the water filled space (intersticial), on the top of sediment (epibenthic) or burrowed in the sediment (infauna; Fig. 1).

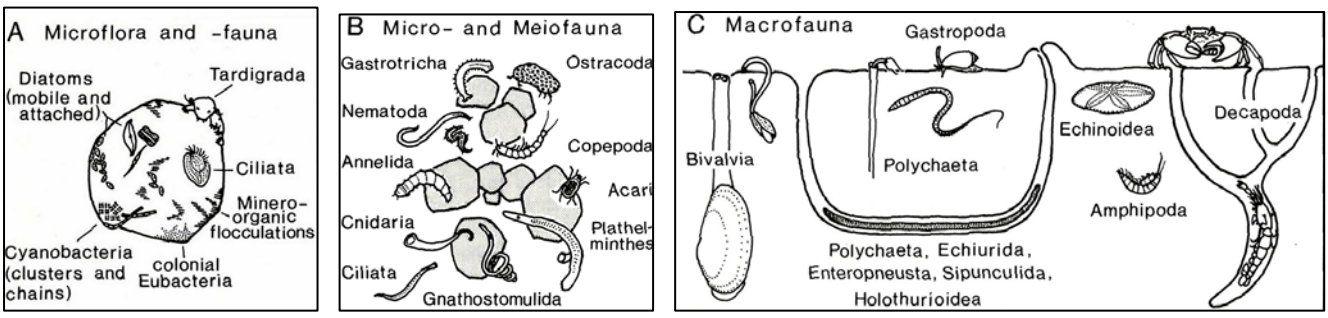


Fig. 1: Representative organisms of the small community in tidal flats. (A) at the scale of a sand grain, (B) at the interstices of sand (C) and at the sediment (from Reise 1985).

The maximum abundance of microalgae, flagellates, ciliates and meiofauna was found in the oxic layer of littoral sediments of medium grain size (Fenchel 1969; Higgins & Thiel 1988; Alongi 1991; Hondeveld, Nieuwland, et al. 1994; Berninger & Epstein 1995; Böttcher et al. 2000). The abundance of protists reaches values of 100 cells cm^{-3} to 10^6 cells cm^{-3} in freshwater and marine sediments (Patterson & Larsen 1991). Nematodes, copepods, plathelminths, polychaetes and ostracods are the major taxa of the meiofauna (McLusky & McIntyre 1988). The benthic macrofauna in soft sediments is usually dominated by crustaceans, molluscs and polychaetes.

The Food web

The food web describes the sequence of organisms within an ecosystem transferring the energy of food from producers to consumers. Charles Elton (1927) first formulated the "food chain" concept at the beginning of the 20th century. He established the pyramid of biomass for an terrestrial system, illustrating the decreasing numbers of individuals with ascending position within the food chain. The steps of a food chain are categorized in so called trophic levels (Greek Τροφη = feeding). Since Elton (1927) first noted that the food chain length (~ 3 to 6) is different in natural systems, ecologists are still debating many explanatory hypotheses (such as productivity hypothesis, ecosystem-size hypothesis, productive-space hypothesis; Post et al. 2000). However, the concept of straight food chains, where just one organism feeds on one other kind of organism, is valid only for a small part of organism relationships. Many organisms feed on different kinds of food. Furthermore direct predatory feeding is not the only way of energy transfer between trophic levels. The use of waste products (as excrests, dead cells) represents another mechanism of matter and energy transfer. The use of waste products makes chemical energy, contained within detrital organic carbon (as dead cells, faeces, exsudates) available to the biota (Wetzel 2001). The realistic trophic structure of communities is therefore much more complex, rather a net-like food web than a system of straight chains of transfer.

The microbial food web

Since the early 1970s new measurement techniques have revealed that microbial organisms are much more abundant and the high microbial biomass and productivity must play a more important role in energy and carbon flow than previously believed. Pomeroy (1974) and later Azam et al. (1983) formulated the concept of the microbial loop for plankton systems (Fig.2). The function of bacteria therewith changed from being mainly remineralizers to a component leading back dissolved organic carbon (DOC) to the larger-sized microorganisms, in the form

of particulated organic carbon (POC). Protists and ciliates, representing the larger-sized heterotrophic microorganisms are grazed by metazoans and thus act as a trophic link between the microbial food web and the larger metazoan food web.

Trophic interactions including the microbial loop concept in a pelagic food web

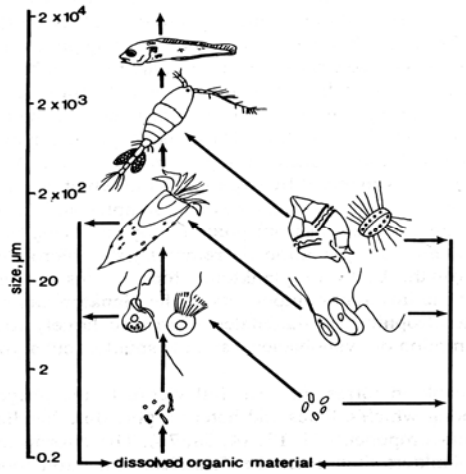


Fig. 2: Schematic trophic interactions of a pelagic food web, including the concept of Azam et al. (1983) for the microbial loop (from Fenchel 1988).

More recently it has been established that the model of the plankton microbial loop should be considered as one of the components of a larger plankton microbial food web including procaryotic and eucaryotic unicellular organisms both auto- and heterotrophic (Sherr & Sherr 1988; Caron & Finlay 1993). Sherr & Sherr (1988) revise the simplified plankton food web model and contribute more detailed relationships between the microbial components by including the various different feeding types of different ciliate and flagellate species (Fig. 3).

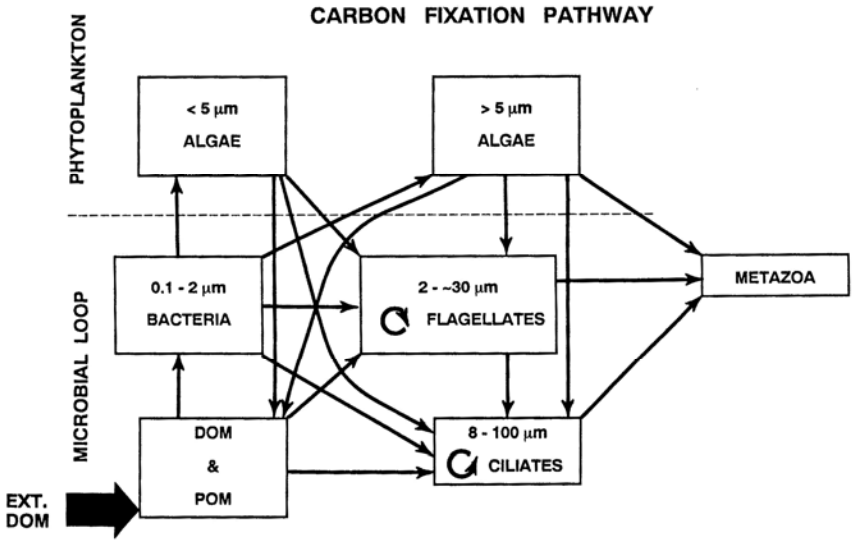


Fig. 3: Trophic interactions within the microbial food web, which is separated here into phytoplankton and "microbial loop" components. Note the many direct links among heterotrophic and autotrophic microbes, as well as ingestion of bacteria by mixotrophic algae. The curved arrows in the flagellate and ciliate compartments indicate the further predator-prey interactions within these broad classes of organisms. (from Sherr & Sherr 1988).

Studies on the function of protists within the benthic microbial food web in soft sediment systems are rare. In ecological microbenthic food web studies on benthic communities, flagellates and ciliates were regarded as homogeneous groups of either heterotrophs or phototrophs and occasionally mixotrophs. However, concerning their feeding interactions and thus their functional ecological role, these groups are in no way homogenous. Kuipers et al. (1981) suggest that the small benthic community, including nano- and microorganisms and small macrofauna, can be regarded as a complete functional unit. Most studies in benthic sediments deal only with selected parts of the whole community, for instance feeding interactions between meiofauna, algae and bacteria on one hand or flagellates and bacteria on the other hand. Hence remarkably little is known about the complex feeding interactions within the small community under consideration of all components and the factors determining the relative importance of direct effects (grazing) and indirect effects (like stimulation or repression).

Control mechanisms

Many studies dealing with communities address the question of which factors predominantly determine the composition, the number of organisms and the structure of trophic levels in a community. Both top-down and bottom-up control mechanisms are described in ecological studies. Impacts on a community starting from the top trophic level of a food web and controlling the abundance of organisms on lower levels are called top-down control effects. Grazing of top predators and resulting cascading effects are the key top-down control effects. On the other hand mechanisms, which start from the bottom of a food web, for instance environmental attributes (such as temperature, grain size, oxygen availability and the amount of nutrients) or the amount of primary production are called bottom-up control effects. The discussion whether resources (bottom-up mechanisms) or predators (top-down mechanisms) perform the primary control of the community (trophic structure, biomass) is still under debate. Power (1992) suggests that a clear top-down control can be expected when short-lived primary producers quickly balance predation by rapid growth and when a single species or guild has direct effects on the trophic level beneath. Manini et al. (2003) found that the amount of available organic matter plays a primary role in benthic microbial loop functioning in coastal lagoons constituting a typical bottom-up control. However, these direct effects (such as grazing, predation or prey availability) acting as clear top-down or bottom-up mechanisms are not the only ones affecting the dynamics of a community. Organisms can affect each other also by indirect effects caused by their physical presence. Bioturbation and

sloppy feeding for instance can enhance nutrients concentration and O_2 saturation and might stimulate the production of other organisms. Inhibition, as a negative indirect effect, may result from competition, enhanced turbulence by bioturbation or exudation of inhibitory waste products. Such indirect effects can distinctly control the dynamics of a community. Tilman (1999) argues that ecosystem dynamics and functions are regulated by species composition, driving ecological processes by individual traits. This implies that the regulatory and selective mechanisms such as competition, predation and disturbance dominantly affect productivity and biomass (Hagerthey et al. 2002). Smetacek & Nicol (2005) assume that the depletion of top predators (e.g. whales) and the decrease of their feeding pressure causes significant changes in the food web structure on the one hand by trophic cascading and on the other hand by the lack of fertilisation by the top predators faeces. Fretwell (1977) predicts that the fundamental attributes of biotopes determine, via bottom-up processes, the biotic basics, such as total biomass and production. The trophic structure however is formed by top-down processes and indirect effects, for instances grazing, competition or stimulation. Thus all control mechanisms, top-down, bottom-up, direct and indirect can act simultaneously in biotopes.

Study sites

Germany

The climate of Germany is temperate with characteristic cold winters (mean temperature in January in Dorum +3°C), warm summers (mean temperature in July in Dorum +18°C) and moderate springs and autumns. Precipitation is sufficient for the growth of green meadows and boreal forests. Sea ice-coverage during severe winters is rare (~six to ten times within the last 60 years).

Fig. 4: Map of the sampling sites in Germany.

Dorum is located northern of Bremerhaven, Germany (53°42`N; 8°29`E) in the national park "Niedersächsisches Wattenmeer" in the North Sea (Fig. 4). The sampling site was located within an intertidal mudflat approx 500 m off the high tide shore line. The tides in this area have a cycle of 12 h with a level difference of 3 m and the whole area drops slowly so the sampling field was submerged for a time of only 3 h by 1 m deep water.

Sylt is an island in the North Sea in Germany (Fig. 4). The **Königshafen** is a sheltered intertidal bay of 4.8 km^2 in the North of the island (55°02`N; 8°25`E). The sampling site was located within the bay between 100 m and 400 m from the shoreline. Like in Dorum the tides are semidiurnal with a level difference of around 2 m. Depending on the position of the sampling site within the bay, the sediment is exposed to the air for 3 h and 6 h per cycle respectively. A general description of the area is given by Reise (1985).

Both areas are situated within a tidal mudflat where the temperature can range between -5°C and 35°C with salinities between 5 and 32 PSU. The sediment in both areas was macroscopically dominated by wave ripple marks (about 2 cm high) and fecal strings of marine Polychaets (such as *Arenicola marina*). Other highly abundant macroinvertebrates were the mudsnail *Hydrobia ulvae* Pennant 1777 and the sand hopper *Corophium* sp. Pallas 1766. Macroalgae such as *Ulva* sp. or vascular plants of the genus *Zostera* were only sparsely present or as in Sylt at places closer to the beach. The salinity is variable which is typical for an intertidal mudflat with freshwater input nearby.

The **Schöhsee** in Plön Germany (54°09`N; 10°26`E) is a small freshwater lake developed at the end of the Weichsel ice age (Fig. 4). The lake covers an area of 0,78 km^2 and has an maximum depth at 29.4 m (medium depth 10.9 m). The lake is categorised as an oligotrophic lake of low productivity and intensely used for recreation and tourism. The sampling area was located on a small sandy beach surrounded by forest on the peninsula "Kleiner Warder" with a water depth of around 0.8 m.

The **Fühlinger See**, near Cologne, Germany (50°58`N; 74°01`E) is a 20 years old complex of meso-eutrophic lakes connected by channels for local recreation (as a boat-racing course, diving) (Fig. 4). The surrounding is covered by a park with lawns and small forests. The water level of the mainly groundwater fed lake is influenced by the water levels of the nearby river Rhein which causes a seasonal fluctuation of about 2 m. The sampling area was located in a side lake part which covers around 4 ha with maximum depth of 14 m. The lake is dimictic and stratified between April and October with a thermocline in a depth between 5 m and 8 m (Auer et al. 2003). Sediment was taken from near the shore in about 30 cm to 50 cm deep water.

North America

The sampling sites in Northern America were located in New Jersey and Pennsylvania. This temperate region exhibits typical cold winters (mean temperature in January in NJ –1.6°C), warm summers (mean temperature in July in NJ 22°C) and moderate inter seasonal periods. Precipitation is sufficient for the growth of green meadows and boreal forests (Fig. 5).

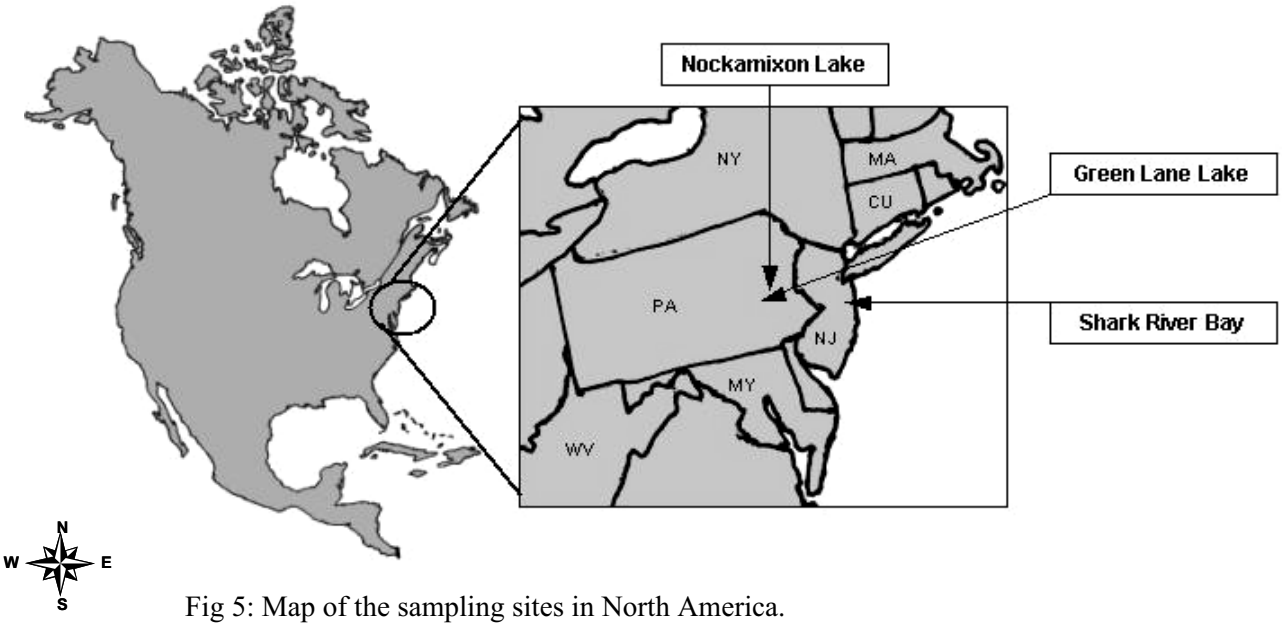


Fig 5: Map of the sampling sites in North America.

The **Shark River Bay** near Belmar, New Jersey USA (40°10`N; 74°01`E) is an estuarine bay or a tidal basin with upland freshwater tributaries coming from the east and south of the bay (Fig. 5). Ocean's tides flushing in and out through the Shark River Inlet, a small 1.5 km long channel connecting the bay with the ocean. The average tidal water level change is about 1.8 m in a semidiurnal cycle. The region is highly frequented by human activity (shipping and fishing) and adjacent towns, harbors and roads empty their waste waters into the bay, therefore the pollution is high (www.waterwire.net). The sampling area was located on the Shark River Island 2 m from high tide line. Water and air temperature vary with the season of an typical temperate maritime climate.

The **Green Lane Lake** Pennsylvania, USA (40°20`N; 75°27`E) is a 25 years old reservoir of a small river surrounded by a recreation park (Fig. 5). The beach of this lake is man-made consisting of coarse sand. On the sampling day the air temperature was –2°C, the water temperature was 1°C. Due to the low temperature the surface water near the beach was frozen to a 1-3 mm thick ice layer. The samples were taken near shore in 30 cm deep water from under the ice layer, the sediment was not frozen.

The **Nockamixon Lake** in Pennsylvania, USA (40°28`N; 75°13`W) is located in a state park near Quakertown. Nocka-miska-ing is an Indian name and means “the place of soft soil” (Fig. 5). The brownish yellow color of the water indicate that the water was containing a high amount of humid acids from the surrounding forest. The lake came into existence by the Nockamixon Dam (31 m high, built 1973) which flooded the Tohickon creek valley. The lakewater covers 5.2 km^2 with a maximum depth of 27 m with summer statification. The whole side is intensely used as recreation area. Vicinal towns and farms are responsible for a high supply of waste water and fertilizers effecting a high nutrient content and eutrophic conditions (http://reference.allrefer.com; www.pennridge.org/works/Nockamixonhm.html).

North East Greenland

The investigated sites in an arctic climate were located on the north-east coast of Greenland, north of the 10°C July isotherm and even north of the Arctic Circle, located within continuous permafrost with pack ice in winter (roughly from October to May; Fogg 1998; Fig. 6). Literature about regions at the latitude of the Arctic regions document 24 h of daylight from end of April to mid of August with monthly mean temperatures rising up to +5°C, and the absence of light (24 h night) from the end of October to mid of February with monthly mean temperatures of around -24°C (Fogg 1998; Jokat 2004). The soil in this region is permanently frozen and the ocean is covered with pack ice from October to May (Stonehouse 1989; Fogg 1998). The precipitation in arctic regions is known to be slight and consequently the vegetation represents a typical tundra, dominated by moss, draft-willows (*Salix arctica*) and lichens.

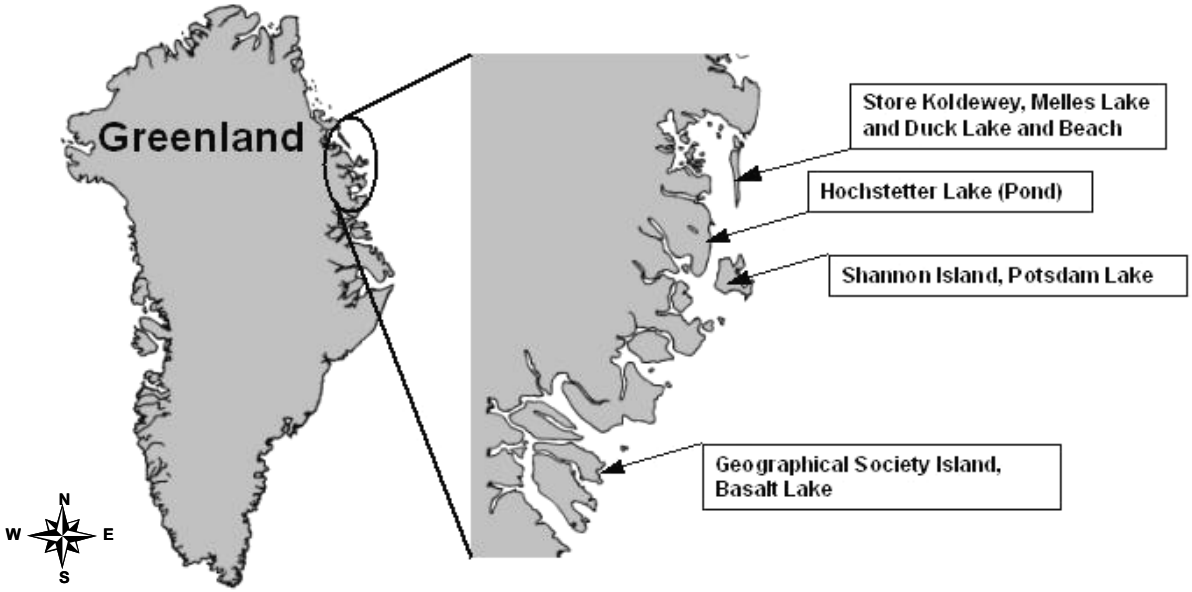


Fig. 6: Map of the sampling sites in North East Greenland.

Store Koldewey is an island in the North East of Greenland (76°06.82`N; 18°30.51`W; Fig. 6). The sampling site was located on an eastern seaside beach. Landwards the beach was surrounded by a glacier. The sediment in this area consisted of coarse sand with a very low content of silt and

clay. No data were available as to the slope of the ocean´s floor on a small scale and as to the tidal range. The appearance of the open ocean shore line, the steep mountain slope close to the shoreline as well as the low amount of silt and clay indicate that the tidal change did not play an important role in this area. The wave shock however seemed to be high.

The arctic **Melles Lake** (76°07' N; 8°37' W) is located on Store Koldewey (Fig. 6). The lake is filled with ultra oligotrophic meltwater from glaciers and has a small outlet to the sea. In the middle of the lake a milky flag of sediment filled water could be seen. Maximum depth is 72 m (Jokat 2004).

The **Duck Lake** (76°25' N; 8°45' W) is an arctic lake located on Store Koldewey (Fig. 6). The maximum water depth is 6.4 m. The lake is filled by melt water (Jokat 2004).

The **Potsdam Lake** (75°03 48`N; 18°45.86'W) is a shallow lake on the north-west coast of Shannon Island (Fig. 6). The lake has a maximal depth of 0.7 m and is fed by melt water in spring (Hubberten 1995). The surrounding area was covered by vegetation. On the sampling area feces were seen, which indicated a former resting of geese, consequently a fertilizing effect had to be expected.

The beach on **Shannon Island** (76°06.82'N; 18°30.51'W) at the North East of Greenland is located in a Bay, so the beach is protected against open ocean waves. The sediment in this area consisted of volcanic basalt. The amount of silt and clay was high (17.3%) and the sediment was not well sorted.

The **Basalt Lake** (72°43.48`N; 22°27.60`W) is situated on the southeastern island Geographical Society (Fig. 6). The main inflow enters the lake at its eastern shore, fed by melt water from farther glaciers. The longish lake (length 2.3 km, width 1 km) has a maximum depth of 22 m (Wagner 2000). The surrounding was densely covered with small plants.

The **Hochstätter Lake** (75°37.31`N; 19°44.12`W) is a small lake situated on the foreland between Shannon Sund and Hochstetterbugten (Fig. 6). No data about maximum depth and covered area were available, but with regard to the surrounding area it is supposed to be a shallow pond. The sediment surface at the sampling site near the shore in about 30 cm deep water had a fluffy layer probably consisting of plant detritus.

Large Ice floes (77°08.98`N; 01°12.06`W) in the Arctic Greenland Sea originate either from sea ice developed near the North Pole or from glacial land ice. Both were floating for several years within the ocean and melt slowly while floating southward. The sediment on the sampled ice floe consisted of silt to clay from Greenland enclosed for years within glacial ice. We sampled the sediment out of a 10 cm deep melt water pond. The salinity in this melt water was 5 PSU.

Aims and outlines of the thesis

Several studies on plankton communities have proven the importance of protists as a link between the microbial- and the large food web. The function of protists in benthic food webs has not been as thoroughly investigated. Hence this study aims at investigating the following:

1. The dynamics of a small benthic food web in an intertidal mudflat over a year.
2. The influence of environmental attributes on the structure of small benthic food webs.
3. The trophic relationships in-between small benthic community components.
4. The influence of small macro fauna on the dynamics of the small community in different soft sediment systems.

The seasonal development of the components of the microbial food web in an intertidal mudflat in Dorum was investigated and presented in **Study 1**. The focus was laid on the investigation of abundance and biomass of diatoms, cyanobacteria, PNF (phototrophic nanoflagellate), bacteria and HNF (heterotrophic nanoflagellate). For a better understanding of the factors controlling the development of the organisms´ abundance and dynamics, possible inter-guild relationships between the components of the small food web as well as the potential influence of prevailing environmental parameters were analysed and discussed. Finally**,** a possible model of matter and energy flow for four seasonal phases exhibiting the potential contribution of each component was presented.

The structures of small benthic communities from marine and freshwater systems in Germany, USA and North East Greenland were investigated to identify similarities and differences which could be attributed to the geographic position, climate and salinity (**Study 2**). The focus was placed on abundance and biomass of diatoms, cyanobacteria, PNF, bacteria and HNF.

The seasonal dynamics as well as the spatial distribution of ciliate species composition and diversity and meiofauna groups was analysed in **Study 3**. Ciliates and meiofauna seasonal dynamics were investigated in the temperate mudflat of Dorum as well as in sediments from marine and freshwater systems in Germany, USA and North East Greenland. Further, the influence of environmental parameters and prey type availability on the ciliate species composition were analysed and discussed.

The influence of small macro fauna on the dynamics of the small community in different soft sediment systems was analysed in laboratory experiments with sediment from marine and freshwater systems in Germany, USA and Greenland (**Studies 4 and 5**). The main focus was placed on the top-down effects and indirect effects caused by small macro fauna on the abundance of the microbial components. Laboratory experiments were carried out to analyse the influence of the absence of macro fauna and the presence of single species of macrofauna (such as *Hydrobia ulvae, Corophium* sp., *Bathyporeia* sp., mixed polychaets, *Gammarus* sp. and Chironomid larvae) on the microbial components. During a long term in situ experiment in the mudflat of the island of Sylt the influence of the long term exclusion of *Arenicola marina* on the components of the microbial components of the small benthic food web was analysed.

In **Study 4** the influence of small macro fauna during laboratory experiments and the long term exclusion of *Arenicola marina* on the abundance and biomass of diatoms, cyanobacteria, PNF, bacteria and HNF were analysed. The influence of small macro fauna during laboratory experiments and the long term exclusion of *Arenicola marina* on ciliate species composition and diversity as well as meiofauna groups were investigated in **Study 5**.

List of Studies

This dissertation is based on five studies prepared for submission. The contribution of the authors is specified.

Study 1

K. Stumm, U.-G. Berninger

Seasonal changes of a benthic microbial community in an intertidal fine sediment.

The concept was developed by U.-G. Berninger and K. Stumm. Samples were collected and analysed by K. Stumm. The manuscript was prepared by K. Stumm.

Study 2

K. Stumm, Y. Lei, U.-G. Berninger

Community structure and biomass partitioning of benthic microbial communities

The concept was developed by U.-G. Berninger and K. Stumm. Samples were collected by K. Stumm. Samples were analysed by K. Stumm. The manuscript was prepared by K. Stumm.

Study 3

Y. Lei, K. Stumm, S.A. Wickham, U.-G. Berninger

Temporal dynamics and spatial distributions of ciliate community structure and meiofauna in sediments.

The concept was developed by K. Stumm. Samples were collected and prepared by K. Stumm. Data were analysed by Y. Lei (ciliates and meiofauna) and K. Stumm (environmental parameters and microbes). The manuscript was prepared by Y. Lei in cooperation with S.A. Wickham and U.-G. Berninger.

Study 4

K. Stumm, N. Volkenborn, U.-G. Berninger

Functional role of small macrofauna in small benthic communities

The concept was developed by K. Stumm. Samples were collected by K. Stumm and N. Volkenborn. Samples were analysed by K. Stumm. The manuscript was prepared by K. Stumm.

Study 5

Y. Lei, K. Stumm, S. A. Wickham, U.-G. Berninger

Control of benthic ciliates and meiofauna by grazers in marine, brackish and freshwater sediments: a cross system comparison.

The concept was developed by K. Stumm. Samples were collected and prepared by K. Stumm. Data were analysed by Y. Lei (ciliates and meiofauna) and K. Stumm (environmental parameters and microbes). The manuscript was prepared by Y. Lei in cooperation with S.A. Wickham and U.-G. Berninger.

Study 1

Seasonal changes of a benthic microbial community in an intertidal fine sediment

K.Stumm[1)], U.-G. Berninger[2)]

[1)] Alfred -Wegener - Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

[2)] University of Salzburg, Hellbrunner Strasse 34, 5020 Salzburg, Austria

Abstract

Protists play an important role in the nutrient cycle of a pelagic microbial food webs as a link between the microbial and the "classic" food web. Detailed information about the function of protists in benthic microbial food webs is rare, although it is assumed that protists play an important role in benthic habitats. This study aimed at investigating the seasonally changing composition and the controlling factors of the microbial community in an intertidal mudflat.
Samples of the upper 3 mm of the sediment were collected over a period of one year. Bacteria, cyanobacteria, diatoms and nanoflagellates (phototrophic = PNF and heterotrophic = HNF, both split in different size classes) were counted using epifluorescence microscopy. During summer diatoms were heavily grazed by large HNF (>10 µm) and an increasing number of ciliates and meiofauna. An enhanced activity of deposit feeders and thus an intensified positive feed-back to primary production was assumed to happen at the same time, whereas cyanobacteria and bacteria production appeared to be favoured by temperature and organic carbon input. Significant correlations between abundance of diatoms, bacteria and HNF led to the assumption that small HNF (2-5 µm) feed mainly on macromolecules, HNF 5-10 µm on bacteria and on smaller nanoflagellates (HNF and PNF 2-5 µm). The results of this study indicated that the benthic microbial systems of fine sediments went through a seasonal cycle with a primacy of bottom up control in winter and spring followed by an increasing top-down control (influence of grazing) from spring to summer. These results completed by literature data were assembled to a probable model of the major carbon and energy flow in the main seasons.

1. Introduction

The microbial components of benthic ecosystems represent an often underestimated factor in food webs (Fenchel 1967; 1968; 1969; 1982; Dietrich & Arndt 2000). Especially in shallow intertidal habitats the activities of bacteria, microphytobenthos and protists play a major role in organic matter deposition, in nutrient flux and in the biogeochemical dynamics (Epstein et al. 1992; Danovaro et al. 1999; Manini et al. 2003; Cook et al. 2004). Moreover microbes form an important link to the metazoan food web (Hamels et al. 2001; Pinckney et al. 2003; Cook et al. 2004).

Benthic habitats are generally characterised by steep changes in physical and chemical properties across the discontinuity layer (Barnes & Hughes 1999). Especially in shallow habitats, wave action and water current control many abiotic factors (Knox 2000). In particular, intertidal sediments form unstable habitats due to permanently changing conditions such as moving sand grains, alternating water current and changing salinity and temperature. Such highly unstable conditions are in general unfavourable for most organisms. However, intertidal mudflats are known to be highly productive with high abundance of microbial organisms (Bak & Nieuwland 1989; Cammen 1992; Berninger & Epstein 1995; Barranguet et al.1997; Hondeveld 1998). Maximum abundance of benthic microalgae, flagellates, ciliates and meiofauna organisms are indeed found within the narrow oxic layer (Böttcher et al. 2000).

Studies on food webs often address the subject of bottom-up or top-down control of abundance and biomass. Power (1992) states that the respective conclusions are often predetermined by the focus of the studies: either on energy and nutrient fluxes (bottom-up) or on trophic relationships between the organisms (top-down). Most communities however are probably controlled by a mixture of predation, disturbance, recruitment and environmental conditions such as resource limitation. Thus bottom-up and top-down control are not mutually exclusive alternatives but act simultaneously or alternately (Hondeveld et al. 1994).

So far most studies regarding benthic sediments deal only with selected parts of the whole food web, for instance feeding interactions between meiofauna, algae and bacteria on one hand or flagellates and bacteria on the other hand. Hence remarkably little is known about the complex feeding interactions within the microbial food web under consideration of all components.

Furthermore in most ecological food web studies, flagellates and ciliates are regarded as homogeneous groups of either heterotrophs or phototrophs and occasionally mixotrophs. But concerning their feeding interactions and so their functional ecological role, these groups are

in no way homogenous. For instance ciliates consist of different species which are feeding either as carnivores, herbivores, bacterivores or omnivores. In the same sense heterotrophic flagellates, usually considered as the main bacterivores, are able to feed on a wide spectrum of other prey types (such as HNF, microalgae; Fenchel 1968; 1987; Cleven 1996; Premke & Arndt 2000). These investigations dealing with selected parts of the food web only and under the assumption of homogenous groups, make it difficult to identify complex interactions between the components and therewith to understand the function of the food web system.

This study aimed at the identification of prevailing mechanisms controlling abundance changes and component relationships in a microbial food web of an intertidal mudflat. Therefore the seasonal dynamics of the abundance of bacteria, cyanobacteria, diatoms, phototrophic and heterotrophic flagellates, the two last species split into size classes, and environmental data were investigated over a period of two years.

2. Materials and Methods

Study site

This study was carried out in a tidal mudflat near Dorum, Germany (53°42`N; 8°29`E), part of the national park "Niedersächsisches Wattenmeer" in the North Sea, from August 2001 to May 2003 (Fig. 1). The region is a typical temperate zone with cold winters, warm summers and moderate springs and autumns. The sampling site was located within an intertidal mudflat approximately 500 m from the high tide shore line where the tides have a cycle of about 12 h with a level difference of 3 m. The downward slope of the whole area is slight, so the sampling field was submerged for only 3 h of the complete 12 h tidal cycle in 1 m deep water. The sampling was carried out within 1 h before to 1 h after low tide at all sampling dates. The sediment in this area is macroscopically dominated by fecal strings of marine Polychaets (such as *Arenicola marina*) and wave ripple marks about 2 cm high. In addition the mudsnail *Hydrobia ulvae* Pennant 1777 and the sand hopper *Corophium volutator* Pallas 1766 were observed as highly abundant grazers. Due to the tidal currents and missing settlement areas (e.g. rocks, shells) macroalgae such as *Ulva* sp. or vascular plants of the genus *Zostera* were rare.

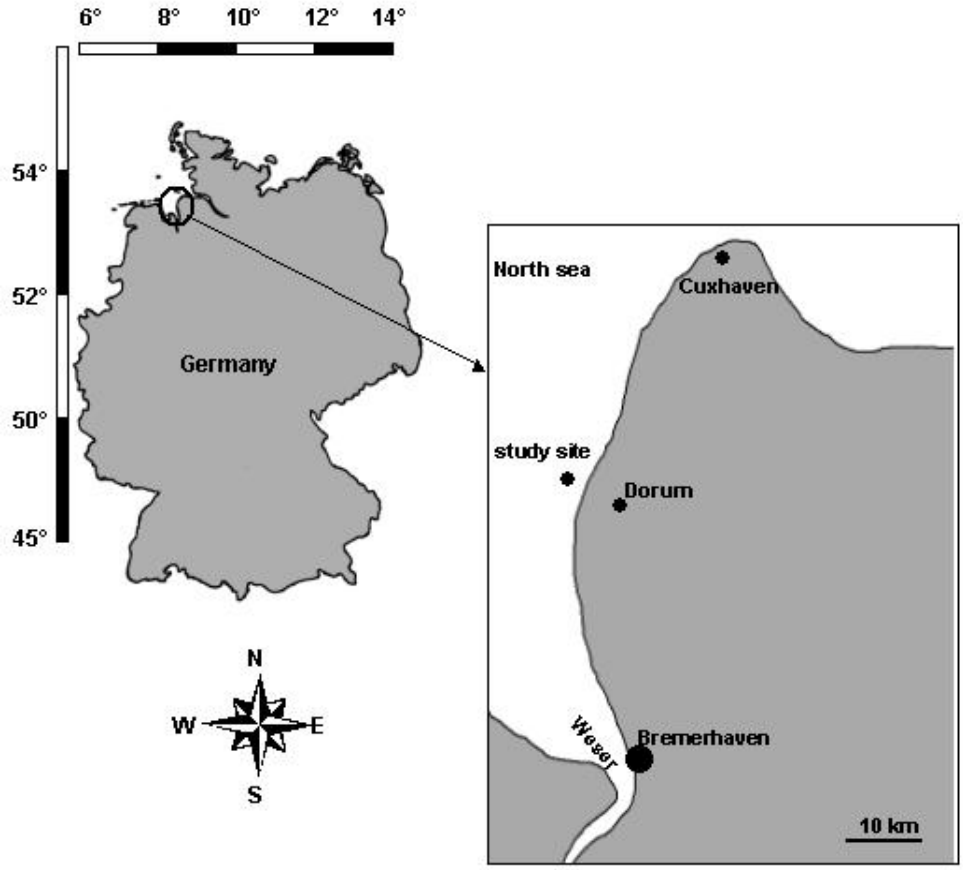


Fig. 1: Sampling site in Dorum at 53°42`N; 8°29`E North Sea coast.

Sampling

During each sampling the temperature of air, water and sediment (1 cm deep) as well as the salinity were measured in the field. Sediment samples were taken during low tide, when the sediment was exposed to the air at a place, where the water depth in high tide situations was between 0.3 m and 1 m. The upper 3 mm (within the oxic layer) of sediments were collected using round plexiglass cores (inner diameter 36 mm) or with a flat shovel. Three samples were taken per station. The samples were transported to the laboratory in a dark cooled container and processed within 4 h.

Sediment analysis

The samples for the sediment analysis (50 ml of surface sediment) were frozen in tubes immediately after returning to the lab (max 2 h) at -20°C and stored for the measurement of photosynthetic pigments, organic carbon, total carbon and total nitrogen content as well as grain size distribution.

Carbon and nitrogen content determination

140-160 mg of frozen, dried and milled sediment were weighed into an annealed pottery saggar. The contents of total carbon and total nitrogen were analyzed by combustion (1600°C in a pure O_2 atmosphere) combined with gas-chromatography, infrared absorption spectroscopy and thermal conductivity measurement (Leco CNS 2000 determinator). 140-160 mg of standard soil (Leco soil calibration sample for CHNS) were used for calibration purposes. For the analysis of the organic carbon content, 30 – 50 mg of frozen, dried, milled sediment were weighed into an annealed pottery saggar. 0.5 ml of concentrated hydrochloric acid and three drops of alcohol were added. The sample was heated up to 250°C for 2 h in order to dissolve inorganic carbonates. A spattle spoon of tungsten and a small spoon of iron filings were added to increase temperature and to catalyse the combustion in pure oxygen. The amount of organic carbon was measured by an infrared and thermal conductivity detector (CS – 125 carbon sulfur determinator). The carbonate content was calculated according to the equation:

CaCO3 (wt.%) = (total C – organic C) * 8.333.

In order to identify organic carbon sources, C/N ratios were calculated by using the measured values of organic C and total N.

Grain size distribution, water content, porosity

The grain size distribution was determined by adding 100 ml of H_2O_2 (10%) to 50 ml of sediment in order to oxidise all organic content (waiting time 3 h to 6 days) (Buchanan 1984). Afterwards the biotic carbon was dissolved by adding 50 – 100 ml of acetic acid (f.c. 25%). After 12 h, the wet sediment was sieved through a 63 µm sieve (Buchanan 1984). The sieve residue was dried in an oven at 80°C and weighed. Silt and clay (<63 µm) were separated by sedimentation. Water content (wc) was calculated as follows:

wc = (wet - dry sediment weight) / wet sediment weight (Dell'Anno et al. 2002) .

Porosity (P) was determined by the equation:

$$P = (wc/1.02) / [(1-wc)/2.64 + wc/1.02]$$

where 1.02 g cm^{-3} is the density of 35 PSU salt water and 2.64 g cm^{-3} is the density of quartz (Buchanan 1984; Dell'Anno et al. 2002).

Photosynthetic pigments

3 to 8 g of wet sediment were suspended in acetone (f.c. 90%; dilution 1:7 to 1:100) and sonicated on ice for 90 s (Branson Sonifier 250; pulse; level 6) in order to extract chlorophyll-derived pigments (chlorophyll-*a* and phaeopigments). Pigments were extracted over 24 h at 4°C under no light conditions and subsequently separated from the sediment by centrifugation (2000 g for 5 to 10 min). The chlorophyll-*a* content was measured with a fluorometer (430 nm excitation wavelength and 665 nm fluorescence wavelength). The phaeopigment content was measured by adding 200 µl of 0,1 N HCl to the extract. The amounts of pigments were calculated. This methode is described in detail in Lorenzen (1976) and Daemen (1986).

The amount of pigments was calculated according to the equation of Lorenzen (1976):

$$\text{Chlorophyll-}a = F_d*(\tau/\tau-1)*(R_b-R_a)*(V_e/V_f)$$
$$\text{Phaeopigment} = F_d*\tau/\tau-1)*(\tau R_a-R_b)*(V_e/V_f)$$

with:

Chlorophyll-*a*	[µgC/g]	Chlorophyll *a* content
Phaeopigment	[µgC/g]	Phaeopigment content
F_d		correlation factor (here after calibration with standard chlorophyll-*a* solution; 0,84)
R_b		emission at wavelength 665 nm before acidifying
R_a		emission at wavelength 665 nm after acidifying
τ		max. ratio R_b/R_a of phaeo free Chl-*a* (Chl*a* = 2,2)
V_e	[l]	volume of acetone
V_f	[g]	volume of extracted sediment

Bacteria, nanoflagellates, cyanobacteria and diatom abundance and biomass

An equivalent of 1 - 3 g wet sediment or 1 to 2 ml of the upper layer (less than 3 mm from the surface) were transferred into tubes containing 2 ml filtered (0.2 µm) seawater each, fixed with cold (~ 0°C) glutardialdehyd (f.c. 2%) and stored at 4°C in darkness until further analysis (max. one week). For the quantification of organisms samples were diluted in artificial seawater (5 to 20 ml). The organisms were detached chemically with PPI (Tetrasodiumpyrophosphate, f.c. 0.5-10 mM) and Tween 80 (f.c.1-10 µg ml^{-1}) and detached physically by gentle sonification on ice (Branson, Sonifer 250, pulses for 30 s at 60 W). The supernatant was filtered using a black polycarbonate filter (Osmonics 0.2 µm) and stained with DAPI (4`.6-Diamino-2-phenylindol, working solution 50 µg ml^{-1}, f.c. of supernatant 5 µg ml^{-1}). Finally, the filter was embedded in fluorescence-free immersion oil (AppliChem) and stored at -20°C until microscopical analysis (Sherr et al. 1993; Velji & Albright 1993; Epstein & Rossel 1995). The slides were examined using epifluorescence microscopy (Zeiss, Axioscop2 plus, *1000 magnification). For the determination of bacteria abundance a minimum of 500 cells per slide on 24 fields were counted. For the count of cyanobacteria, diatoms and PNF abundance a minimum of 30 random fields were evaluated using the autofluorescence of the photosynthetic pigments (Waterbury et al. 1986; Maclassac & Stoeckner 1993). For the count of HNF abundance only cells with a definite nucleus were counted on a minimum of 30 random fields, while cells with irregular shapes were excluded (Sherr et al. 1993). PNF and HNF were split in size groups according to their lengths, 2 - 5 µm = “small HNF and PNF”, 5 -10 µm = “medium sized PNF and HNF”, >10 µm = “large HNF and PNF”. This size split was introduced in order to allow the study of cell size related interactions of the organisms with the other components of the food web. The cell sizes of organisms of all different groups were measured and the biovolume was estimated using simple geometrical shapes from the literature (Edler 1979). The biomass was calculated by converting cell biovolume using different conversion factors from the literature (Table 1).

Table 1: Measurements of mean cell sizes, geometrical forms and conversion factors used for the estimation of biovolume and biomass of counted organisms.

	length*width*depth [µm]	shape (Edler 1979)	conversion factor	reference
Diatoms	15 * 4.2 * 3	parallelepiped	$0.288*Vol^{0.811}$ pgC $cell^{-1}$	Menden-Deuer & Lessard 2000
Cyanobacteria	3.2 * 2.6 * 2.6	rotational ellipsoid	310 fgC μm^{-3}	Caron et al. 1991
PNF 2-5 µm	2.8 * 2.6 * 2.6	rotational ellipsoid	220 fgC μm^{-3}	Ekebom 1999; Borsheim & Bratbak 1987; Fry 1990
PNF 5-10 µm	6.1 * 4 * 4	rotational ellipsoid		-« -
PNF >10µm	18.9 * 9.5 * 9.5	rotational ellipsoid		-« -
Bacteria	0.6 * 0.6 * 0.6	sphere	19.8 fgC $cell^{-1}$	Lee & Patterson 2002
HNF 2-5µm	1.9 *1.6 * 1.6	rotational ellipsoid	220 fgC μm^{-3}	Ekebom 1999; Borsheim & Bratbak 1987; Fry 1990
HNF 5-10µm	6.5 * 5.6 * 5.6	rotational ellipsoid		-« -
HNF >10µm	20.6 * 11.5 * 11.5	rotational ellipsoid		-« -

Statistical analysis

For a better understanding of the relationships between the components of the microbial benthic food web and environmental attributes, non parametric Spearman rank correlations (able to correlate linear as well as non linear relations) were calculated. Correlations were regarded as significant, if R (correlation coefficient) was larger than 0.5 or smaller than -0.5 (R^2 (coefficient of determination) > 0.25) and p (significance level) < 0.05. Correlations were regarded as highly significant if R > 0.7 or < -0.7 (R^2 > 0.5) and p<0.01.

3. Results

Seasonal changes of environmental variables and phytopigment concentrations

During the sampling period salinity ranged between 10 and 31 PSU depending on tides, weather (insolation, evaporation or dilution by rain) and wind direction, pushing a freshwater tongue of the nearby river Weser against the land and displacing saltwater (Table 2). The surface sediment at the sampling site was composed of well sorted fine quartz sand (mean grain size 63 - 125 µm) with less than 7 (± 3)% of silt and clay, which is typical for an intertidal shallow mud flat with a moderate water current. The calculated porosity was 0.5 and during the sampling period from August 2001 to May 2003 the grain size distribution did not change (Table 2). The amount of total carbon in the sediment did not change between seasons (annual mean 57.4 mg C (g d.w.)$^{-1}$; Table 2). No obvious seasonal change was observed in the amount of organic carbon (annual mean 13.4 mgC g $^{-1}$; 13% of total carbon). No seasonal change could be measured in the amount of total nitrogen (annual mean 2.05 mgN (g d.w.)$^{-1}$). The TOC:TN ratio ranged between 5 and 10, where the highest value was measured during late spring. The amount of phytopigments in the sediment showed high variability during the sampling period (chlorophyll-*a*: 2.3 – 14.5 µgC g^{-1}, phaeopigment: 1.1 – 2.8 µg g^{-1}). Chlorophyll-*a* peaked in June and August 2002, whereas the phaeopigment exhibited only a small peak in August 2002. The ratio of phaeopigment to chlorophyll-*a* varied between 0.2 and 0.5.

Table 2: Main environmental parameters. Content of nitrogen and carbon (total and organic) in mg (g dry weight)$^{-1}$, chlorophyll-*a* and phaeopigment in µg (g wet weight)$^{-1}$, sd = standard deviation (n=3); nd = no data. d.w. = dry weight; w.w. = wet weight.

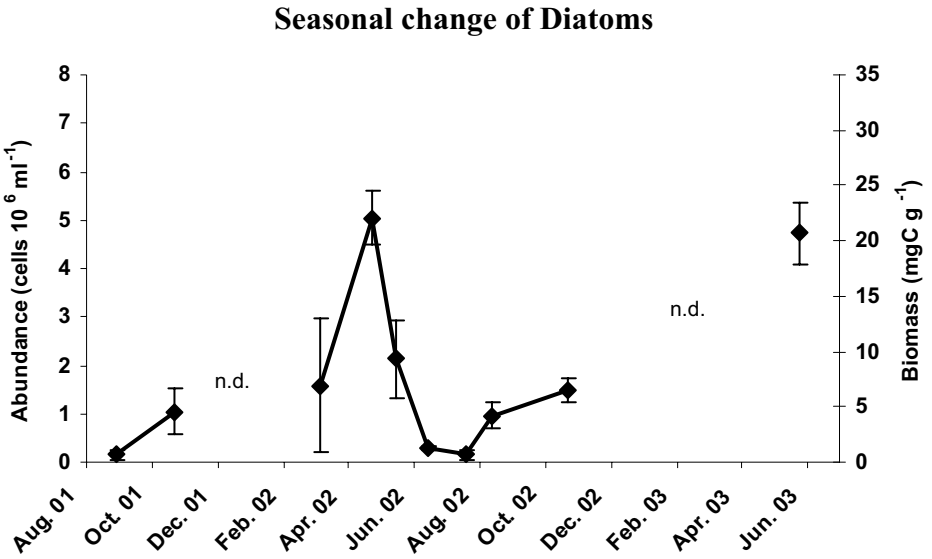
Season	date	temperature		salinity	poriosity	nitrogen total	cabon total	carbon organic
		air	water			TON	TOC	Corg
		°C		PSU		mgN/g d.w.	mgC/g d.w.	mgC/g d.w
Summer	28.08.2001	12	18	31	0.53	0.16	4.82	0.85
Autumn	22.10.2001	15	11	18	0.49	0.18	5.29	1.01
Winter	05.03.2002	8	6	17	0.49	0.14	5.55	0.88
Spring	22.04.2002	12	17	26	0.5	0.17	5.35	1.09
Spring	14.05.2002	15	16	21	0.5	0.18	5.33	1.36
Spring	13.06.2002	15	16	25	0.51	0.21	5.55	2.04
Summer	18.07.2002	14	17	10	0.51	0.16	5.51	0.9
Summer	12.08.2002	21	21	10	0.5	0.28	6.6	1.87
Autumn	22.10.2002	12	10	15	0.49	0.32	7.25	1.69
Spring	25.05.2003	18	28	25	0.5	0.21	5.79	1.46

date	ratio	Chl-*a*		Phaeo	
	Corg:TOC	µg/g w.w	sd	µg/g w.w	sd
28.08.2001	5.31	4.5	0.2	2.3	0.2
22.10.2001	5.61	6.6	0.7	1.3	0.7
05.03.2002	6.29	n.d		n.d	
22.04.2002	6.41	2.3	0.4	1.1	0.2
14.05.2002	7.56	3.4	0.1	1.6	0.1
13.06.2002	9.71	8.3	0.7	2.5	0.5
18.07.2002	5.63	6.5	1.2	2.8	0
12.08.2002	6.68	11.7	2.4	4	0.2
22.10.2002	5.28	3.3	0.1	1.3	0.1
25.05.2003	6.95	14.5	1.6	2.5	1.6

Seasonal changes of autotrophic organisms

Diatoms exhibited a typical seasonal trend with highest abundance during spring (April and May 2002; 4.7 - 5.0 x 10^6 cells ml^{-1}) and lowest abundance during summer (July 2002; 1.5 x 10^5 cells ml^{-1}; Fig. 2a). The diatom biomass ranged between 0.6 and 19.2 µgC g^{-1} during the study period. The cell numbers of cyanobacteria varied between 1.0 x 10^5 and 5.0 x 10^6 cells ml^{-1} where the highest abundance was found during autumn and the lowest during spring (Fig. 2b). The biomass of cyanobacteria ranged between 0 and 1 µgC g^{-1}.

a)



b)

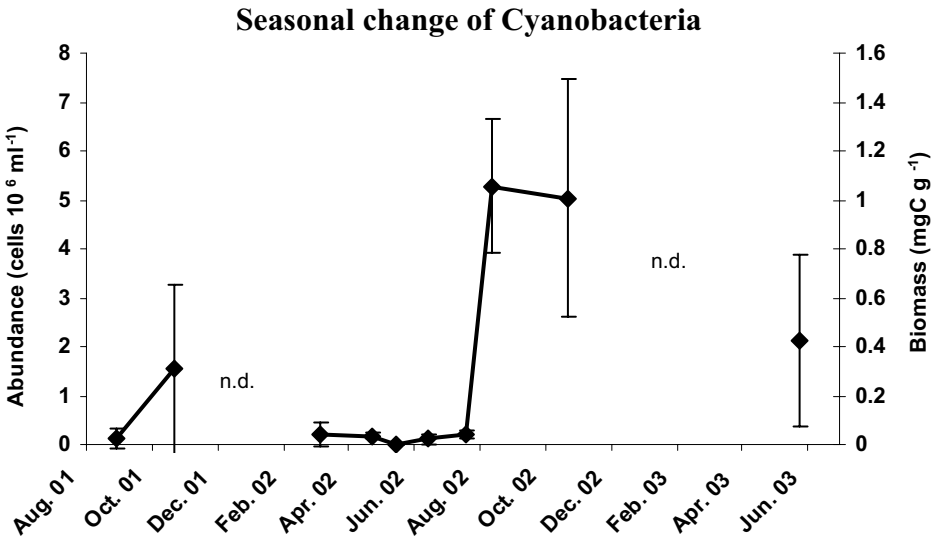


Fig.2: Seasonal change of diatoms (a) and cyanobacteria (b) shown as abundance (cells ml^{-1} wet sediment) and biomass (µg C g^{-1}) with standard deviation (n=3); n.d.= no data available.

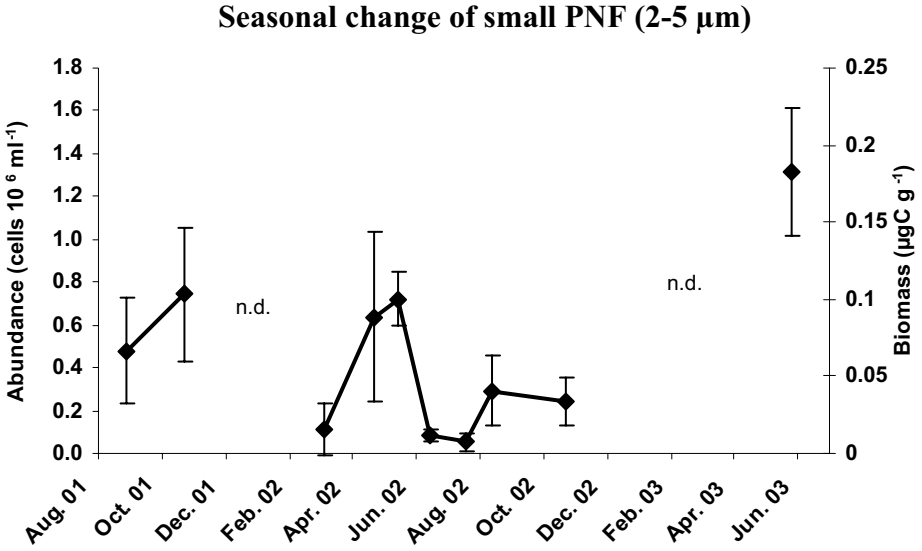
Abundance of all PNF size classes followed a typical seasonal trend with a bloom during spring, a smaller one during autumn and with lowest abundance during summer and winter (Fig. 3a-c). The numbers of small PNF ranged from 6.3 to 1.3 x 10^6 cells ml^{-1} during spring whereas the numbers of cells were much lower (7.4 x 10^5 cells ml^{-1}) during autumn. Lowest abundance were found in March (1.1 x 10^5 cells ml^{-1}) and July 2002 (5.1 x 10^4 cells ml^{-1}; Fig. 3a). The biomass of the small PNF ranged from 0.01 to 0.2 µgC g^{-1} during the study period. The mean annual abundance of small PNF was 56% ± 13% of the total PNF abundance whereas their biomass accounted for 18% ± 13% of total PNF biomass (Fig . 4).

The abundance of medium PNF varied between 2.9 x 10^4 cells ml^{-1} and 7.5 x 10^5 cells ml^{-1} during the study period (Fig. 3b). The number of medium PNF cells accounted for 39% ± 9%

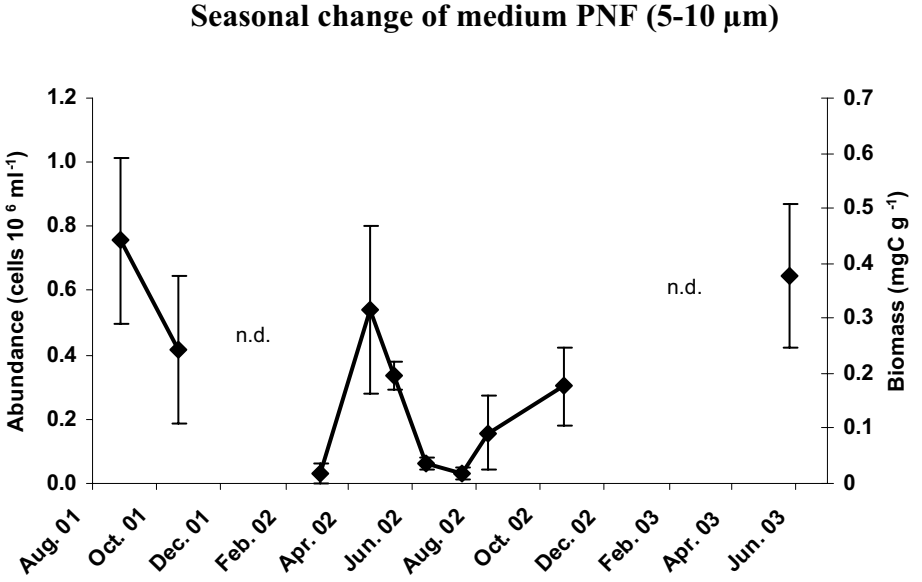
of the PNF (Fig. 4). The biomass of medium PNF ranged between 0.02 and 0.4 µgC g^{-1} which accounted for an average of 47% ± 26% of the total PNF biomass.

The abundance of large PNF always ranged below those of small and medium PNF. A small bloom during spring and autumn could be observed (Fig. 3c). In contrast to small and medium PNF with highest abundance in spring, large PNF had a maximum abundance during their late summer/autumn bloom (August 2001; 1.5 x 10^5 cells ml^{-1}; Fig.3c). The calculated biomass of large PNF reached values up to 1.7 µgC g^{-1}. The proportion of the large PNF of total PNF abundance had maximum values of only 4.6%, but due to their size the biomass represented up to 60% of the PNF biomass (Fig. 4).

a)



b)



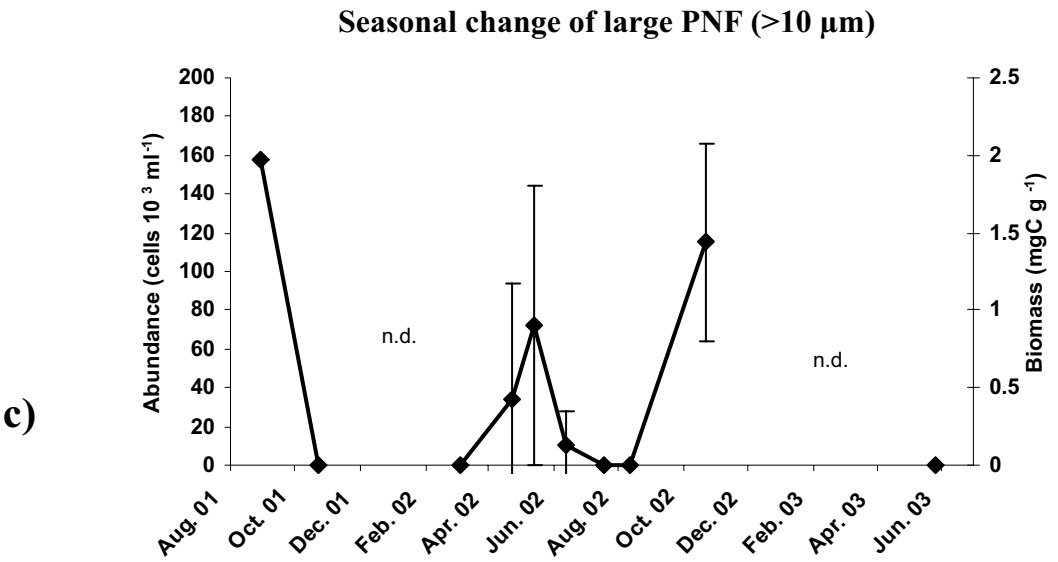


Fig. 3: Seasonal change of small (a), medium (b) and large (c) phototrophic nanoflagellates (PNF) shown as abundance (cells ml^{-1} wet sediment) and biomass (µg C g^{-1}) with standard deviation (n=3); n.d.= no data available.

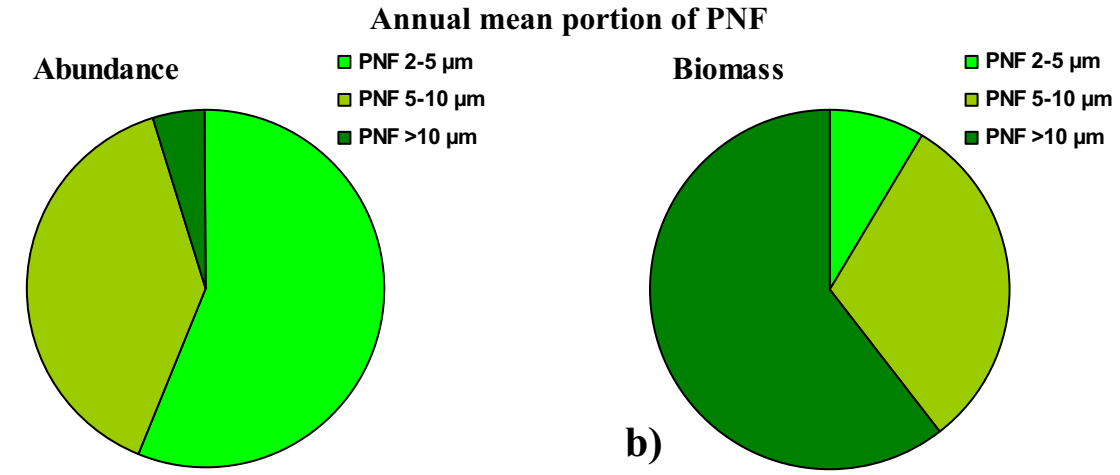


Fig. 4: Annual mean portion of abundance (a) and biomass (b) of phototrophic nanoflagellates (PNF) in different size classes.

Seasonal changes of bacteria

The abundance of bacteria revealed seasonal blooms in spring and autumn, ranging between 8.8 x 10^7 cells ml^{-1} and 1.7 x 10^9 cells ml^{-1} (Fig. 5). Lowest values were counted in early summer and winter. The biomass of bacteria ranged between 0.1 and 19.2 µgC g^{-1}.

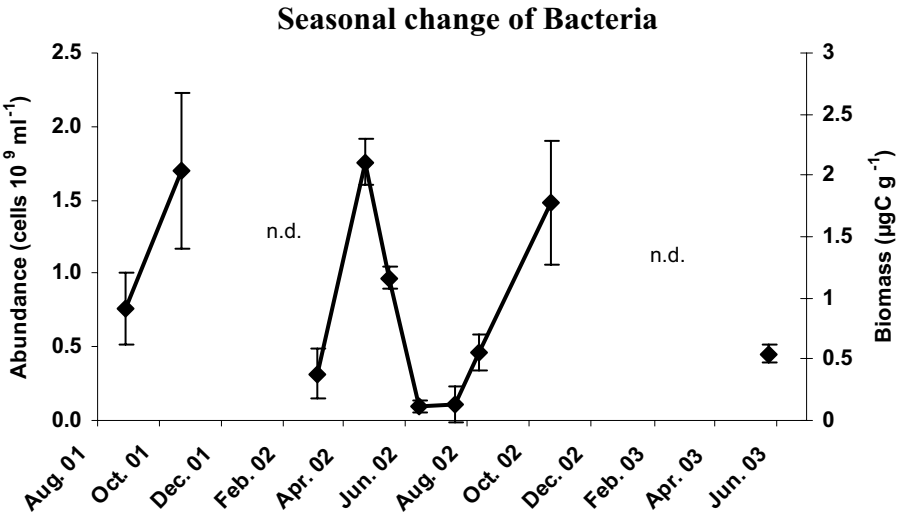
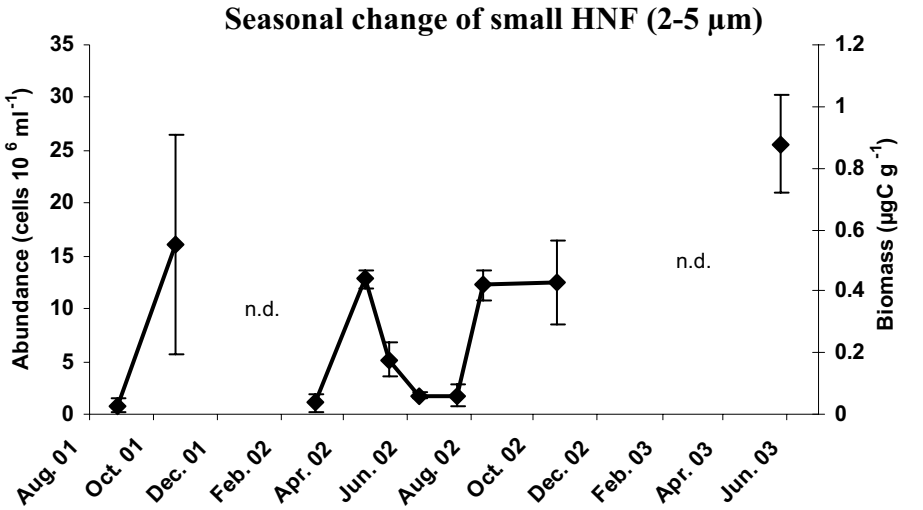


Fig. 5: Seasonal change of bacteria shown as abundance (cells ml^{-1} wet sediment) and biomass (µg C g^{-1}) with standard deviation (n=3); n.d.= no data available.

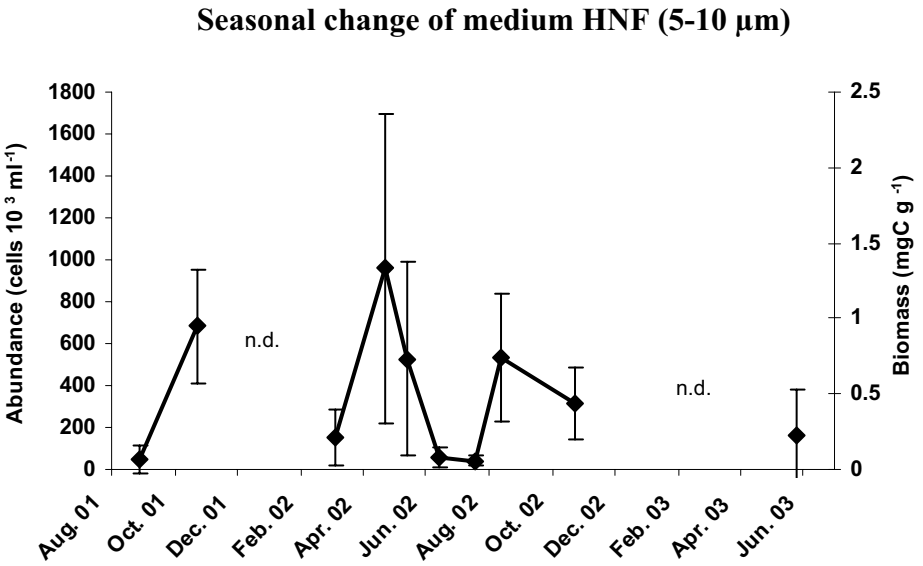
Seasonal changes of heterotrophic nanoflagellates

The abundance of HNF (all sizes) peaked during spring and late summer whereas the lowest numbers were counted during winter and mid summer (Fig. 6 a-c). The abundance of small HNF covered a wide range between summer lows and seasonal blooms (8.5 x 10^5 to 2.5 x 10^7cells ml^{-1}; Fig. 6a). The calculated biomass of small HNF during the study period ranged from 0.03 to 0.8 µgC g^{-1}. The annual mean percentage of small HNF of total HNF abundance was 95%, where their annual mean biomass represented only 11.3% of total HNF biomass (Fig. 7). The medium HNF were also most abundant in late autumn and spring but their total abundance range (4.02 x 10^4 - 9.58 x 10^5 cells ml^{-1}) was much narrower than that of the small HNF (Fig. 6b). The medium HNF biomass ranged from 0.05 to 1.2 µgC g^{-1}. Hence the annual mean percentage of medium HNF on total HNF abundance was only 3.7%. In comparison to the low proportion of medium HNF abundance, their annual mean biomass accounted for 17.5% of HNF biomass (Fig. 7). The abundance of large HNF ranged up to 5.27 x 10^5 cells ml^{-1} during spring, whereas no large HNF could be found during summer (May to July 2002; Fig. 6c). The biomass of large HNF during spring was found to be 9 µgC g^{-1}. Their mean proportion of total HNF biomass was found to be 71.2% where their maximum proportion of total HNF abundance had maximum values of 10% (annual mean 1%; Fig. 7).

a)



b)



c)

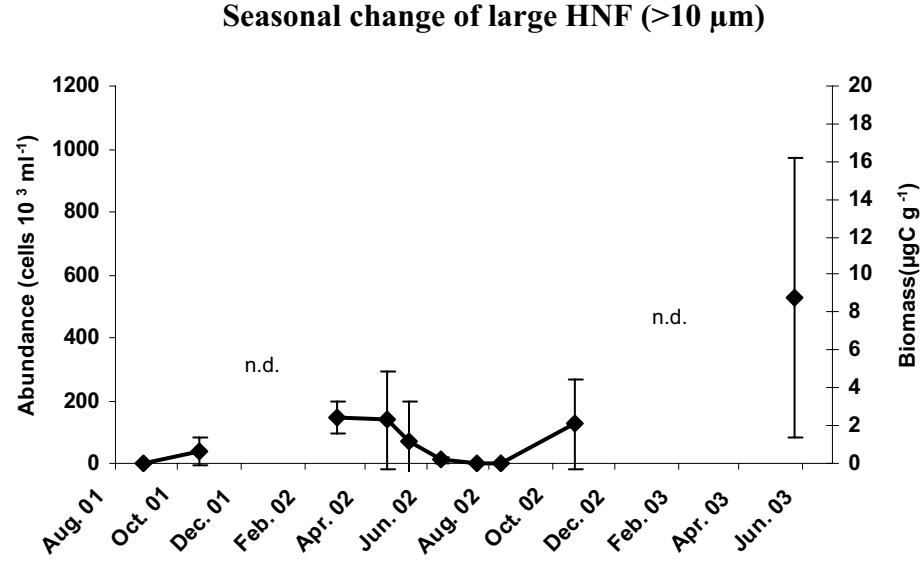


Fig. 6: Seasonal change of small (a), medium (b) and large (c) heterotrophic nanoflagellates (HNF) shown as abundance (cells ml^{-1} wet sediment) and biomass (µg C g^{-1}) with standard deviation (n=3); n.d.= no data available.

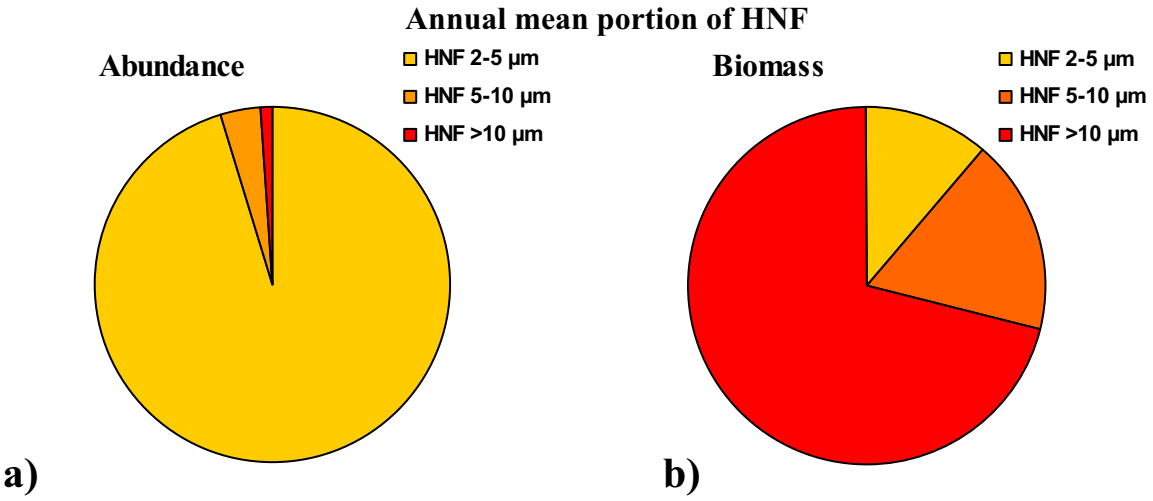


Fig. 7: Annual mean portion of abundance and biomass of heterotrophic nanoflagellates in different size classes.

Seasonal change of biomass partitioning

Diatoms represented up to 75.7% of the total microbial benthic biomass during spring and as little as 17.2% during summer (mean 58%; Fig. 8). Cyanobacteria represented up to 15.8% of the total microbial benthic biomass in August 2002 with an annual mean of 3.6%. The PNF represented an annual mean of 10.1% with a marginal portion of 0.4% in late winter and the largest one of 58.5% in August 2001. The percentage of bacteria of total microbial biomass varied between 3.5% in March and 21.4% in August 2001 with an annual mean of 9.9%. The portion of HNF varied between 2.3% in August 2001 and 32.3 in May 2003 with an annual mean of 18.4%.

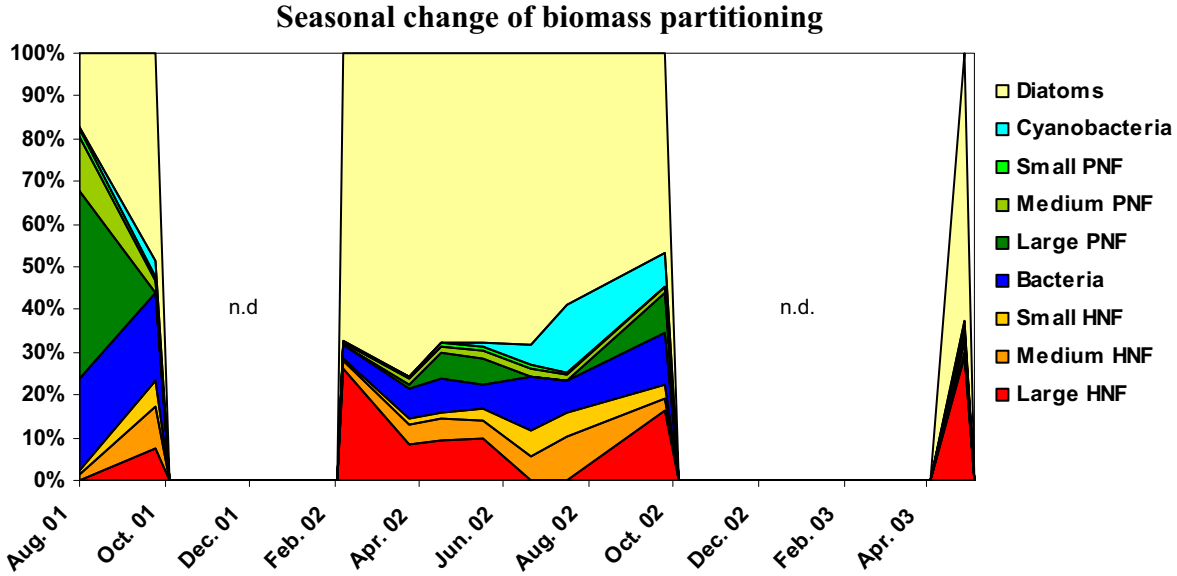


Fig. 8: Seasonal change of the proportions of diatoms, cyanobacteria, phototrophic and heterotrophic nanoflagellates of the total microbial biomass in Dorum. Annual mean microbial carbon biomass was 11.09 ± 9.7 µgC g^{-1} wet sediment. n.d.= no data available.

Correlations

Positive correlations were found between cyanobacteria and total carbon and nitrogen (Table 3). Small HNF correlated significantly with organic carbon and total nitrogen. Surprisingly, no correlation was found between the numbers of autotrophic organisms (diatoms, cyanobacteria and PNF) and phytopigments (chlorophyll-*a* and phaeopigment). Strong negative correlations were found between phytopigments and bacteria. Significant positive correlations were found between different organism groups: diatoms correlated with bacteria, small and large HNF and small PNF. Bacteria correlated with small and medium PNF as well as with medium HNF. In addition to the correlation with bacteria, small PNF correlated with medium PNF and small HNF. The small HNF correlated additionally with medium HNF.

Table 3.: Spearman rank correlation coefficients of abundance data. The lower half of the table exhibits only p-levels as simplified display. n.s. = not significant. N_{total} = total nitrogen; C_{total} = total amount of carbon, Chl-*a* = chlorophyll-*a*; Phaeo = phaeopigment. Significant correlations were defined as * $p < 0.05$; ** $p < 0.01$, and *** $p < 0.001$ with $n = 33$, blanks = not significant.

	N_{total}	C_{total}	C_{org}	Chl-*a*	Phaeo	Diatom	Cyanos	PNF 1-5 µm	PNF 5-10 µm	PNF >10 µm	Bacteria	HNF 1-5 µm	HNF 5-10 µm	HNF >10 µm
N_{total}		0.66 ***	0.84 ***				0.55 **					0.67 ***		
C_{total}	***		0.74 ***	0.52 **			0.63 ***							
C_{org}	***	***		0.43 **								0.53 **		
Chl-*a*		**	**		0.92 ***						-0.78 ***			
Phaeo				***							-0.79 ***			
Diatom								0.58 ***			0.50 **	0.70 ***		0.61 ***
Cynobacteria	**	***												
PNF 1-5 µm						***			0.73 ***		0.59 ***	0.56 ***		
PNF 5-10 µm								***			0.61 ***			
PNF >10 µm														
Bacteria				***	***	**		***	***				0.56 ***	
HNF1-5 µm	***		**			***		***					0.50 **	
HNF 5-10 µm											***	**		
HNF >10 µm						***								

4. Discussion

The analysis of the functional role of the components within the microbenthic food web used the models of Fretwell (1977) and Odum (1971) as theoretical background approaches. The model of Fretwell (1977) predicts that the fundamental attributes of biotopes determine, via bottom-up processes, the biotic basics such as total biomass and production. The trophic structure however is formed by top-down processes, as grazing, competition or stimulation. The benthic habitat in an intertidal mudflat is characterised by high variability of the fundamental attributes of the biotope due to the continuously changing environment. Odum (1971) describes shallow tidal areas as systems with pulse stability, where the tidal oscillations maintain the living community in a young, relatively fertile stage. Consequently the trophic structure, concerning species composition, is relatively unstable. But based on the short generation times and high productivity the total biocenosis exploits the seasonally variable offer of the biotope in a steady way. Thus these populations in sandy shallow benthic habitats can be called r-selected and the biocenosis is characterised by low resistance but high resilience.

Biotope attributes

The sampling site was a typical shallow mudflat with well sorted sand of medium grain size. The content of total and organic carbon (TC and TOC) as well as total nitrogen (TN) were high with no obvious seasonal trend. Other studies dealing with different topics in shallow marine sediments report comparable values (Van Duyl et al. 1993; Böttcher et al. 2000; Dietrich & Arndt 2000). The origin of TOC in marine sediments is the end result of a balance between allochthonous inputs, production, degradation, mineralisation and export. The calculation of the TOC:TN ratio is an easy way to get a first reference to the sources of organic material. A high ratio (>12) thereby points to terrestrial sources (plant material), whereas a low ratio (6-8) indicates undigested phytoplankton and microphytobenthos. Several authors show that the organic material in shallow marine sediments is dominated by terrestrial sources (Reineck 1983; Van Duyl et al. 1993; Van Duyl & Kop 1994; Cook et al. 2004). In Dorum the TOC:TN ratio ranged from 5 to 10. On the one hand this ratio suggested that the organic matter originated from mainly undigested material either from sedimented phytoplankton or microphytobenthos. On the other hand we did not observe a seasonal cycle of TOC and TN, which could have been expected as a consequence of typical algae blooms. The measured data could not explain this inconsistency. But the possibly small contribution of

sedimented algae blooms could have been masked by the relatively large amount of total TOC. Dell'Anno et al. (2002) also found TOC concentrations without seasonal changes, but by measuring the composition of TOC in detail (proteins and carbohydrates) the authors could identify seasonal changes of specific components.

Organisms' abundance

Phototrophic organisms

Diatoms, cyanobacteria and phototrophic flagellates represented the phytobenthic primary producers in the intertidal sediment community in Dorum. These phototrophic organisms exhibited more than 56% of the total microbial biomass at all sampling times, diatoms always dominating the phototrophic group. Diatoms are known to dominate the microphytobenthic community also in other investigated silty and sandy sediments (Agatz et al. 1999). The typical annual cycles with blooms in spring and autumn and decreasing cell numbers during summer and winter in Dorum corresponded well with those found in other studies. In Dorum the abundance of diatoms had an annual mean value of 1.7 x 10^6 cells ml^{-1}. In other studies of tidal sediments diatoms abundance range between 8.95 x 10^4 cells g^{-1} and 4.42 x 10^6 cells g^{-1} (Epstein 1997; Lee & Patterson 2002; Aberle-Malzahn 2004).

Cyanobacteria were most abundant during late summer and autumn with cell numbers up to 5.27 x 10^6 ml^{-1} sediment. Bostroem et al. (1989) also found highest values of the cyanobacterial biomass in October in a shallow sediment of a Swedish lake.

The number of PNF in the Dorum sediment showed a typical seasonal cycle with blooms in spring and autumn as to be expected for phototrophic organisms. The abundance of all size classes of PNF varied between 8.1 x 10^4 and 1.9 x 10^6 cells ml^{-1}. Several other authors found comparable numbers of PNF in shallow intertidal sediments (Sundbäck et al. 1996; Lee & Patterson 2002). Interestingly the bloom of large PNF identified in this study followed the cyanobacteria bloom in autumn. Detailed literature data on the abundance of benthic cyanobacteria and different size classes or species of PNF, based on counting such as in this study, are rare. Detailed pigment data (e.g. zeaxanthine and lutein) can indirectly bring some information about biomass, but the absolute values read from these pigment data are difficult to compare to those based on counting (Barranguet et al. 1997; Lucas & Holligan 1999). However, relative data describing seasonal trends can be used for comparison. Like at Dorum, Barranguet et al. (1997) reports that cyanobacteria (zeaxanthin) are present all over the year in a marine tidal sediment and reach their maximum in September followed by high numbers of large PNF (high values of lutein; Barranguet et al. 1997; Lucas & Holligan 1999; Cook et al.

2004). The factors controlling this shift are not well understood. A possible explanation for this shift is high grazing by ciliates, meiofauna and small macrofauna. The increase of microphytobenthic production from March to July, reported in several studies by Cadée & Hegeman (1977) and Asmus & Asmus (1985) and the relative minimum of microphytobenthic abundance can be explained by an increase of the grazing pressure during summertime.

Bacteria

The abundance of bacteria in Dorum covered a wide range (8.8×10^7 to 1.7×10^9 cells ml^{-1}) following the typical seasonal cycle of the autotrophs. Other authors also observed a wide range of bacteria abundance in tidal marine sediments (6.8×10^7 to 1×10^{10} cells ml^{-1}), but less information is available about factors influencing these values (Cammen & Walker 1986; Hondeveld et al. 1992; Epstein 1997; Böttcher et al. 2000; Dietrich & Arndt 2000; Lee & Patterson 2002; Manini et al. 2003). Most bacteria in sediments are heterotrophic and feed on organic carbon sources such as polysaccharides and dissolved amino acids (as from exudates; Wolter 1982; Bak et al. 1995; Kirschner & Velimirov 1997; Danovaro et al. 1999; Dietrich & Arndt 2000; Passow 2002). It seems therefore likely that bacteria abundance followed the seasonal cycle of the autotrophic organisms or rather the exudates released for instance by diatoms and PNF. The low bacteria biomass during summer is discussed later.

HNF

The HNF followed the seasonal cycle of the phototrophs with peaks in spring and late summer. The counted abundance were high compared to other studies from shallow marine sediment (Alongi 1991; Hondeveld et al. 1992; Hondeveld et al. 1994; Dietrich & Arndt 2000; Lee & Patterson 2002; Manini et al. 2003). A reason for these high numbers may be found in the applied method. The applied method to determine cell numbers (fixing, sonication, staining and fluorescence microscopy) in this study may lead to an overestimation of HNF by including amoeba and funghi cells with regular shapes into the count. Other authors using this method also found abundance of large flagellates (>20 µm) ranging from 10^2 to 10^5 cells ml^{-1} and of nanoflagellates (< 20 µm) from 10^5 to10^6 cells ml^{-1} (Alongi 1991; Hondeveld et al. 1992; Hondeveld et al. 1994; Beardsley et al. 1997; Berninger & Huettel 1997; Dietrich & Arndt 2000; Lee & Patterson 2002; Manini et al. 2003). Thus our results compared to those in literature.

Interactions and controlling mechanisms

Microphytobenthos, ciliates and meiofauna

Ciliates and meiofauna data, presented in detail in study 3, were included into the following analysis of biomass partitioning of the benthic microbial food in order to complete the picture of seasonal changes and interactions.

The seasonal development of microphytobenthos as well as of ciliates and meiofauna biomass exhibited remarkable maximums during spring and summer, respectively (Fig. 9). During their blooming times they clearly dominated the community biomass, microphytobenthos during springtime with about 70% and meiofauna and ciliates during summer with about 90% of the total biomass (Fig. 10). Blooming of phytobenthic algae during spring is typical for temperate regions.

The dominant portion of meiofauna and ciliates biomass in comparison to the lowest biomass of microphytobenthos during summer can be explained by a grazing pressure on microphytobenthos, increasing from spring to summer. The high biomass of meiofauna and ciliates during summer were dominated by meiofauna (diatom feeding copepods), whereas the smaller peak during spring was caused by ciliates, mainly feeding on diatoms (study 3, Giere 1993). It seems likely that an increase of their biomass was connected with a higher grazing, in other words to a top-down control on microphytobenthos.

Seasonal change of community structure including meiofauna and ciliates

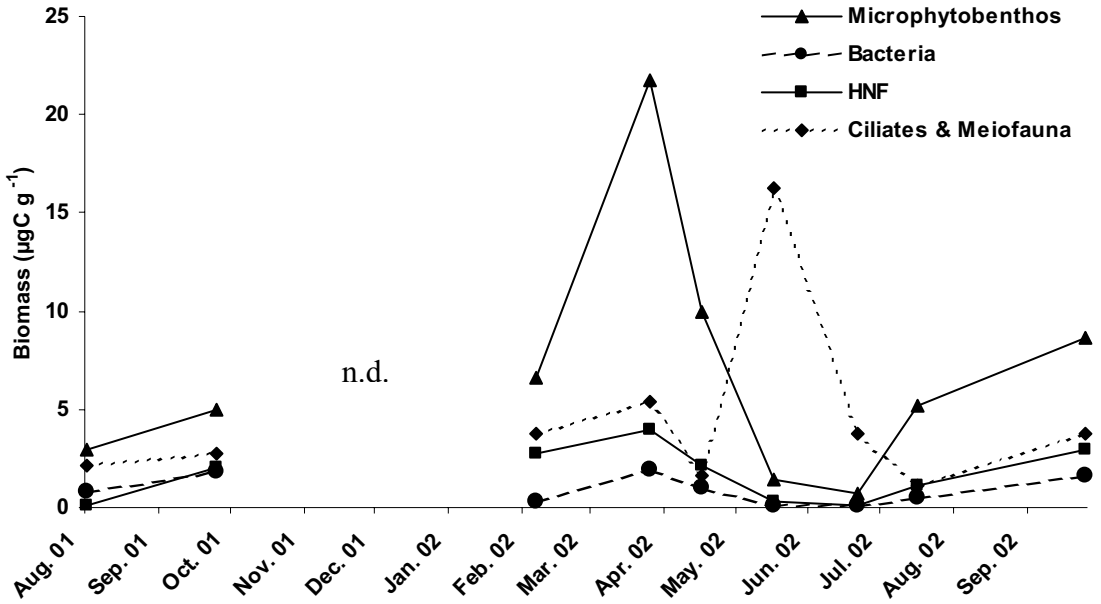


Fig. 9: Seasonal change of biomass (mgC g^{-1}) of the benthic microbial food web including microphytobenthos (diatoms, cyanobacteria and phototrophic nanoflagellates); bacteria; HNF = heterotrophic nanoflagellates, ciliates and meiofauna. . n.d.= no data available.

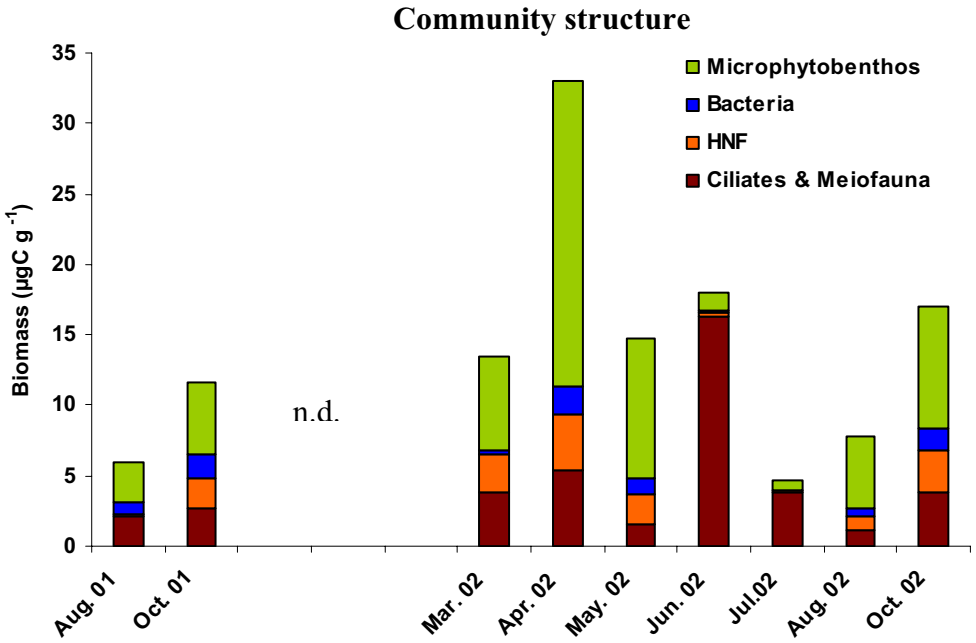


Fig. 10: Biomass partitioning (mgC g^{-1}) of the benthic microbial food web including microphytobenthos = diatoms, cyanobacteria and phototrophic nanoflagellates; bacteria; HNF = heterotrophic nanoflagellates, ciliates and meiofauna. n.d.= no data available.

According to Cadée & Hegeman (1974 and 1977) and Asmus & Asmus (1985) the microphytobenthic production in intertidal sediments increases from winter to spring and remains on a high level during summer. Consequently cells should accumulate during the period of high production. During spring a bloom of microphytobenthic biomass was indeed observed. During this period diatomivorous ciliates peaked too, but their grazing seemed not to be high enough to control micophytobenthic abundance. While literature data show that microphytobenthic production is high during summer, their biomass in this study was low. At the same period meiofauna biomass was extremely high. This indicated a top-down control of meiofauna on microphytobenhos during summer time. Other studies also suggest strong top-down effects on the microphytobenthic community during summer (Gray 1981; Rudnick et al. 1985; Epstein et al. 1992; Montagna et al. 1995; Sundbäck et al. 1996; Epstein 1997 ; Sigee et al. 1999 ; Middelburg et al. 2000; Pinckney et al. 2003).

The heavily grazed phytobenthic abundance recovered during late summer and autumn and a small bloom appeared. This might have happened because of decreasing meiofauna, mortality or encystment and habitat change of temporal meiofauna. At the same time less grazed phytobenthic species might have grown, such as cyanobacteria, PNF and other diatom species (Fig. 2, 3 and 4; Aberle-Malzahn 2004). Consequently the high grazing pressure, in other words the top-down control, decreased towards autumn.

Bacteria

The seasonal change of bacteria biomass was similar to the development of the microphytobenthic biomass with a bloom during springtime and autumn. This might be attributed to the increasing temperature and increasing availability of exudates from autotrophs. The bacterial production in marine sediments increases towards summer and decreass towards winter, similar to microphytobenthos primary production (Van Duyl & Kop 1990; Van Duyl 1992; Van Duyl et al. 1993; Epstein 1997; Ekebom 1999). Bacteria biomass was found indeed to be low during summer. According to Hondeveld et al. (1992; 1995; 1999) HNF are feeding on bacteria. A significant positive correlation was found between the abundance of medium size HNF and bacteria, explainable as feeding of medium size HNF on bacteria. However, according to literature data grazing of HNF appears to play a minor role in controlling the abundance of bacteria throughout the year (Bak & Nieuwland 1989; Kemp 1990; Bak et al. 1991; Gasol & Vaque 1993; Ekebom 1999; Wieltschnig et al. 2001). Thus the low bacteria biomass during summer could not sufficiently be explained by HNF grazing. Bacteria were also grazed by meiofauna, ciliates and small macrofauna in sediments, which were most abundant during summertime (study 3). However, according to Kemp (1987; 1990) small macrofauna removes only a few percent of bacterial production. Consequently it seems unlikely that macrofauna can effectively control bacteria abundance. Several studies suggest that temperature and organic matter availability have a considerable influence on bacteria abundance at least in winter, spring and late summer (Meyer-Reil 1983; Alongi 1985; Cole et al. 1988; Van Duyl et al. 1993; Pace & Cole 1994;Van Duyl & Kop 1994; Kirschner & Velimirov 1997; Manini et al. 2003). Based on these findings the low bacteria biomass during summertime might thus have been caused by a lack of organic matter availability, but this is a clear contradiction to the above mentioned high bacterial production (Van Duyl & Kop 1990; Van Duyl 1992; Van Duyl et al. 1993; Epstein 1997; Ekebom 1999). If this high bacterial production held true at the Dorum site, at least one different mechanism bringing down bacteria abundance has to be considered. Virus attacks could have been such a possible controlling factor at Dorum. According to Tuomi et al (1999); Middelboe et al. (2003) and Mei & Danovaro (2004) between 55% and 100% of total bacterial production in sediment are indeed subject to lysis by viral infection.

HNF

Several positive correlations were found between the abundance of different size groups of HNF and other components of the microbial food web. As pointed out before a significantly

positive correlation was found between the abundance of medium HNF and bacteria, which points to a predator-prey relationship. The seasonal change of the medium HNF biomass development was similar to the bacteria biomass development and clearly indicated by the positive significant correlation. This represented a bottom-up control of medium HNF by bacteria. These results are in accordance with literature showing that HNF are the main bacterivores within microbial food webs, but in contrast to our studies no size classification is made there (Hondeveld et al. 1992; Hondeveld et al. 1995; Hondeveld 1998; Hondeveld et al. 1999). In plankton habitats HNF are known to control bacteria abundance. However, in benthic habitats HNF grazing appears to play a minor role in controlling bacteria abundance throughout the year (Bak & Nieuwland 1989; Kemp 1990; Bak et al. 1991; Gasol & Vaque 1993; Ekebom 1999; Wieltschnig et al. 2001). Several positive correlations between the different size classes of HNF and various other components were found (Table 3). Medium HNF also correlated positively with small HNF, which indicated that medium HNF are feeding on smaller flagellates in addition to bacteria. The correlation between large HNF and diatoms indicated a predator-prey relationship. The correlation between small HNF and diatoms was interpreted in a way that small HNF fed on the exudates of diatoms, as it seems unlikely that small HNF (mean size 3 μm^3) fed on diatoms (mean size 189 μm^3) directly. Even if some specialized species were able to feed on prey larger than themselves, the use of polysaccharides exudates as from diatoms and of organic detritus in environments such as at the investigated study site is more likely (Fenchel 1987; Sherr 1988; Sanders 1991; Tranvik et al. 1993; Christoffersen et al. 1996; Jacobson 1999; Cleven & Weisse 2001). The positive correlation between small HNF and small PNF indicated either a predator-prey relationship or the use of PNF exudates by HNF.

These complex feeding interactions, based on significant correlations, were supported by literature data. Studies on the food spectrum of HNF exhibit that these organisms feed rather omnivorously on algae and other protists than on bacteria (Sanders 1991; Dietrich & Arndt 2000). Furthermore HNF can incorporate and use macromolecules such as polysaccharides (Sherr 1988; Sanders 1991; Tranvik et al. 1993; Christoffersen et al. 1996; Jacobson 1999; Cleven & Weisse 2001). These considerations combined with literature data exhibited a wide variety of HNF feeding preferences therefore HNF might not be regarded as a homogenous group of bacterivores.

Hypothetical carbon and energy flow

The data and findings of this study were assembled to a carbon and energy flow model for the four seasonal periods. We assumed that the benthic microbial systems in the intertidal fine sediment of Dorum followed a seasonal cycle with a primacy of bottom-up control during winter and spring with an increasing influence of grazing towards summer. The grazing influence then decreased again towards winter. Benthic microbial systems were very complex due to feedback mechanisms between prey and predators, allochthonous input and phenomena such as omnivory.

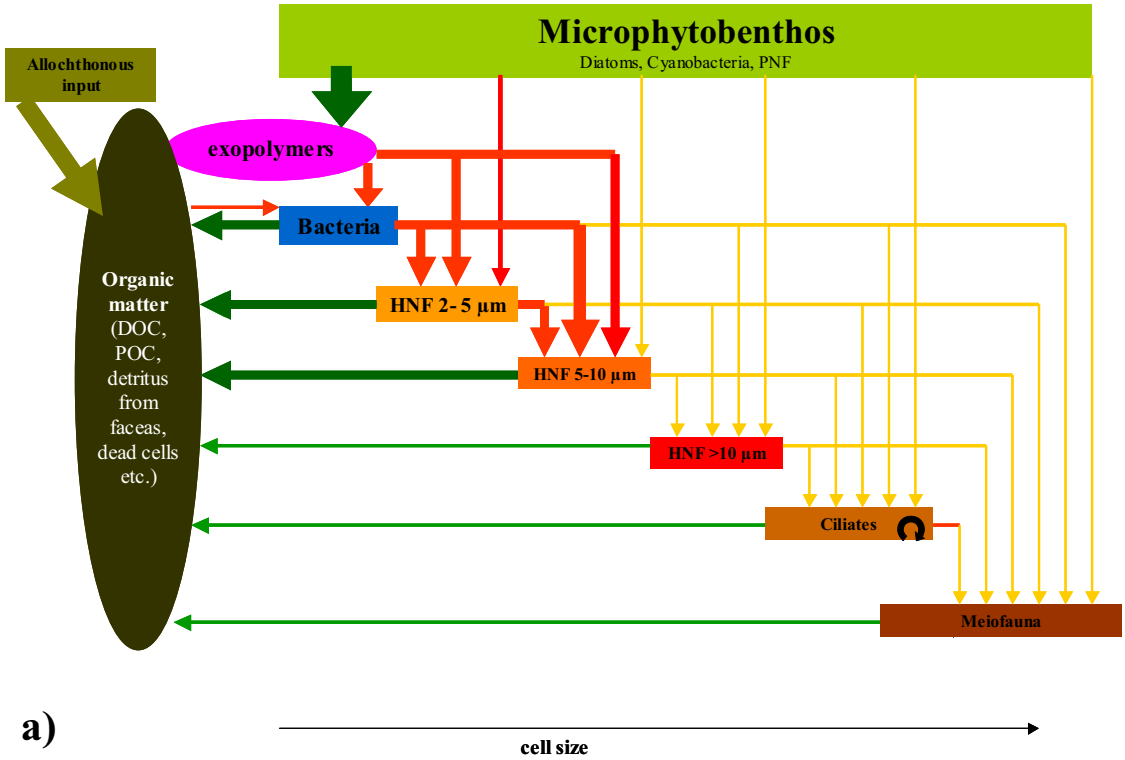
The growth season started during spring, when temperature and light intensity increased (Fig. 11a). Phytoplankton and phytobenthos, including diatoms, cyanobacteria and PNF, increased their production. This led to a higher abundance of these organisms and to a higher concentration of released exopolymers. This fresh organic input with simultaneously rising temperatures stimulated bacterial and small HNF production, followed by an increase of larger predators (larger HNF and Ciliates; Fig. 11a).

The primary production remained on a high level during summer. This consequently kept the organic input in shallow sediments on a high level also and therefore bacterial production remained on a high level (Fig. 11b). Benthic and planktonic metazoans contributed additionally to this input of organic carbon through “sloppy feeding”. An added bacterial production stimulus resulted from a positive feedback by heavy feeding of all bacterivores and their deposit to the organic matter pool (Lopez & Levinton 1987). Although detritus feeders rather fed on bacteria and diatoms than detritus, they simultaneously concentrated nutrients in their deposit which in turn kept bacterial production on a high level. These effects increased with rising numbers of deposit feeders (e.g. meiofauna and growing metazoan larvae) and ciliates during summer. At the end of summer the grazing pressure was high because of increasing numbers of predators and the phytobenthic population collapsed.

During autumn the heavily grazed phytobenthic abundance recovered and a small bloom occured (Fig. 11c). The decrease of predators due to mortality, encystment and the change of habitat by temporal meiofauna may have been one reason. The growth of other less grazed phytobenthic species (species shift) may have been instead or in addition another reason for this recovery. The increasing phytobenthic abundance was followed again by an increase of a rapid development of predators such as large HNF. At the same time temperature and light intensity decreased, consequently the primary production and its releases of expolymers were reduced and with it both bacteria and HNF reduced their abundance and production.

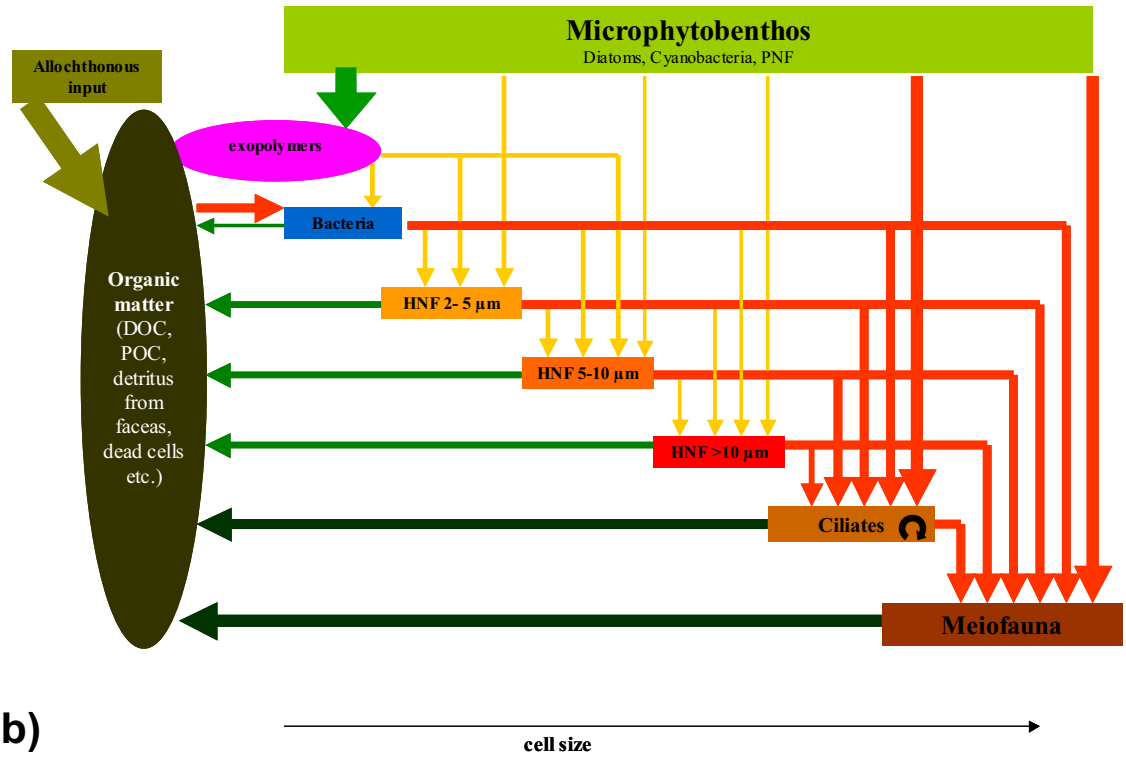
During winter, primary production as well as bacterial production were low, because of low light and low temperatures, respectively (Fig. 11d). Decreasing numbers of micro-, meio- and macrofauna caused additionally a lower grazing pressure.

Major carbon and energy flow in Dorum during springtime

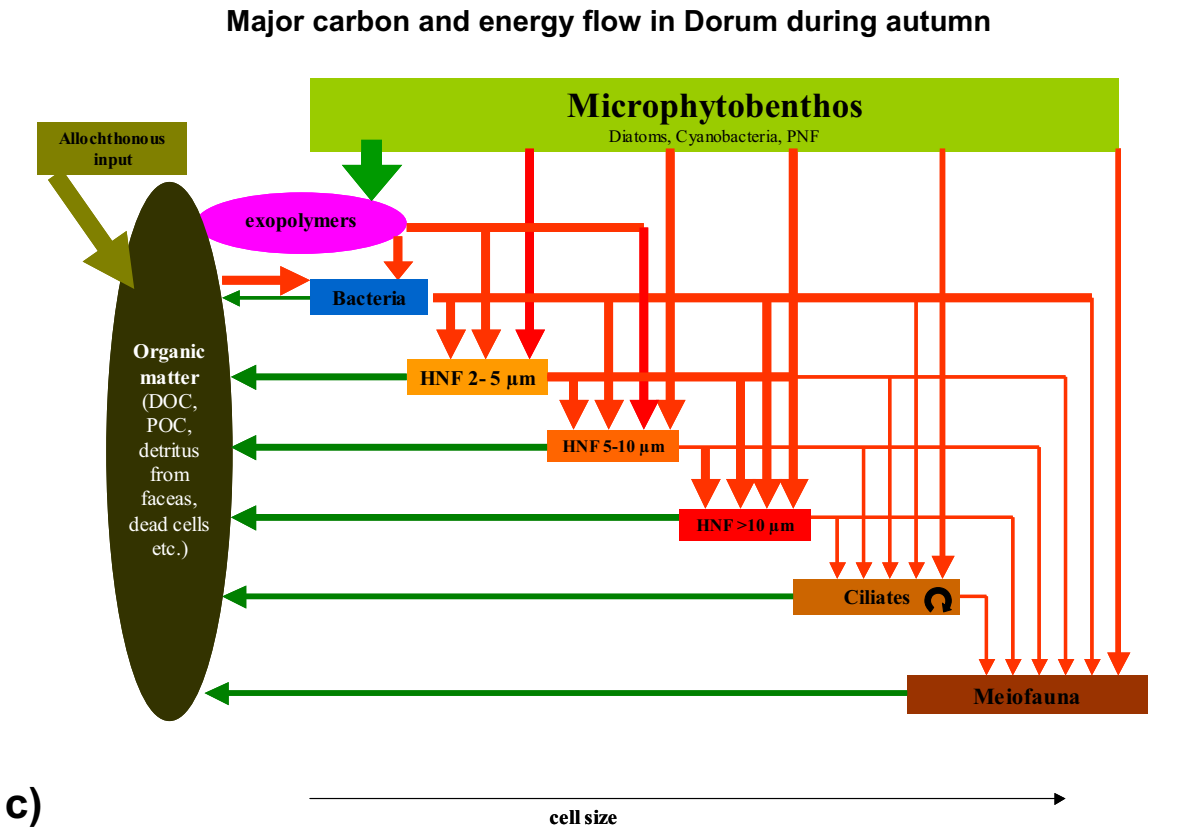


a)

Major carbon and energy flow in Dorum during summertime



b)



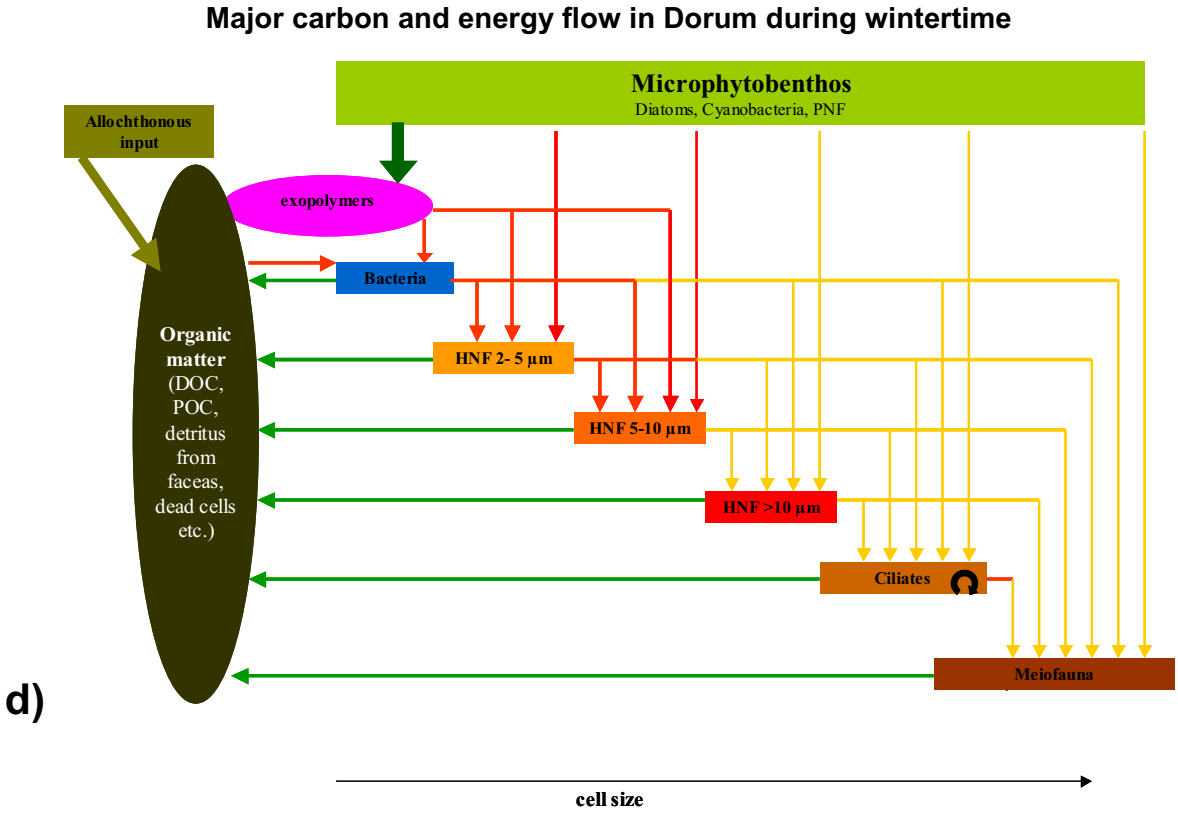


Fig. 11: Simplified scheme of major carbon and energy flow in the microbial benthic community of Dorum during (a) springtime, (b) summertime, (c) autumn and (d) winter. In order to visualize differences, box and arrow sizes and colour-saturation are proportional to their potential contribution to biomass and carbon flows.

Critical remarks

Measurement of Phytopigments

In the following remarks some difficulties concerning the determination of microphytobenthic biomass and phytopigment concentration in sediments will be discussed. A common method to convert the measured chlorophyll-*a* values into the biomass of primary producers uses a multiplication of chlorophyll-*a* with a conversion factor (e.g. 40; Manini et al. 2003 and references therein). We found a large discrepancy between the phototrophic biomass determined by counting organisms (0.7 - 21.7 µgC g^{-1}) and by converting chlorophyll-*a* values (92 - 580 µgC g^{-1}). This is also reported by others (Aberle-Mahlzahn 2004; Klein per. com.). Moreover no correlation could be identified between the chlorophyll-*a* concentration and the abundance of all primary producers. This excess biomass suggested additional important sources of chlorophyll-*a*. In coastal shallow sediments chlorophyll-*a* origins from diatoms, phototrophic euglenids, cryptophytes and dinoflagellates, different chlorophytes, seaweed leaves and cyanobacteria but also from freshly sedimented phytoplankton, incompletely digested cells in fecal pellets of herbivores and shreddered plant material. Indeed because of the method used (epifluorescent cell counts on 0.2 µm filters) we neither counted common large (>50 µm) phototrophic flagellates such as large Eugelnides, dinoflagellates and diatoms (>200 µm), nor pieces of undegraded macrophytes, shreddered plant material and sedimented phytoplankton nor undigested primary producer residuals in fecal pellets. These large cells might have an influence on the chlorophyll-*a*, but random counted samples and the calculation of their biomass revealed that they could not explain the discrepancy between chlorophyll-*a* and counted biomass (pers. observation). The ratio of phaeopigment : chlorophyll-*a* was calculated to estimate the freshness of the material. The values ranged between 0,2 to 0,5 and therefore indicated fresh and undigested material. The contribution of the "uncounted" groups to chlorophyll-*a* and phaeopigment seemed to play an important role in structuring the benthic microbial community.

Surprisingly, maximum peaks of chlorophyll-*a* were found in June and August when all counted phytobenthic organisms were low. For a better understanding it seems useful to mention that within sediments, chlorophyllides, phaeopigments (e.g. phaeophytin, pyrophaeophytin) and fluorescent chlorophyll catabolites (rcc = red chlorophyll catabolite, pfcc = primary fluorescent chlorophyll catabolite and fcc = fluorescent chlorophyll catabolite) are degradative products of different chlorophyll-*a* sources (e.g. Hoertensteiner et al. 2000). Furthermore chlorophyllide seem to be the major degradative products in marine sediments (Pace et al. 1979). The method of the acentone extraction and following fluorometric

measurement did not differentiate between fluorescent chlorophyll-*a* and fluorescent degradative products of chlorophyll-*a*. Converting the chlorophyll-*a* values of such a measurement to primary benthic biomass is therefore an inadequate method for the determination of living microphytobenthic biomass.

P:B (production:biomass) ratio

The sole analysis of biomass does not provide sufficient data to understand the dynamics in the food web. The P:B ratio represents an important indicator for the estimation of the contribution of a component to the energy and matter flow. A component with a high production can, even with a relatively low biomass, virtually fuel and sustain an entire system. This is known for instance from phytoplankton in upwelling systems where the P:B ratio is 1:0.2 d^{-1}. In systems with higher biomass the phytoplankton P:B ratios are found to be 1:0.036 d^{-1}, 1:0.08 d^{-1} and 1:0.14 d^{-1} for the Sylt-Rømø Bight, the Baltic Sea and Chesapeake Bay, respectively (Barnes & Hughes 1999 and references therein, Baird et al. 2004). Literature data on the production of microphytobenthos of the North Sea sandy shallow sediments vary with season between 22 and 2100 mgC m^{-2} d^{-1} (Cadée & Hegeman 1977; Sundbäck et al. 1996). The results of this study revealed a seasonal variation of microphytobenthos biomass by a factor of 10 to 20. Consequently the P:B ratio should increase by a factor of 10 to 20 from winter to summer. This supports the assumption that during summer low microphytobenhtic biomass could fuel the high herbivorous biomass of ciliates and meiofauna.

Acknowledgments

The authors thank the following people for their help: Dr. J. Matthiessen for assistance in measuring environmental parameters; Dr. W. Wosniock in statistical questions; Dr. W. Stumm for reading the proofs. Dr. S. Schultes and Dr. U. Schneider for comments on previous versions of the manuscript. The present work has been financed by the DFG (Deutsche Forschungs Gemeinschaft) grant number BE 2279/3-1, by the Alfred-Wegener-Institute for Polar and Marine Research and by the German Social Security System.

References

Aberle-Malzahn, N., 2004. The microphytobenthos and its role in aquatic food webs. Ph.D. Thesis, Christian-Albrechts-Universität Kiel, Germany, unpublished.

Agatz, M., Asmus, R.M., Deventer, B., 1999. Structural changes in the benthic diatom community along a eutrophication gradient on a tidal flat . Helgol. Mar. Res. 53, 92-101.

Alongi, D.M., 1985. Effect of physical disturbance on population dynamics and trophic interactions among microbes and meiofauna. J. Mar. Res. 43, 351-364.

Alongi, D.M., 1991. Flagellates of benthic communities: characteristics and methods of study. In: Patterson, D.J., Larsen, J. (Eds.), The biology of free-living heterotrophic flagellates. Systematics association by Clarendon Press, Oxford, England, pp. 57-75.

Asmus, H., Asmus, R., 1985. The importance of grazing food chain for energy flow and production in three intertidal and sand bottom communities of the northern Wadden Sea. Helgol. Wiss. Meeresunters. 39, 273-302.

Bak, R.P.M., Nieuwland, G., 1989. Seasonal fluctuations in benthic Protozoan populations at different depth in marine sediments. Neth. J. Sea. Res. 24, 37-44.

Bak, R.P.M., Van Duyl, F.C., Nieuwland, G., 1995. Organic sedimentation and macrofauna as forcing factors in marine benthic nanoflagellate communities. Microbial. Ecol. 29, 173-182.

Bak, R.P.M., Van Duyl, F.C., Nieuwland, G., Kop, A.J., 1991. Benthic heterotrophic nanoflagellates in North Sea field/mesocosm bottoms and their response to algal sedimentation. Ophelia 33, 187-196.

Barranguet, C., Herman, P.M.J., Sinke, J.J., 1997. Microphytobenthos biomass and community composition studied by pigment biomarkers: importance and fate in the carbon cycle of a tidal flat. J. Sea. Res. 38, 59-70.

Barnes, R.S.K., Hughes, R.N., 1999. An introduction to marine ecology. Blackwell Science Ltd, London, pp286.

Beardsley, C., Gieseke, A., Wickham, S., Berninger, U.-G., 1997. The role of benthic ciliates in intertidal sediments - vertical distribution and trophic interactions. J. Euk. Microbiol., Abstract.

Berninger, U.-G., Epstein, S.S., 1995. Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea sediment. Aquat. Microb. Ecol. 9, 229-236.

Berninger, U.-G., Huettel, M., 1997. The impact of flow on the oxygen dynamics in photosynthetically active sediments. Aquat. Microb. Ecol. 12, 291-302.

Borsheim, K.Y., Bratbak, G., 1987. Cell volume to cell carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar. Ecol. Prog. Ser. 36, 171-175.

Bostroem, B., Pettersson, A.-K., Ahlgren, 1989. I. Seasonal dynamics of a cyanobacteria-dominated microbial community in surface sediments of a shallow, eutrophic lake. Aquatic Science. 51(2), 153-178.

Böttcher, M.E., Hespenheide, B., Llobet-Brossa, E., Beardsley, C., Larsen, O., Schramm, A., Wieland, A., Böttcher, G., Berninger, U.-G., Amann, R., 2000. The biogeochemistry, stable isotope geochemistry, and microbial community structure of a temperate intertidal mudflat: an integrated study . Cont. Shelf Res. 20[12-13], 1749-1769.

Buchanan, J.B., 1984. Sediment analysis. In: Methods for study on marine benthos. Eds: Holme, N.A., McIntyre, A.D., Blackwell science, pp. 41-65.

Cadée, G.C., Hegeman, J., 1974. Primary production of the benthic microflora living on tidal flats in the Dutch Wadden Sea. Neth. J. Sea Res. 8, 260-291.

Cadée, G.C., Hegeman, J., 1977. Distribution of primary production of the benthic microflora and accumulation of organic matter on the tidal flat area, Balgzand, Dutch Wadden Sea. Neth. J. Sea Res. 11, 24-41.

Cammen, L.M., 1992. Annual bacterial production in relation to benthic microalgae production and sediment oxygen uptake in an intertidal sandflat and an intertidal mudflat. Mar. Ecol. Prog. Ser. 71, 13-25.

Cammen, L.M., Walker, J.A., 1986. The relationship between bacteria and micro-algae in the sediment of a Bay of Fundy mudflat. Estuar. Coast. Shelf Sci. 22, 91-99.

Caron, D.A., Lim, L.E., Miceli, G., Waterbuty, J.B., Valois, F.W., 1991. Grazing and utilization of chroocoid cyanobaceria and heterotrophic bacteria by protozoa in laboratory cultures and a coastal plankton community. Mar. Ecol. Prog. Ser. 76, 205-217.

Christoffersen, K., Bernard, C., Ekebom, J., 1996. A comparison of the ability of different heterotrophic nanoflagellates to incorporate dissolved macromolecules. Adv. Limnol. 48, 73-84.

Cleven, E.-J., 1996. Indirectly flourescently labelled flagellates (IFLF): a tool to estimate the predation on free-living heterotrophic flagellates. J. Plankton Res. 18, 429-442.

Cleven, E.-J., Weisse, T., 2001. Seasonal succession and taxon-specific bacterial grazing rates of heterotrophic nanoflagellates in Lake Constance. Aquat. Microb. Ecol. 23, 147-161.

Cole, J.J., Finlay, S., Pace, M.L., 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. Mar. Ecol. Prog. Ser. 43, 1-10.

Cook, P.L.M., Butler, E.C.V., Eyre, B.D., 2004. Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. I. Benthic metabolism . Mar. Ecol. Prog. Ser. 280, 28-38.

Cook, P.L.M., Revill, A.T., Butler, E.C.V., Eyre, B.D., 2004. Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling. Mar. Ecol. Prog. Ser. 280, 39-54.

Cook, P.L.M., Revill, A.T., Clementson, L.A., Volkman, J.K., 2004. Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. III. Sources of organic matter. Mar. Ecol. Prog. Ser. 280, 55-72.

Daemen, E.A.M.J., 1986. Comparison of methods for the determination of Chorophyll in estuarine sediments. Neth. J. Sea Res. 20, 21-28.

Danovaro, R., Marrale, D., Della Croce, N. , Parodi, P., Fabiano, M., 1999. Biochemical composition of sedimentary organic matter and bacterial distribution in the Aegean Sea: trophic state and pelagic-benthic coupling. J. Sea Res. 42, 117-129.

Dell'Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Mar. Pollut. Bull. 44, 611-622.

Dietrich, D., Arndt, H., 2000. Biomass partitioning of benthic microbes in a Baltic inlet: relationships between bacteria, algae, heterotrophic flagellates an ciliates. Mar. Biol. 136, 309-322.

Edler, L., 1979. Recommendations for marine biological studies in the Baltic Sea: phytoplankton and chlorophyll. The Baltic Marine Biologists 5, 1-37.

Ekebom, J., 1999. Heterotrophic nanoflagellates and bacteria in sediment of a brackish water sill basin in the Baltic Sea. Hydrobiologia 393, 151-161.

Epstein, S.S., 1997. Microbial food webs in marine sediments. II Seasonal changes in trophic interactions in a sandy tidal flat community. Microb. Ecol. 34, 199-209.

Epstein, S.S., Burkovsky, I.V., Shiaris, M.P., 1992. Ciliate grazing on bacteria, flagellates, and microalgae in a temperate zone sandy tidal flat: ingestion rates and food niche partitioning. J. Exp. Mar. Biol. Ecol. 165, 103-123.

Epstein, S.S., Rossel, J., 1995. Enumeration of sandy sediment bacteria: search for optimal protocol. Mar. Ecol. Prog. Ser. 117, 289-298.

Fenchel, T., 1967. The ecology of marine microbenthos I. The quantitative importance of Ciliates as compared with Metazoans in various types of sediment. Ophelia 4, 121-137.

Fenchel, T., 1968. The ecology of marine Microbenthos II. The food of marine benthic Ciliates. Ophelia 5, 73-121.

Fenchel, T., 1969. The ecology of marine Microbenthos IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated Protozoa. Ophelia 6, 1-182.

Fenchel, T., 1982. Ecology of heterotrophc Microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar Ecol. Prog. Ser. 9, 35-42.

Fenchel, T., 1987. Ecology of Protozoa. The biology of free-living phagotrophic Protists. Science Tech, Wisconsin, pp197.

Fretwell, S.D., 1977. The regulation of plant communities by food chains exploiting them. Perspectives in Biology and Medicine 20, 169-185.

Fry, J.C., 1990. Direct methods and biomass estimation. In: Grigorova, R., Norris, J.R. (Eds). Methods in microbiology. pp. 41-85. Academic Press, London.

Gasol, J.M., Vaque, D., 1993. Lack of coupling between heterotrophic nanoflagellates and bacteria: a general phenomenon across aquatic systems? Limnol. Oceanogr. 38, 657-665.

Gray, J.S., 1981. The ecology of marine sediments. Cambridge University Press, Cambridge, pp185.

Giere, O., 1993. Meiobenthology: the microscopic fauna in aquatic sediments. Springer Berlin, pp328.

Hamels, I., Moens, T., Muylaert, K., Vyverman, W., 2001. Trophic interactions between ciliates and nematodes from an intertidal flat. Aquat. Microb. Ecol. 26, 61-72.

Hoertensteiner, S., Chinner, J., Matile, P., Thomas, H., Donnison, I.S., 2000. Chlorophyll breakdown in *Chlorella protothecoides*: characterization of degreening and cloning of degreening-related genes. Plant Mol. Biol. 42, 439-450.

Hondeveld, B.J.M., 1998. Heterotrophic nanoflagellates and their role as bacterivores in marine sediments. Ph.D. Thesis, Universiteit van Amsterdam, Texel, Netherlands, unpublished.

Hondeveld, B.J.M., Bak, R.P.M., Raaphorst, W.V., Van Duyl, F.C., 1999. Impact of grazing by benthic eucaryotic organisms on the nitrogen sediment-water exchange in the North Sea. J. Sea Res. 41, 255-268.

Hondeveld, B.J.M., Bak, R.P.M., Van Duyl, F.C., 1992. Bacterivory by heterotrophic nanoflagellates in marine sediments measured by uptake of flourescently labeled bacteria. Mar. Ecol. Prog. Ser. 89, 63-71.

Hondeveld, B.J.M., Nieuwland, G., Van Duyl, F.C., Bak, R.P.M., 1994. Temporal and spatial variations in heterotrophic nanoflagellate abundance in North Sea sediments. Mar. Ecol. Prog. Ser. 109, 235-243.

Hondeveld, B.J.M., Nieuwland, G., Van Duyl, F.C., Bak, R.P.M., 1995. Impact of nanoflagellate bacterivory on benthic bacterial production in the North Sea. Neth. J. Sea Res. 34, 275-287.

Hoppenrath, M., 2000. Taxonomische und ökologische Untersuchung von Flagellaten mariner Sande. Ph.D. Thesis, Universität Hamburg, Germany, unpublished.

Jacobson, D.M., 1999. A brief history of dinoflagellate feeding research. J. Eukaryot. Microbiol. 46, 376-381.

Kemp, P. F. Potential impact on bacteria of grazing by a macrofaunal deposit-feeder, and the fate of bacterial production. Mar. Ecol. Prog. Ser. 1987; 36:151-161.

Kemp, P.F., 1990. The fate of benthic bacterial production. Aquat. Sci. 2, 109-124.

Kirschner, A.K.T., Velimirov, B., 1997. A seasonal study of bacterial community succession in a temperate backwater system, indicated by variation in morphotype numbers, biomass, and secondary production . Microb. Ecol. 34, 27-38.

Knox, G.A., 2000. The ecology of seashores. CRC Press, London

Lee, W.J., Patterson, D.J., 2002. Abundance and biomass of heterotrophic flagellates, and factors controlling their abundance and distribution in sediments of Botany Bay. Microb. Ecol. 43, 467-481.

Lee, W.J., Patterson, D.J., 2002. Optimising the extraction of bacteria, heterotrophic protists and diatoms, and estimating their abundance and biomass from intertidal sandy sediments. Korean Soc. Oceanogr. 37[2], 58-65.

Lopez, G.R., Levinton, J.S., 1987. Ecology of deposit-feeding animals in marine sediments. Q. Rev. Biol. 62, 235-260.

Lorenzen, C.J., 1976. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnol. Oceanogr. 12, 343-346.

Lucas, C.H. Holligan, P.M., 1999. Nature and ecological implications of algal pigment diversity on the Molenplaat tidal flat (Westerschelde Estuary, SW Netherlands). Mar. Ecol. Prog. Ser. 180, 51-64.

Maclassac, E.A., Stoeckner, J.G., 1993. Enumeration of phototrophic picoplancton by autofluorescence microscopy. In: Handbook of Methods in Aquatic Microbial Ecology. Eds: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. Lewis Boca Raton, FL, USA, pp 187-97.

Manini, E., Fiordelmondo, C., Gambi, C., Pusceddu, A., Danovaro, R., 2003. Benthic microbial loop functioning in coastal lagoons: a comparative approach. Oceanol. Acta. 26, 27-38.

Mei, M. L., Danovaro, R., 2004. Virus production and life strategies in aquatic sediments. Limnol. Oceanogr. 49(2), 459-470.

Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationship for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr. 45, 569-579.

Meyer-Reil, L.-A., 1983. Benthic response to sedimentation events during autumn to spring at a shallow water station in the Western Kiel Bight II. Analysis of benthic bacterial populations. Mar. Biol. 77, 247-256.

Middelboe, M., Glud, R. N., Finster, K., 2003. Distribution of viruses and bacteria in relation to diagenetic activity in an estuarine sediment. Limnol. Oceanogr. 48(4), 1447-1456.

Middelburg, J.J., Barranguet, C., Boschker, H.T.S., Herman, P.M.J., Moens, T., Heip, C.H.R., 2000. The fate of intertidal microphytobenthos carbon: An in situ super(13)C-labeling study. Limnol. Oceanogr. 6, 1224-1234.

Montagna, P.A., Blanchard, G.F., Dinet, A., 1995. Effect of production and biomass of intertidal microphytobenthos on meiofaunal grazing rates. J. Exp. Mar. Biol. Ecol. 185, 149-165.

Odum, E.P., 1971. Fundamentals of ecology. Saunders Company, London, pp574.

Pace, M.L., Shimmel, S., Darley, W.M., 1979. The effect of grazing by a gastropod, *Nassarius absoletus*, on the benthic microbial community of a salt marsh mudflat. Estuar. Coast. Mar. Sci. 9, 121-134.

Passow, U., 2002. Production of transparent exopolymer particles (TEP) by phyto- and bacterioplankton. Mar. Ecol. Prog. Ser. 236, 1-12.

Pinckney, J.L., Carman, K.R., Lumsden, S.E., Hymel, S.N., 2003. Microalgal-meiofaunal trophic relationships in muddy intertidal estuarine sediments . Aquat. Microb. Biol. 31[1], 99-108.

Power, M.E., 1992. Top-down and bottom-up forces in food webs: do plants have primacy. Ecology 73, 733-746.

Premke, K., Arndt, H., 2000. Predation on heterotrophic flagellates by protists: Food selectivity determined using a live-staining technique. Arch. Hydrobiol. 150, 17-28.

Reineck, H.-E., 1983. Sediment and dynamical processes. In: Ecology of the Wadden Sea. Eds: Wolff, W.J., A. A. Balkema, Rotterdam, pp 49.

Rudnick, D.T., Elmgren, R., Frithsen, J.B., 1985. Meiofaunal prominence and benthic seasonality in a coastal marine ecosystem. Oecologia 67, 157-168.

Sanders, R.W., 1991. Trophic strategies among heterotrophic flagellates. In: The biology of free-living heterotrophic flagellates. Eds: Patterson, D.J., Larsen, J. Systematics association by Clarendon press, Oxford, UK, pp 21-38.

Sherr, E., Sherr, B., 1988. Role of microbes in pelagic food webs: A revised concept. Limnol. Oceanogr. 33, 1225-1227.

Sherr, E.B., 1988. Direct use of high molecular weight polysaccharide by heterotrophic flagellates. Nature 335, 348-351.

Sherr, E.B., Caron, D.A., Sherr, B.F., 1993. Staining of heterotrophic protists for visualization via epiflourescence microscopy. In: Handbook of Methods in Aquatic Microbial Ecology. Eds: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. Lewis Boca Raton, Fl, USA, pp. 213-27.

Sigee, D.C., Andrews, M.J., Bellinger, E.G., Butler, R.D., Epton, R.D., Hendry, R.D., 1999. Biological control of cyanobacteria: principles and possibilities. Hydrobiologia 395/396, 161-172.

Sundbäck, K., Nilsson, P., Nilsson, C., Jönsson, B., 1996. Balance between autotrophic and heterotrophic components and processes in microbenthic communities of sandy sediments: a field study. Estuar. Coast . Mar. Sci. 43, 689-706.

Tranvik, L.J., Sherr, E.B., Sherr, B.F., 1993. Uptake and utilization of `colloidal DOM´ by heterotrophic flagellates in seawater. Mar. Ecol Prog. Ser. 92, 301-309. 1993.

Tuomi, P., Lundsgaard, C., Ekebom, J., Olli, K., and Kuennis, K., 1999. The production and potential loss mechanisms of bacterial biomass in the southern Gulf of Riga. J. Mar. Syst. 23(1-3), 185-196.

Van Duyl, F.C., 1992. Mesocosm experiments: mimicking seasonal developments of microbial variables in North Sea sediments. Hydrobiologia 235/236, 267-281.

Van Duyl, F.C., Kop, A.J., 1990. Seasonal patterns of bacterial production and biomass in intertidal sediments of the western Dutch Wadden Sea. Mar. Ecol Prog. Ser. 59, 249-261.

Van Duyl, F.C., Kop, A.J., 1994. Bacterial production in North Sea sediments: Clues to seasonal and spatial variations. Mar. Biol. 120, 323-337.

Van Duyl, F.C., Van Raaphorst, W., Kop, A.J., 1993. Benthic bacterial production and nutrient sediment-water exchange in sandy North Sea sediments. Mar. Ecol. Prog. Ser. 100, 85-95.

Velji, M.I., Albright, L.J., 1993. Improved sample preparation for enumeration of aggregated aquatic substrate bacteria. In: Handbook of Methods in Aquatic Microbial Ecology. Eds: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. Lewis Boca Raton, FL, USA, pp139-42.

Waterbury, J.B., Watson, S.W., Valois, F.W., Franks, D.G., 1986. Biological and ecological characterization of the marine unicellular cyanobacterium Synechococcus. Can. Bull. Fish. Aquat. Sci. 214, 71-120.

Wieltschnig, C., Kirschner, A.K.T., Steitz,A., Velimirov,B., 2001. Weak coupling between heterotrophic nanoflagellates and bacteria in a eutrophic environment. Microb. Ecol. 42, 159-167.

Wolter, K., 1982. Bacterial Incorporation of organic substances released by natural phytoplankton populations. Mar. Ecol. Prog. Ser. 7, 287-295.

Study 2

Structure and biomass partitioning of benthic microbial communities in temperate and arctic climate

K. Stumm[1)], Y. Lei[2, 3)], U.-G. Berninger[3)]

[1)] Alfred - Wegener - Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

[2)] Institute of Oceanology, Chinese Academy of Science, 266071 Qingdao, PR China

[3)] University Salzburg, Hellbrunner Strasse, 34, 5020 Salzburg, Austria

Abstract

Little information is available about the trophic structure of benthic microbial communities and the influences of the environmental attributes. Systematic comparisons of microbial communities in soft sediments in different climatic and geographic regions and salinity situations are rare. Therefore the structures of small benthic communities in marine and freshwater systems in Germany, USA and North East Greenland were investigated in order to identify interactions and causal connections between microbial communities and environmental attributes. The abundance of bacteria, cyanobacteria, diatoms, heterotrophic and phototrophic nanoflagellates in sediments from beaches and lakes in the above named areas were enumerated and biomass were calculated. Environmental parameters in the sediment (water content, grain size, total carbon content, organic carbon content, nitrogen content, chlorophyll-*a* content) were measured and their correlations and eventual relationships with organisms´ biomass and community structures were analysed and discussed.

Abundance and biomass at different sites revealed considerable differences, whereby geographical position, climate and salinity were not the main structuring factors. The study revealed substantial influences of the amount of easily available carbon and of regional particularities (such as wave shock) on microbial communities. The contribution of bacteria and HNF to the communities' heterotrophic yield, in the sense of Fenchel (1974), added up to 93% ± 10% in all investigated sites regardless of their contribution to the heterotrophic biomass. The importance of bacteria and HNF in the carbon and energy flow seemed therefore to be less diverse at different investigated sites as initially assumed on base of the observed differences in biomass and abundance. The functional role of microbes in benthic communities proved to be similar at all investigated sites.

1. Introduction

Several studies exhibit the importance of microbial components for the cycle of organic matter and the transfer of carbon and energy to higher trophic levels (Fenchel 1969; Azam et al. 1983; Porter et al. 1985; Hondeveld et al.1994; Epstein 1997; Dietrich & Arndt 2000; Manini et al. 2003; study 1). However, in general the functional role and the trophic structure of benthic microbial communities have not been as thoroughly investigated.

Light, temperature, the presence of liquid water, the concentration of gas and ions are considered among others to be the most important environmental factors, governing the distribution of organisms (Odum 1971; Begon et al. 1996).

The light and temperature depending biomass of phototrophic primary producers are expected to be lower in polar regions than in the temperate areas at least in terrestrial habitats. The abundance of benthic microorganisms is furthermore known to be highly dependent on the grain size distribution, oxygen availability and organic matter availability in the sediment (Fenchel 1969.; Manini et al. 2003). Normally the thickness of the oxic layer ranges from millimetres up to centimetres in soft sediments depending on grain size (Gray 1981; Berninger & Huettel 1997). According to literature data the maximum abundance of microbial organisms (nano- micro- and meiofauna and microphyobenthos) is found in the oxic layer in littoral sediments of medium grain size (0.063 to 0.25 mm; Fenchel 1969; Alongi 1991; Hondeveld et al. 1994; Berninger & Epstein 1995). The organic matter availability depends on the composition of components. Humic substances of plant material (lignin and cellulose) are of high molecular weight, often dark coloured and persistent against microbiodegradadation (Wetzel 2001). The turnover rates are low and their percentage of freshwater sediment´s organic matter is higher than in marine sites (Capone & Kiene 1988). Organic matter availability and the abundance of benthic microorganisms are therefore expected to be clearly different between marine and freshwater systems.

Salinity plays an important role in the osmotic physiology of organisms which are spending high energy to keep the proper salt concentration within their cells. For many protozoan species the salinity seems however to play a minor role when a slow adaptation of these organisms can take place (Fenchel 1969; 1987; Patterson & Larsen 1991). Many flagellate and ciliate species are known to live in salinities ranging from 0 - 40 PSU (Fenchel 1969).

In general, harsh environmental conditions (to be expected with increasing latitude) as well as a high rate of perturbation and instability are known to decrease species abundance and diversity. However, many protozoan species are known to be cosmopolitan and many of them

are adapted to a broad spectrum of environmental factors. The importance of different factors structuring the benthic microbial communities in a given environment is less known, systematic investigations on microbial communities in different climatic and geographic regions and salinity conditions are rare.

This study aimed at the identification of interactions and causal connections between microbial communities and environmental parameters in different habitats. Freshwater and marine microbial communities in different climatic and geographic regions were therefore investigated. The abundance and biomass of bacteria, cyanobacteria, diatoms, heterotrophic and phototrophic flagellates in littoral sediments from marine regions and freshwater lakes in Germany, North America and North East Greenland were determined and relationships with environmental parameters were analysed and discussed.

2. Materials and Methods

Study sites

This study was carried out in shallow marine and freshwater sediments in Germany, the East coast of USA and the North East Greenland from August 2001 to December 2003.

Germany

The climate of Germany is temperate with characteristic cold winters (mean temperature in January in Dorum +3°C), warm summers (mean temperature in July in Dorum +18°C) and moderate springs and autumns. Precipitation is sufficient for the growth of green meadows and boreal forests. Sea ice-coverage during severe winters is rare (~six to ten times within the last 60 years).

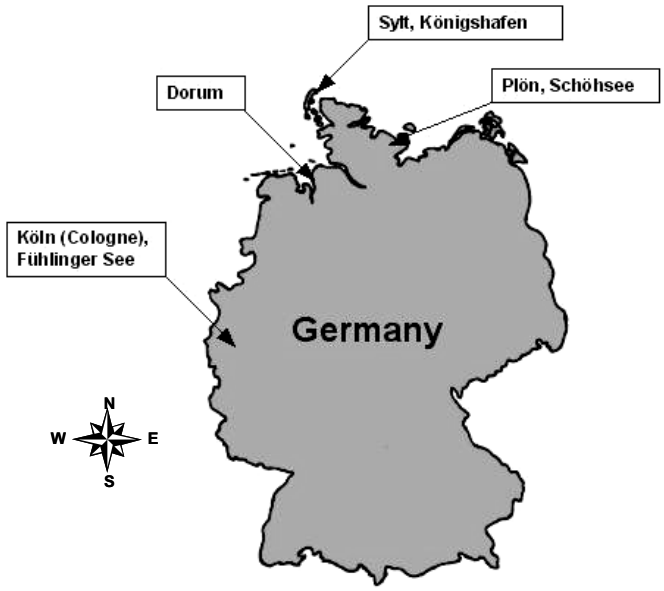


Fig. 4: Map of the sampling sites in Germany

Dorum is located northern of Bremerhaven, Germany (53°42`N; 8°29`E) in the national park "Niedersächsisches Wattenmeer" in the North Sea (Fig. 4). The sampling site was located within an intertidal mudflat approx 500 m off the high tide shore line. The tides in this area have a cycle of 12 h with a level difference of 3 m and the whole area drops slowly so the sampling field was submerged for a time of only 3 h by 1 m deep water.

Sylt is an island in the North Sea in Germany (Fig. 4). The **Königshafen** is a sheltered intertidal bay of 4.8 km^2 in the North of the island (55°02`N; 8°25`E). The sampling site was located within the bay between 100 m and 400 m from the shoreline. Like in Dorum the tides are semidiurnal with a level difference of around 2 m. Depending on the position of the

sampling site within the bay, the sediment is exposed to the air for 3 h and 6 h per cycle respectively. A general description of the area is given by Reise (1985).

Both areas are situated within a tidal mudflat where the temperature can range between –5°C and 35°C with salinities between 5 and 32 PSU. The sediment in both areas was macroscopically dominated by wave ripple marks (about 2 cm high) and fecal strings of marine Polychaets (such as *Arenicola marina*). Other highly abundant macroinvertebrates were the mudsnail *Hydrobia ulvae* Pennant 1777 and the sand hopper *Corophium* sp. Pallas 1766. Macroalgae such as *Ulva* sp. or vascular plants of the genus *Zostera* were only sparsely present or as in Sylt at places closer to the beach. The salinity is variable which is typical for an intertidal mudflat with freshwater input nearby.

The **Schöhsee** in Plön Germany (54°09`N; 10°26`E) is a small freshwater lake developed at the end of the Weichsel ice age (Fig. 4). The lake covers an area of 0,78 km^2 and has an maximum depth at 29.4 m (medium depth 10.9 m). The lake is categorised as an oligotrophic lake of low productivity and intensely used for recreation and tourism. The sampling area was located on a small sandy beach surrounded by forest on the peninsula “kleiner Warder” with a water depth of around 0.8 m.

The **Fühlinger See**, Germany (50°58`N; 74°01`E) is a 20 years old complex of meso-eutrophic lakes connected by channels for local recreation (as a boat-racing course, diving) (Fig. 4). The surrounding is covered by a park with lawns and small forests. The water level of the mainly groundwater fed lake is influenced by the water levels of the nearby river Rhein which causes a seasonal fluctuation of about 2 m. The sampling area was located in a side lake part which covers around 4 ha with maximum depth of 14 m. The lake is dimictic and stratified between April and October with a thermocline in a depth between 5 m and 8 m (Auer et al. 2003). Sediment was taken from near the shore in about 30 cm to 50 cm deep water.

North America

The sampling sites in Northern America were located in New Jersey and Pennsylvania. This temperate region exhibits typical cold winters (mean temperature in January in NJ –1.6°C), warm summers (mean temperature in July in NJ 22°C) and moderate inter seasonal periods. Precipitation is sufficient for the growth of green meadows and boreal forests (Fig. 5).

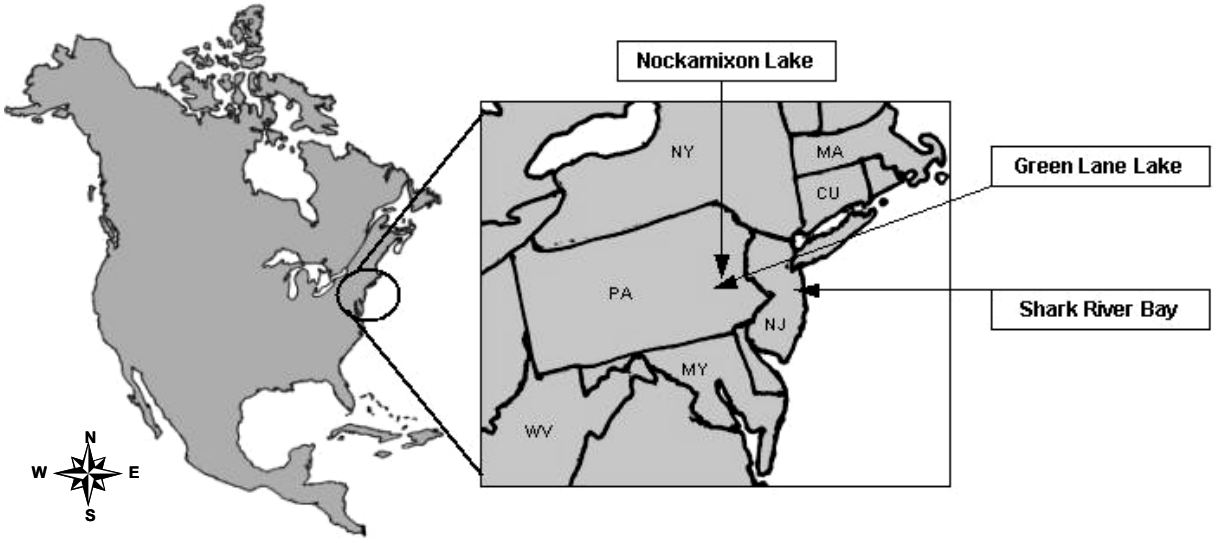


Fig 5: Map of the sampling sites in North America

The **Shark River Bay** near Belmar, New Jersey USA (40°10`N; 74°01`E) is an estuarine bay or a tidal basin with upland freshwater tributaries coming from the East and South of the bay (Fig. 5). Ocean's tides flushing in and out through the Shark River Inlet, a small 1.5 km long channel connecting the bay with the ocean. The average tidal water level change is about 1.8 m in a semidiurnal cycle. The region is highly frequented by human activity (shipping and fishing) and adjacent towns, harbors and roads empty their waste waters into the bay, therefore the pollution is high (www.waterwire.net). The sampling area was located on the Shark River Island 2 m from high tide line. Water and air temperature vary with the season of an typical temperate maritime climate.

The **Green Lane Lake** Pennsylvania, USA (40°20`N; 75°27`E) is a 25 years old reservoir of a small river surrounded by a recreation park (Fig. 5). The beach of this lake is man-made consisting of coarse sand. On the sampling day the air temperature was –2°C, the water temperature was 1°C. Due to the low temperature the surface water near the beach was frozen to a 1-3 mm thick ice layer. The samples were taken near shore in 30 cm deep water from under the ice layer, the sediment was not frozen.

The **Nockamixon Lake** in Pennsylvania, USA (40°28`N; 75°13`W) is located in a state park near Quakertown. Nocka-miska-ing is an Indian name and means "the place of soft soil" (Fig. 5). The brownish yellow color of the water indicate that the water was containing a high amount of humid acids from the surrounding forest. The lake came into existence by the Nockamixon Dam (31 m high, built 1973) which flooded the Tohickon creek valley. The lakewater covers 5.2 km^2 with a maximum depth of 27 m with summer statification. The whole side is intensely used as recreation area. Vicinal towns and farms are responsible for a high supply of waste water and fertilizers effecting a high nutrient content and eutrophic conditions (www.pennridge.org/works/Nockamixonhm.html).

North East Greenland

The investigated sites in an arctic climate were located on the North East coast of Greenland, north of the 10°C July isotherm and even north of the Arctic Circle, located within continuous permafrost with pack ice in winter (roughly from October to May; Fogg 1998; Fig. 6). Literature about regions at the latitude of the arctic regions document 24 h of daylight from end of April to mid of August with monthly mean temperatures rising up to +5°C, and the absence of light (24 h night) from the end of October to mid of February with monthly mean temperatures of around –24°C (Fogg 1998; Jokat 2004). The soil in this region is permanently frozen and the ocean is covered with pack ice from October to May (Stonehouse 1989; Fogg 1998). The precipitation in arctic regions is known to be slight and consequently the vegetation represents a typical tundra, dominated by moss, draft-willows (*Salix arctica*) and lichens.

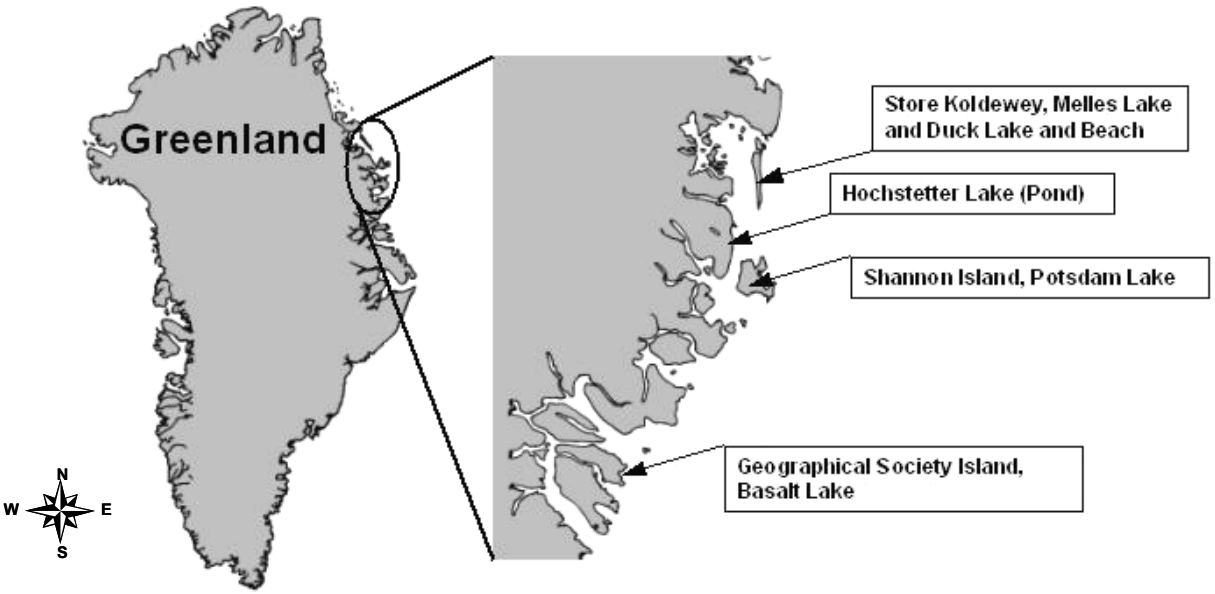


Fig. 3: Map of the sampling sites in North East Greenland

Store Koldewey is an island in the North East of Greenland (76°06.82`N; 18°30.51`W; Fig. 6). The sampling site was located on an eastern seaside beach. Landwards the beach was surrounded by a glacier. The sediment in this area consisted of coarse sand with a very low content of silt and clay. No data were available as to the slope of the ocean´s floor on a small scale and as to the tidal range. The appearance of the open ocean shore line, the steep mountain slope close to the shoreline as well as the low amount of silt and clay indicate that the tidal change did not play an important role in this area. The wave shock however seemed to be high.

The arctic **Melles Lake** (76°07' N; 8°37' W) is located on Store Koldewey (Fig. 6). The lake is filled with ultra oligotrophic meltwater from glaciers and has a small outlet to the sea. In the middle of the lake a milky flag of sediment filled water could be seen. Maximum depth is 72 m (Jokat 2004).

The **Duck Lake** (76°25' N; 8°45' W) is an arctic lake located on Store Koldewey (Fig. 6). The maximum water depth is 6.4 m. The lake is filled by melt water (Jokat 2004).

The **Potsdam lake** (75°03 48`N; 18°45.86'W) is a shallow lake on the north-west coast of Shannon Island (Fig. 6). The lake has a maximal depth of 0.7 m and is fed by melt water in spring (Hubberten 1995). The surrounding area was covered by vegetation. On the sampling area feces were seen, which indicated a former resting of geese, consequently a fertilizing effect had to be expected.

The **Basalt Lake** (72°43.48`N; 22°27.60`W) is situated on the southeastern island Geographical Society (Fig. 6). The main inflow enters the lake at its eastern shore, fed by melt water from farther glaciers. The longish lake (length 2.3 km, width 1 km) has a maximum depth of 22 m (Wagner 2000). The surrounding was densely covered with small plants.

The **Hochstätter Lake** (75°37.31`N; 19°44.12`W) is a small lake situated on the foreland between Shannon Sund and Hochstetterbugten (Fig. 6). No data about maximum depth and covered area were available, but with regard to the surrounding area it is supposed to be a shallow pond. The sediment surface at the sampling site near the shore in about 30 cm deep water had a fluffy layer probably consisting of plant detritus.

Sampling

During each sampling the temperature of air, water and sediment (1 cm deep) as well as the salinity were measured in the field. Samples were taken during low tide in intertidal mudflats, when the sediment was exposed to the air from places where the mean water depth in high tide situations was between 0.3 and 1 m. At freshwater lakes sediment samples were taken from places where the water depth was between 0.3 and 1 m. The upper 3 mm (within the oxic layer) of sediments were collected using round plexiglass cores (inner diameter 36 mm) or with a flat shovel. Three samples were taken per station. The samples were transported to the laboratory in a dark cooled container and processed within 4 h.

Sediment analysis

The samples for the sediment analysis (50 ml of surface sediment) were frozen in tubes immediately after returning to the lab (max 2 h) at –20°C and stored for the measurement of photosynthetic pigments, organic carbon, total carbon and total nitrogen content as well as grain size distribution.

Carbon and nitrogen content determination

140-160 mg of frozen, dried and milled sediment were weighed into an annealed pottery saggar. The contents of total carbon and total nitrogen were analyzed by combustion (1600°C in a pure O_2 atmosphere) combined with gas-chromatography, infrared absorption spectroscopy and thermal conductivity measurement (Leco CNS 2000 determinator). 140-160 mg of standard soil (Leco soil calibration sample for CHNS) were used for calibration purposes. For the analysis of the organic carbon content, 30 – 50 mg of frozen, dried, milled sediment were weighed into an annealed pottery saggar. 0.5 ml of concentrated hydrochloric acid and three drops of alcohol were added. The sample was heated up to 250°C for 2 h in order to dissolve inorganic carbonates. A spattle spoon of tungsten and a small spoon of iron filings were added to increase temperature and to catalyse the combustion in pure oxygen. The amount of organic carbon was measured by an infrared and thermal conductivity detector (CS – 125 carbon sulfur determinator). The carbonate content was calculated according to the equation:

CaCO3 (wt.%) = (total C - organic C) * 8.333.

In order to identify organic carbon sources, C/N ratios were calculated by using the measured values of organic C and total N.

Grain size distribution, water content, porosity

The grain size distribution was determined by adding 100 ml of H_2O_2 (10%) to 50 ml of sediment in order to oxidise all organic content (waiting time 3 h to 6 days) (Buchanan 1984). Afterwards the biotic carbon was dissolved by adding 50 – 100 ml of acetic acid (f.c. 25%). After 12 h, the wet sediment was sieved through a 63 µm sieve (Buchanan 1984). The sieve residue was dried in an oven at 80°C and weighed. Silt and clay (<63 µm) were separated by sedimentation. Water content (wc) was calculated as follows:

wc = (wet - dry sediment weight) / wet sediment weight (Dell'Anno et al. 2002) .

Porosity (P) was determined by the equation: P = (wc/1.02) / [(1-wc)/2.64 + wc/1.02], where 1.02 g cm^{-3} is the density of 35 PSU salt water and 2.64 g cm^{-3} is the density of quartz (Buchanan 1984; Dell'Anno et al. 2002).

Photosynthetic pigments

3 to 8 g of wet sediment were suspended in acetone (f.c. 90%; dilution 1:7 to 1:100) and sonicated on ice for 90 s (Branson Sonifier 250; pulse; level 6) in order to extract chlorophyll-derived pigments (chlorophyll-*a* and phaeopigments). Pigments were extracted over 24 h at 4°C under no light conditions and subsequently separated from the sediment by centrifugation (2000 g for 5 to 10 min). The chlorophyll-*a* content was measured with a fluorometer (430 nm excitation wavelength and 665 nm fluorescence wavelength). The phaeopigment content was measured by adding 200 µl of 0,1 N HCl to the extract. The amounts of pigments were calculated. This methode is described in detail in Lorenzen (1976) and Daemen (1986). The amount of pigments was calculated according to the equation of Lorenzen (1976):

Chlorophyll-*a* = $F_d*(\tau/\tau-1)*(R_b-R_a)*(V_e/V_f)$
Phaeopigment = $F_d*\tau/\tau-1)*(\tau R_a-R_b)*(V_e/V_f)$

With:

Chlorophyll-*a*	[µgC/g]	Chlorophyll *a* content
Phaeopigment	[µgC/g]	Phaeopigment content
F_d		correlation factor (here after calibration with standard chlorophyll-*a* solution; 0,84)
R_b		emission at wavelength 665 nm before acidifying
R_a		emission at wavelength 665 nm after acidifying
τ		max. ratio R_b/R_a of phaeo free Chl-*a* (Chl*a* = 2,2)
V_e	[l]	volume of acetone
V_f	[g]	volume of extracted sediment

Bacteria, nanoflagellates, cyanobacteria and diatom abundance and biomass

An equivalent of 1 - 3 g wet sediment or 1 to 2 ml of the upper layer (less than 3 mm from the surface) were transferred into tubes containing 2 ml filtered (0.2 µm) seawater each, fixed with cold (~ 0°C) glutardialdehyd (f.c. 2%) and stored at 4°C in darkness until further analysis (max. one week). For the quantification of organisms samples were diluted in artificial seawater (5 to 20 ml). The organisms were detached chemically with PPI (Tetrasodiumpyrophosphate, f.c. 0.5-10 mM) and Tween 80 (f.c.1-10 µg ml^{-1}) and detached physically by gentle sonification on ice (Branson, Sonifer 250, pulses for 30 s at 60 W). The supernatant was filtered using a black polycarbonate filter (Osmonics 0.2 µm) and stained with DAPI (4`.6-Diamino-2-phenylindol, working solution 50 µg ml^{-1}, f.c. of supernatant 5

µg ml^{-1}). Finally, the filter was embedded in fluorescence-free immersion oil (AppliChem) and stored at –20°C until microscopical analysis (Sherr et al. 1993; Velji & Albright 1993; Epstein & Rossel 1995). The slides were examined using epifluorescence microscopy (Zeiss, Axioscop2 plus, x1000 magnification). For the determination of bacteria abundance a minimum of 500 cells per slide on 24 fields were counted. For the count of cyanobacteria, diatoms and PNF abundance a minimum of 30 random fields were evaluated using the autofluorescence of the photosynthetic pigments (Waterbury et al. 1986; Maclassac & Stoeckner 1993). For the count of HNF abundance only cells with a definite nucleus were counted on a minimum of 30 random fields, while cells with irregular shapes were excluded (Sherr et al. 1993). PNF and HNF were split in size groups according to their lengths, 2 - 5 µm = "small HNF and PNF", 5 -10 µm = "medium sized PNF and HNF", >10 µm = "large HNF and PNF". This size split was introduced in order to allow the study of cell size related interactions of the organisms with the other components of the food web. The cell sizes of organisms of all different groups were measured and the biovolume was estimated using simple geometrical shapes from the literature (Edler 1979). The biomass was calculated by converting cell biovolume using different conversion factors from the literature (Table 1).

Table 1: Measurements of mean cell sizes, geometrical forms and conversion factors used for the estimation of biovolume and biomass of counted organisms.

	length*width*depth [µm]	shape (Edler 1979)	conversion factor	reference
Diatoms	15 * 4.2 * 3	parallelepiped	$0.288*Vol^{0.811}$ pgC*$cell^{-1}$	Menden-Deuer & Lessard 2000
Cyanobacteria	3.2 * 2.6 * 2.6	rotational ellipsoid	310 fgC*μm^{-3}	Caron et al. 1991
PNF 2-5 µm	2.8 * 2.6 * 2.6	rotational ellipsoid	220 fgC*μm^{-3}	Ekebom 1999; Borsheim & Bratbak 1987; Fry 1990
PNF 5-10 µm	6.1 * 4 * 4	rotational ellipsoid		-« -
PNF >10µm	18.9 * 9.5 * 9.5	rotational ellipsoid		-« -
Bacteria	0.6 * 0.6 * 0.6	sphere	19.8 fgC*$cell^{-1}$	Lee & Patterson 2002
HNF 2-5µm	1.9 *1.6 * 1.6	rotational ellipsoid	220 fgC*μm^{-3}	Ekebom 1999; Borsheim & Bratbak 1987; Fry 1990
HNF 5-10µm	6.5 * 5.6 * 5.6	rotational ellipsoid		-« -
HNF >10µm	20.6 * 11.5 * 11.5	rotational ellipsoid		-« -

Data analysis

For a better understanding of the relationships between the components of the microbial benthic food web and environmental attributes, non parametric Spearman rank correlations (able to correlate linear as well as non linear relations) were calculated. Correlations were regarded as significant, if R (correlation coefficient) was larger than 0.5 or smaller than

-0.5 (R^2 (coefficient of determination) > 0.25) and p (significance level) < 0.05. Correlation were regarded as highly significant if R > 0.7 or < -0.7 (R^2 > 0.5) and p<0.01.

For the evaluation of alikeness of the investigated areas a principal component analysis (PCA) was calculated by using normalized environmental data and organisms' abundance. In order to normalise data, the fourth root of the values was taken (Wosniock per.com).

For the identification of influences of environmental parameters on the abundance of organisms in all sampling sites, a redundance analysis (RDA) was carried out by using CANOCO® Version 4.5 with the abundance of all organisms and environmental data.

Contribution to community carbon and energy flow

The percentages of heterotrophic components of total community yield were calculated according to Fenchel (1974), in order to estimate the component's contribution to community carbon and energy flow. According to Fenchel (1974) the biomass yield (Y) of a population in a relative equilibrium is: Y= N*rm*W with: N = abundance (cells ml^{-1}); W= average body weight (µgC $cells^{-1}$); rm = intrinsic rate of natural increase, $rm = a * W^{-0.27}$ and the constant $a = 10^{-1.94}$ for protozoa and bacteria, $10^{-1.64}$ for metazoan. For the estimation of the component's contribution to the total yield the percentages of each component were calculated.

3. Results

Environmental attributes

The sampling period begun in August 2001, ended in December 2003 and covered 13 locations in the northern hemisphere with very wide ranges of temperature (1-24°C) and salinity (0 to 5 and 26 to 32 PSU; Table 1).

Most of the investigated sediments had fractions of less than 10% of silt and clay, except Potsdam Lake (PO, 40%), the lake near Hochstetter Fjord (HL, 24%) and Melles Lake (M, 32%). The porosity was between 0.4 and 0.53 except at PO (0.7), HL (0.8) and M (0.3). The contents of total nitrogen (0.05 - 1.6 mg g^{-1}) as well as the amount of organic carbon (0.16 - 4.92 mg C g^{-1}) were low in the investigated sediments (Table 1). Only in PO and HL the total nitrogen and organic carbon concentrations were much higher (8.4 mg C g^{-1} and 16.1 mg C g^{-1}). The total carbon concentration exhibited different values. The highest values were found to be approximately 93 mg C g^{-1} (Green Lane Lake, GLL) and the lowest ones 0.56 mg C g^{-1} in the beach sediment of Store Koldewey (KB). In the arctic region the carbonate content (mean 1.8 mg C g^{-1}) was lower than in temperate regions (mean 3.2 mg C g^{-1}).

Table 2: Sampling sites and dates with main environmental parameters and phytopigment concentrations. sd = standard deviation (n=3); d.w. = dry weight of sediment; w.w. = wet weight of sediment.

	sampling site	abbr.	position		date	temp.
			latitude	longitude		°C
t	Shark River Bay/PA, USA	SR	40º 10´N	74° 01´W	Nov.11.2003	12
e	Green Lane Lake/PA, USA	GLL	40º 20´N	75° 27´W	Dec.12.2003	1
m	Nockamixon Lake/PA, USA	N	40º 28´N	75° 13´W	Mar.12.2003	7
p	Fühlinger See/Köln, Germany	K	50º 58´N	74° 01´E	Jun.02.2003	24
e	Dorum, Germany	D8	53º 42´N	8° 29´E	Aug.28.2001	21
r	Dorum, Germany	D10	53º 42´N	8° 29´E	Oct.22.2001	11
a	Dorum, Germany	D4	53º 42´N	8° 29´E	Apr.18.2002	17
t	Dorum, Germany	D6	53º 42´N	8° 29´E	Jun.13.2002	16
e	Schöhsee/Plön, Germany	P	54º 09´N	10° 26´E	Nov.13.2001	4
	List/Island of Sylt, Germany	S	55º 2´N	8° 25´E	Sep.12.2002	21
a	Basalt Lake, Greenland	B	72º 43´N	22° 27´W	Sep.08.2003	9
r	Potsdam Lake/Shannon Island, Greenland	PO	75º 03´N	18° 45´W	Aug.26.2003	7
c	Lake near Hochstetter Fjord, Greenland	HL	75° 37´N	19° 44´W	Sep.06.2003	6
t	Koldewey beach, Greenland	KB	76º 06´N	18° 30´W	Aug.13.2003	2
i	Melles Lake/Island of StoreKoldewey, Greenland	M	76º 07´N	18° 37´W	Aug.13.2003	9
c	Duck Lake/Island of Store Koldewey, Greenland	D	76° 25´N	18° 45' W	Aug.22.2003	10

	salinity	silt and clay	sand	porosity	nitrogen total	cabon total	carbon organic ratio	
	PSU	%	%		mg N x g^{-1}	mg C x g^{-1}	mg C x g^{-1}	C_{org} x N^{-1}
SR	26	3.9	96.1	0.47	0.25	2.87	1.39	6.5
GLL	0	7.4	92.6	0.34	0.11	93.50	0.83	8.5
N	0	2.8	97.2	0.51	0.25	2.91	4.92	22.8
K	0	1.9	98.1	0.45	0.12	3.33	0.63	6.0
D8	25	7.6	92.4	0.53	0.16	4.82	0.85	6.1
D10	25	10.0	90.0	0.49	0.18	5.29	1.01	6.5
D4	26	7.1	92.9	0.50	0.17	5.35	1.09	7.3
D6	25	3.9	96.1	0.51	0.21	5.55	2.04	11.3
P	0	1.3	98.7	0.47	0.44	7.83	3.38	9.0
S	31	0.9	99.1	0.46	0.20	1.96	0.77	4.6
B	5	0.5	99.5	0.43	0.06	0.72	0.16	3.2
PO	3	40.0	60.0	0.71	1.35	12.95	8.47	7.3
HL	4	24.8	75.2	0.89	1.63	21.89	16.14	11.6
KB	36	0.6	99.4	0.40	0.05	0.56	0.24	6.0
M	3	32.3	67.7	0.36	0.13	1.50	1.36	11.9
D	0	5.4	94.6	0.52	0.24	2.75	2.72	13.3

	chlorophyll-*a*		phaeopigment	
	µg * g^{-1} ww	sd	µg * g^{-1} ww	sd
SR	11.19	0.4	3.83	0.1
GLL	2.43	0.5	2.84	0.4
N	0.01	0.0	0.18	0.0
K	1.07	0.3	1.00	0.1
D8	4.5	0.2	2.3	0.2
D10	6.6	0.7	1.3	0.7
D4	2.3	0.4	1.1	0.2
D6	8.3	0.7	2.5	0.5
P	10.36	0.6	4.53	0.1
S	4.18	0.7	1.11	0.2
B	0.34	0.1	0.19	0.1
PO	5.09	0.6	1.99	0.1
HL	0.80	0.4	1.23	0.4
KB	0.04	0.0	0.03	0.0
M	0.36	0.1	0.53	0.0
D	2.05	0.2	1.73	0.1

The principal component analysis (PCA) was used for an easier identification of potential alikeness between the investigated sampling sites in view of their multidimensional environmental data sets. The PCA plot visualises the similarities between data sets in a two or three dimensional plot, where the alikeness between points is inversely related to the distances of the points in this plot. The values of PC1 and PC2 do not reflect any physical or chemical relevance directly, they are designed to display alikeness of data sets.

Only the points of HL and PO were clearly separated from the other sites (Fig. 4). No obvious clusters of freshwater or marine sites could be identified. No clear differences between arctic and temperate sites were found. The points of Dorum (D4, D6, D8, D10) representing samples at different seasons were relatively close together and members of one cluster, which could have been expected. This analysis demonstrated that the habitats represented two clusters: HL and PO in one and all others more or less in a second cluster. In both cases the members of each cluster were relatively similar in their total set of environmental data.

Principal component analysis of environmental parameters

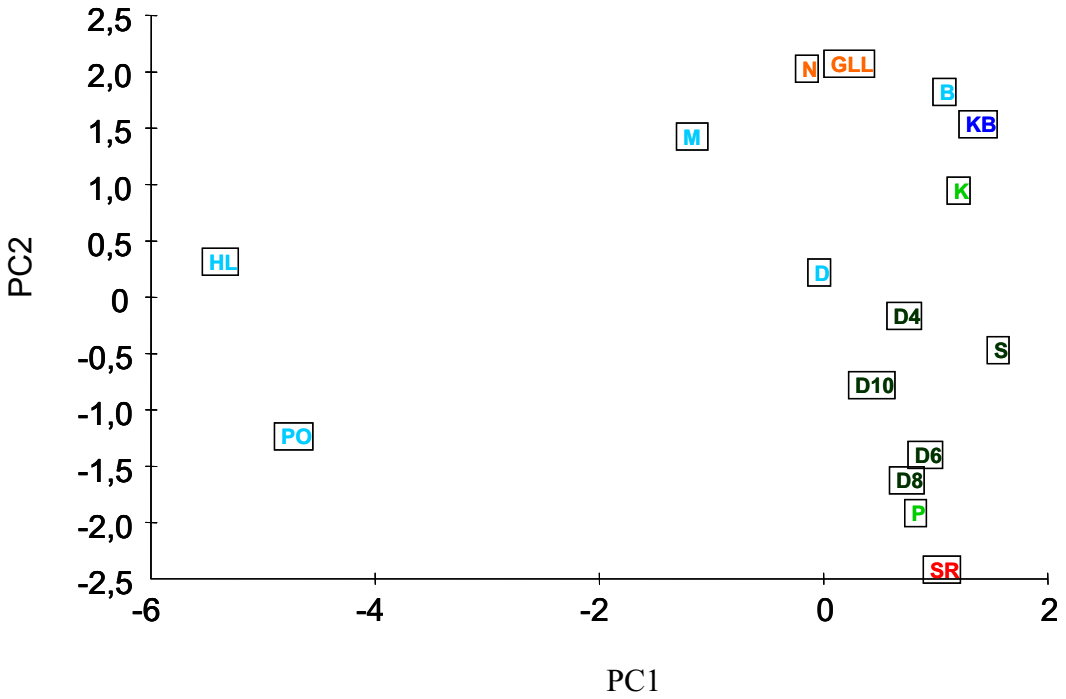


Fig. 4: PCA plot of normalized environmental parameters: temperature, salinity, proportion of silt and clay, proportion of sand, porosity, amount of nitrogen, total and organic carbon. Eigenvalues and variations (%) of PC1 4.22 and 38.4%; PC2 2.24 and 20.4%.

Phytopigment concentrations

The chlorophyll-*a* (0.01 - 11.2 µg g^{-1}) and the phaeopigment (0.03 - 3.4 µg g^{-1}) concentrations showed a high dissimilarity between the study sites (Table 1). A trend to lower chlorophyll-*a* concentrations at arctic sites (mean 1.4 µg g^{-1}) in comparison to temperate regions (mean 5.1 µg g^{-1}) was observed. This trend was not found for the phaeopigment concentrations.

Abundance and biomass of organisms

All measurements at the different sites had to be made inevitably at different seasons. Seasonal influences therefore complicated the direct comparisons of results from the different sites. Hence a statistical analysis of variances (ANOVA) between all sites would not lead to

meaningful results. Instead of that values were sorted along geographic region, climate, salinity and seasonal period of sampling in order to find noticeable patterns in the distribution of abundance and biomass. The lowest abundance of cyanobacteria (8.5 x 10^3 cells ml^{-1}) and diatoms (1.6 x 10^4 cells ml^{-1}) was found in KB (Fig. 5a-b). The highest abundance of cyanobacteria was found in PO (2.8 x 10^7 cells ml^{-1}) and highest abundance of diatoms in Shark river bay (SR) (2.9 x 10^7 cells ml^{-1}) (Fig. 5a-b). The percentage of diatom biomass of total microphytobenthos biomass varied between 24% and 96% (Fig. 6). The percentage of cyanobacteria biomass of total microphytobenthos biomass varied between 0.2% and 54% (Fig. 6). No noticeable patterns of abundance or biomass of diatoms and cyanobacteria fitting to geographic region, climate, season and salinity could be found.

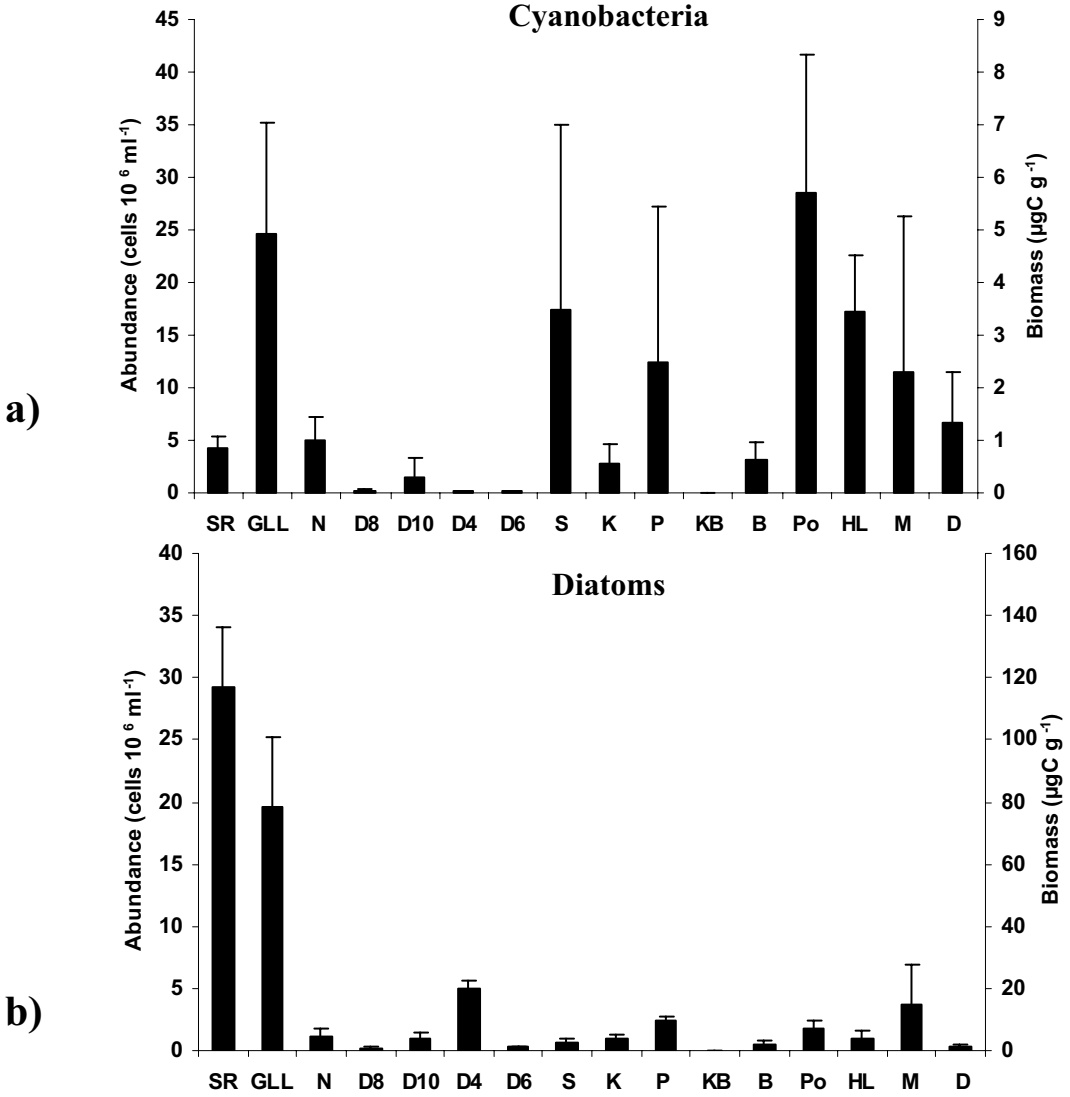


Fig. 5: Mean abundance (cells ml^{-1} sediment) and biomass (µg C g^{-1}) with standard deviation (n = 3) of cyanobacteria (a), and diatoms (b) from Shark River Bay (SR), Green Lane Lake (GLL), Nockamixon Lake (N), Fühlinger Lake (K), Dorum August 2001 (D8), Dorum October 2001 (D10), Dorum April 2002 (D4), Dorum June 2002 (D6), Schöhsee (P), Königshafen (S), Basalt Lake (B), Potsdam Lake (PO), Lake near Hochstetter Fjord (HL), beach on Store Koldewey (KB), Melles Lake (M), Duck Lake (D).

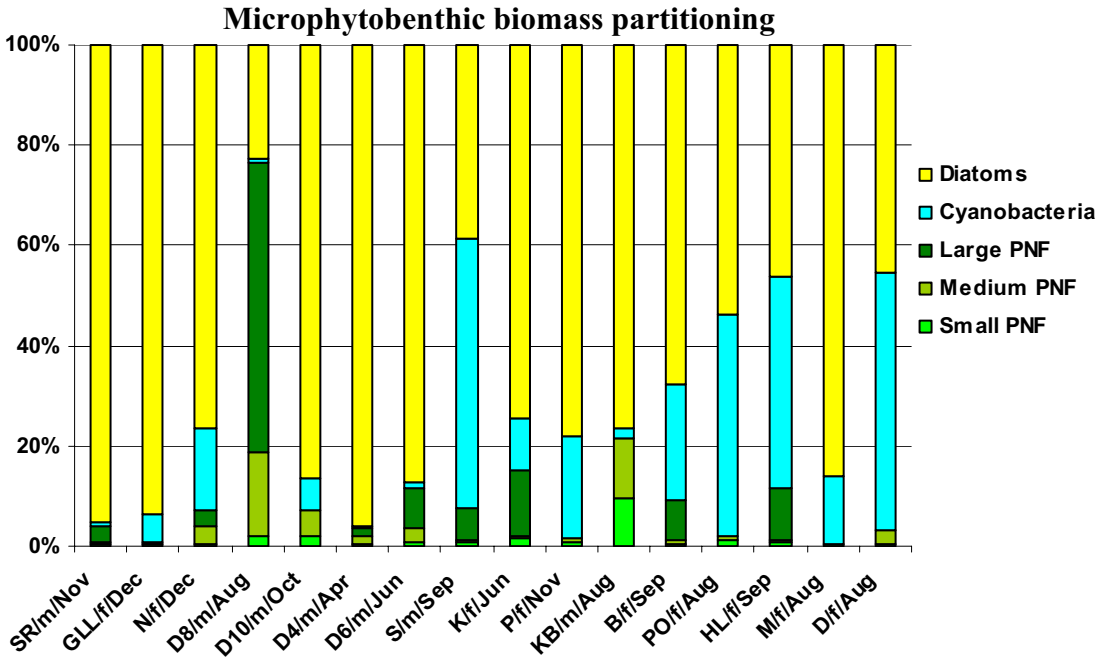
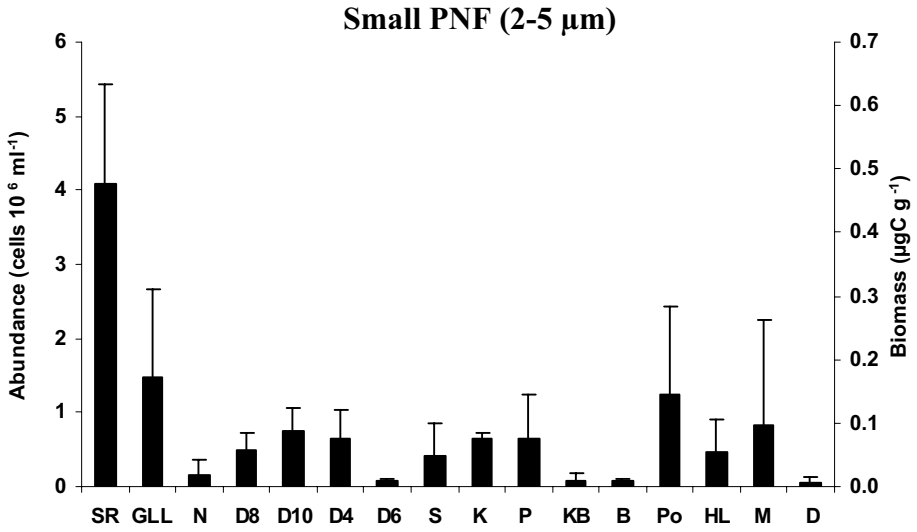


Fig. 6: Microphytobenthic biomass partitioning in %: diatoms cyanobacteria, phototrophic nanoflagellates in different size classes from all investigated sites. Biomass in µgC ml^{-1}, with sampling month and salinity (m = marine, f = freshwater).

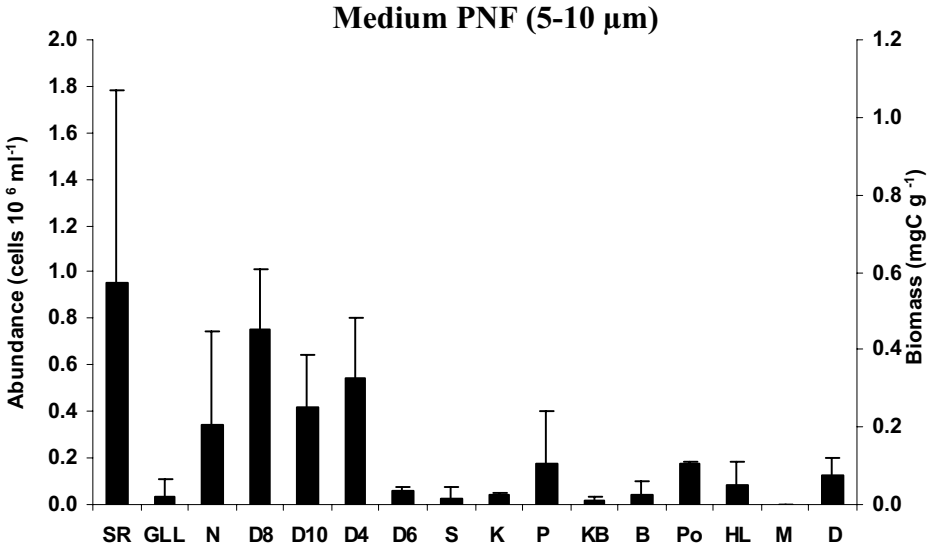
The abundance of phototrophic nanoflagellates (PNF) varied between 8.1 x 10^4 10^6 cells ml^{-1} and 5.4 x 10^6 cells ml^{-1}, and the highest abundance and biomass of all size classes were found in SR (small PNF 4.1 x 10^6 cells ml^{-1}; medium PNF 9.5 x 10^5 cells ml^{-1} and large PNF 3.6 x *10^5 cells ml^{-1}; Fig. 7a-c). The lowest abundance of small PNF was found in the Duck lake (D) sediment (6.1 x 10^4 cells ml^{-1}; Fig. 7a). Melles Lake (M) was the only site where no medium sized PNF were observed (Fig. 7b). The percentage of small, medium and large PNF of total PNF abundance and biomass exhibited high variations (abundance of PNF in the different size classes to total PNF: small 31% - 100%, medium 0% - 65% and large 0% - 15% to total PNF; biomass portion of different sized PNF to total PNF: small 2.7% -100%, medium 0% - 91%, large 0% to 88%; Fig. 8a-b).

The portion of total PNF biomass on total microphytobenthic biomass also exhibited large differences in-between the sites with a minimum of 0.5% (M) and highest values up to 74% (Dorum 2001, D8, Fig. 6). However, no obvious patterns were identified in between abundance and biomass on the one side and geographical region, salinity and sampling season on the other site .

a)



b)



c)

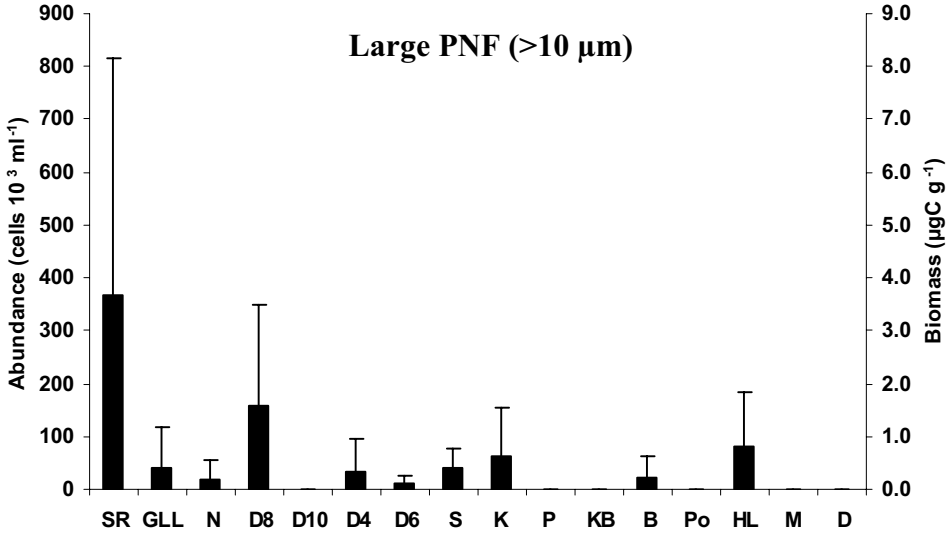
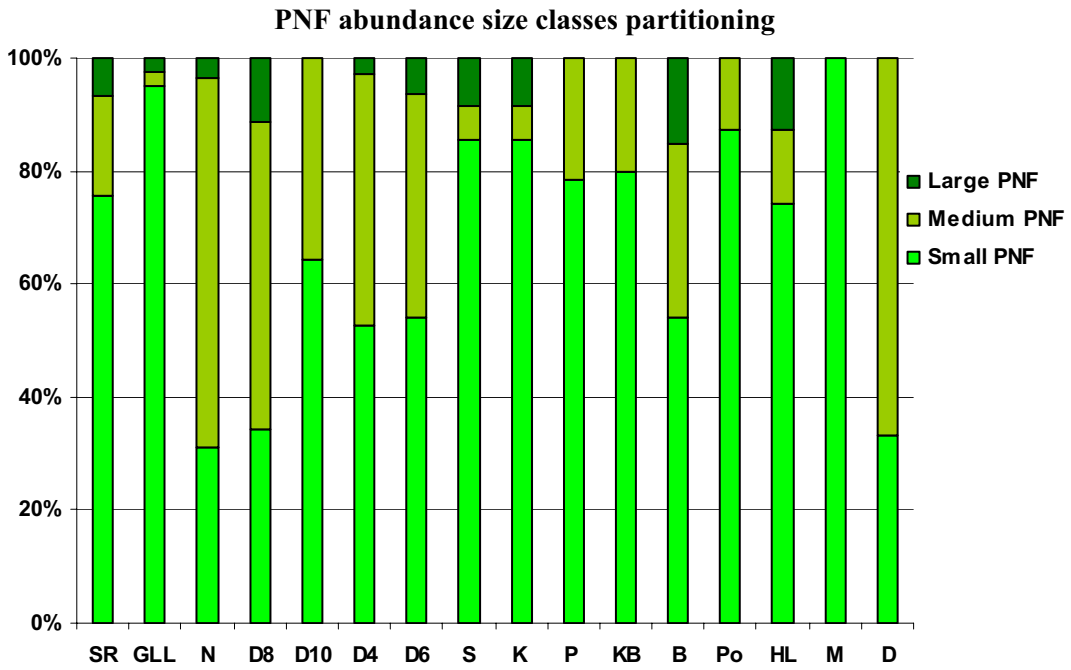


Fig. 7: Mean abundance (cells ml^{-1} sediment) and biomass (µg C g^{-1}) of phototrophic nanoflagellates with standard deviation (n = 3) from all sampling site. Small PNF = 2-5 µm (a), medium PNF = 5-10 µm (b), large PNF = >10 µm (c).

a)



b)

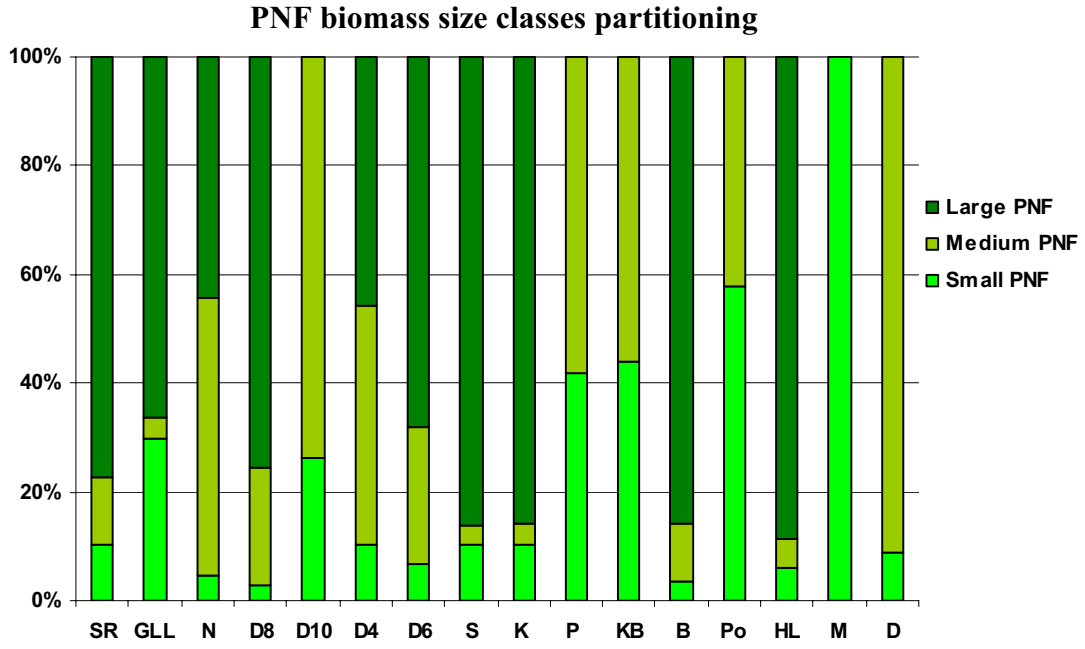


Fig. 8: Abundance and biomass partitioning of PNF size classes. (a) abundance (cells ml^{-1} sediment) and (b) biomass (µgC g^{-1} sediment); 2-5µm = small PNF, 5-10µm = medium PNF; >10µm = large PNF.

The abundance of bacteria within the sediments showed high differences in-between the sites (1.6×10^7 cells ml^{-1} to 2.5×10^9 cells ml^{-1}; Fig. 9). Bacteria were least abundant in KB and most abundant in the SR sediment (Fig. 9). The percentage of bacteria biomass of total microbial biomass (1.4% to 22.6%) also revealed large variations between sites (not shown). Again no obvious patterns in between bacteria abundance and biomass on the one hand and geographical region, salinity, sampling time on the other hand could be identified.

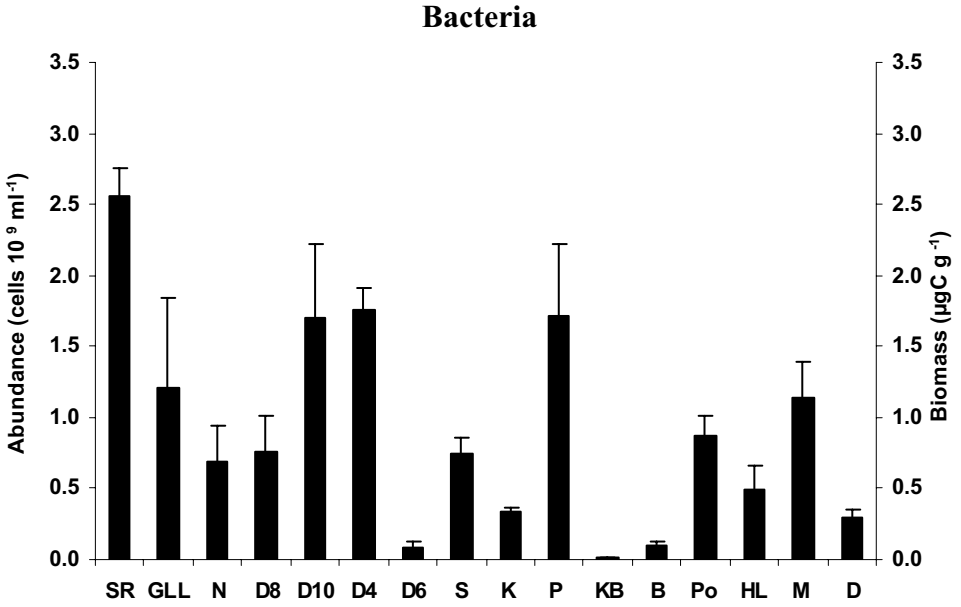
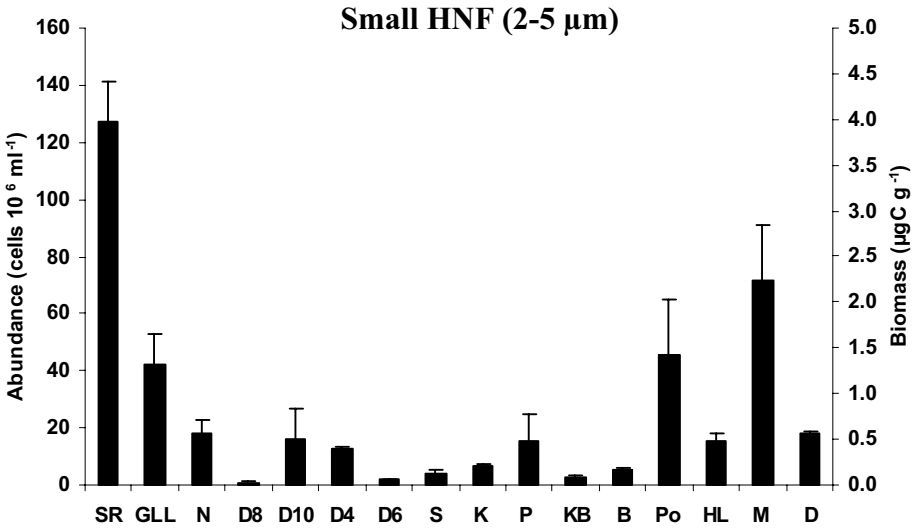


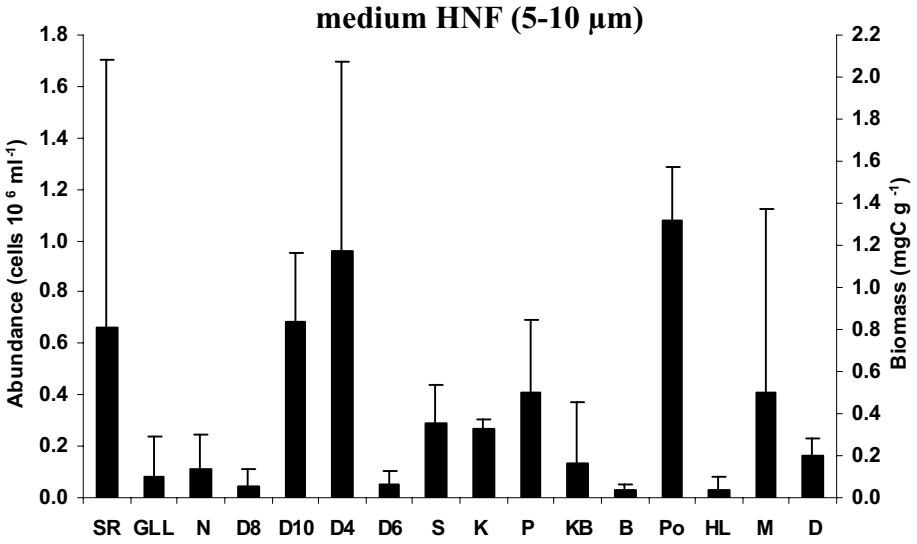
Fig. 9: Mean abundance (cells ml^{-1} sediment) and biomass (µg C g^{-1}) of bacteria with standard deviation (n = 3).

Small HNF were found to be most abundant in SR (1.2×10^8 cells ml^{-1}) and least in D8 (8.4×10^5 cells ml^{-1}; Fig. 10a). The highest abundance of medium sized HNF was observed in PO (1.1×10^6 cells ml^{-1}) and the lowest in HL (2.6×10^4 cells ml^{-1}; Fig. 10b). Large HNF was found in maximum abundance in Dorum April 2002 (D4 $1.3*10^5$ cells ml^{-1}), whereas in several other sediments (KB; M; HL) no cells could be observed (Fig. 10c). The percentage of different HNF size classes of total HNF abundance was quite similar at the investigated sites (small HNF 92% to 99%, medium HNF 0.1% to 6.8% and large HNF 0% to 0.99%), whereas the composition of size classes of HNF biomass was different at the sites (small HNF 10% to 93%, medium HNF 6.6% to 30%, large HNF 0% to 59%; Fig. 11a-b). However, no obvious relationships between HNF abundance and geographic location, climate, season and salinity were recognised by searching for noticeable patterns.

a)



b)



c)

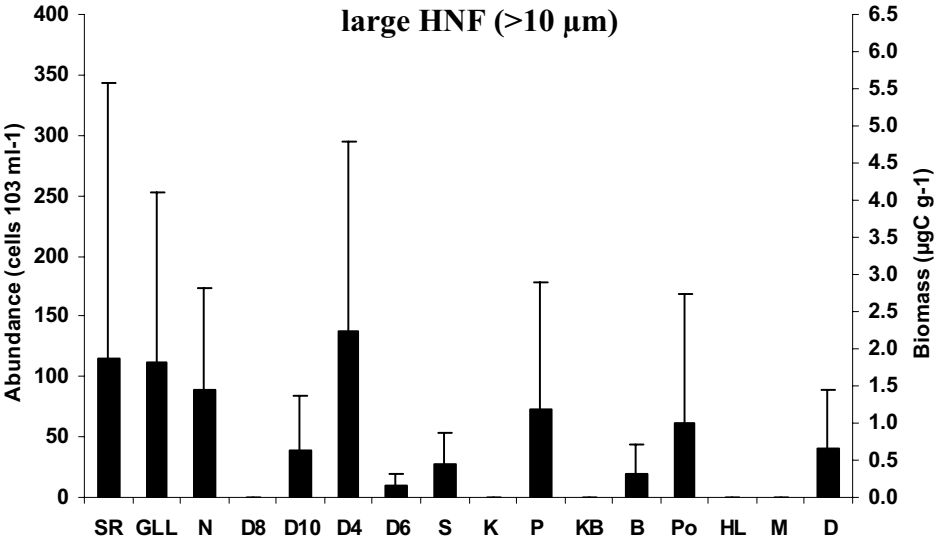
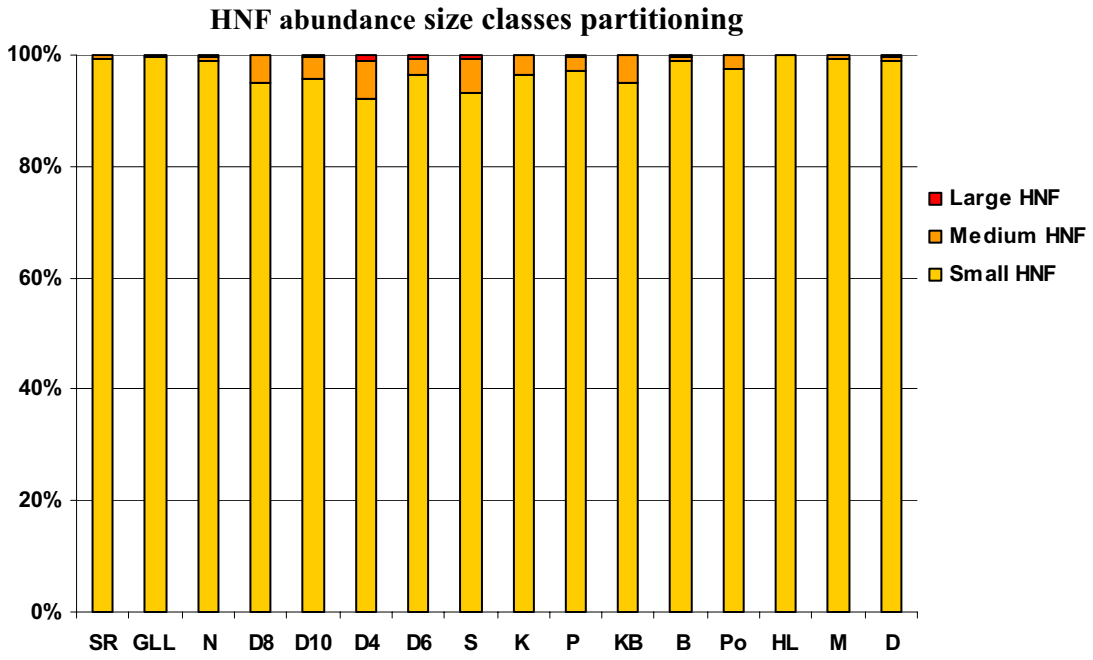


Fig. 10: Mean abundance (cells ml^{-1} sediment) and biomass (µg C g^{-1}) of heterotrophic nanoflagellates with standard deviation (n = 3); (a) small HNF (2-5µm), (b) medium HNF (5-10 µm), (c) large HNF (>10µm) from all investigated sites.

a)



b)

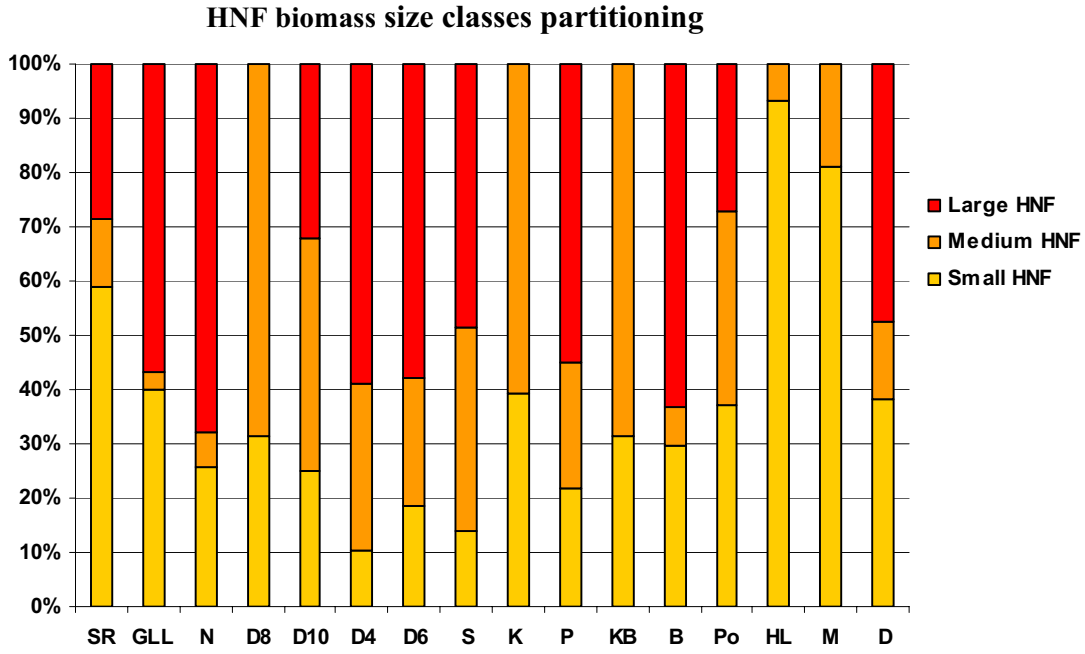


Fig. 11 : Abundance and biomass partitioning of HNF size classes. (a) abundance (cells ml^{-1}) and (b) biomass (µgC g^{-1}); 2-5µm = small HNF, 5-10µm = medium HNF; >10µm = large HNF.

Community structure

The total biomass was calculated by adding the biomass of diatoms, cyanobacteria, small, medium and large PNF, bacteria and small, medium and large HNF, ciliates, other protozoa and meiofauna (Fig. 12). Data for ciliates, other protozoa (comprising amoeba and foraminifera) and meiofauna were taken from study 3. The maximum value of the total biomass was found in SR (257.6 µgC g^{-1}) and the minimum value in KB (0.7 µgC g^{-1}).

Microphytobenthos contributed more than 53% to the total biomass in most investigated sediments. In D6 and S meiofauna, ciliates and other protozoa dominated the communities with 90% and 66% respectively. In KB the HNF had a portion of 63% on the total biomass. In GLL and SR noticeable high portions of diatoms (>80%) could be observed. In D8 the large portion of PNF (~40%) was remarkable, PNF dominated the microbenthic biomass. No obvious pattern based relationships between biomass and climate, salinity or sampling season could be detected.

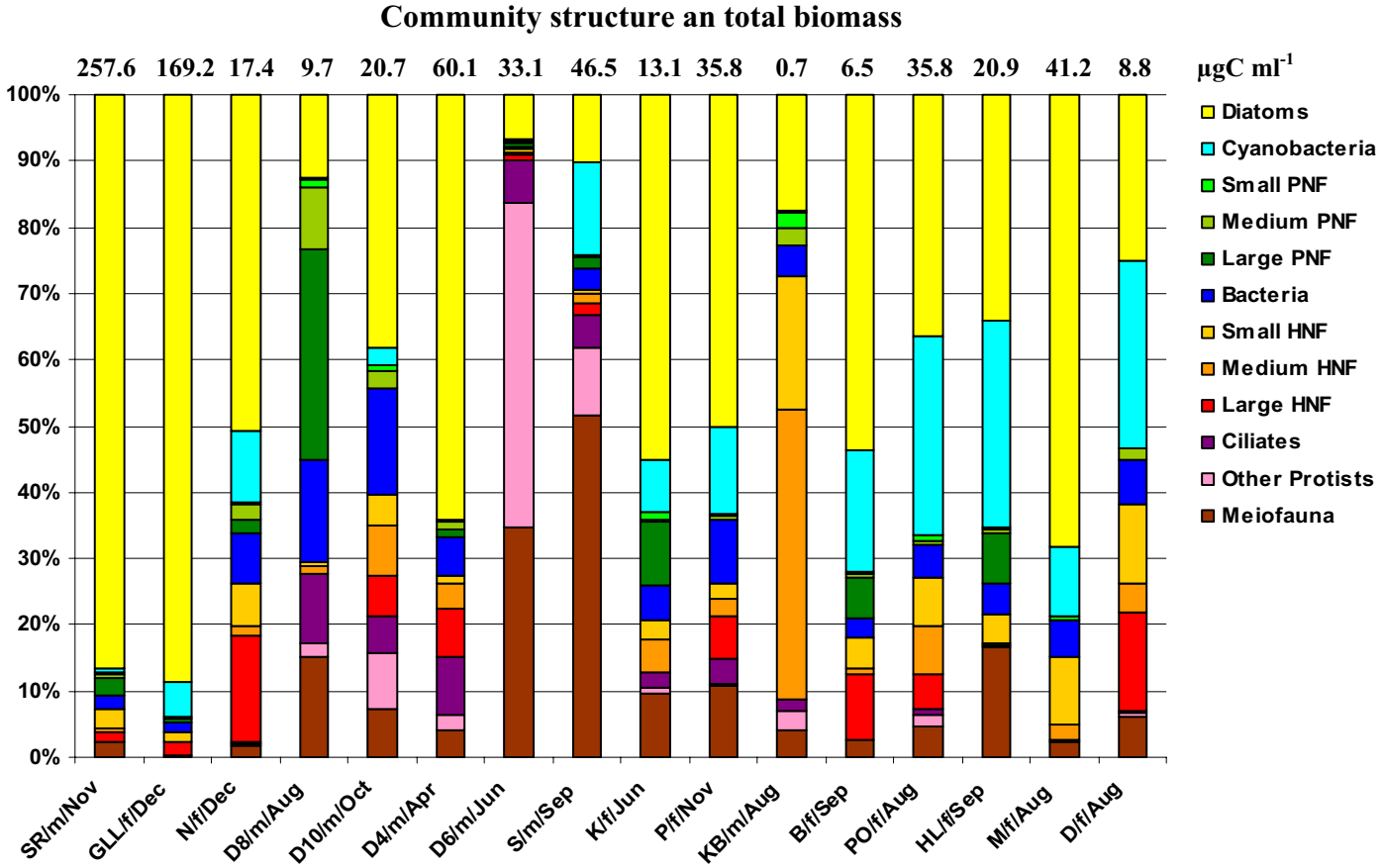


Fig. 12: Total biomass and biomass partitioning of the small benthic communities at all investigated sites. The values for total biomass are given on the top of each column in µgC ml^{-1}. Sampling sites are given with sampling month and salinity (m = marine, f = freshwater): Shark River Bay (SR), Green Lane Lake (GLL), Nockamixon Lake (N), Fühlinger Lake (K), Dorum August 2001 (D8), Dorum October 2001 (D10), Dorum April 2002 (D4), Dorum June 2002 (D6), Schöhsee (P), Königshafen (S), Basalt Lake (B), Potsdam Lake (PO), Lake near Hochstetter Fjord (HL), beach on Store Koldewey (KB), Melles Lake (M), Duck Lake (D).

For the identification of a community structure alikeness between sites the PCA method was used. The percentages of the community components were used to avoid that sites with a high total biomass value (GLL and SR) masked the community structure of sites with small total biomass like KB and D8 (Fig. 13). Most of all sites were found to lie in one cluster. But KB and D8 were clearly separated from the main cluster as well as from each other.

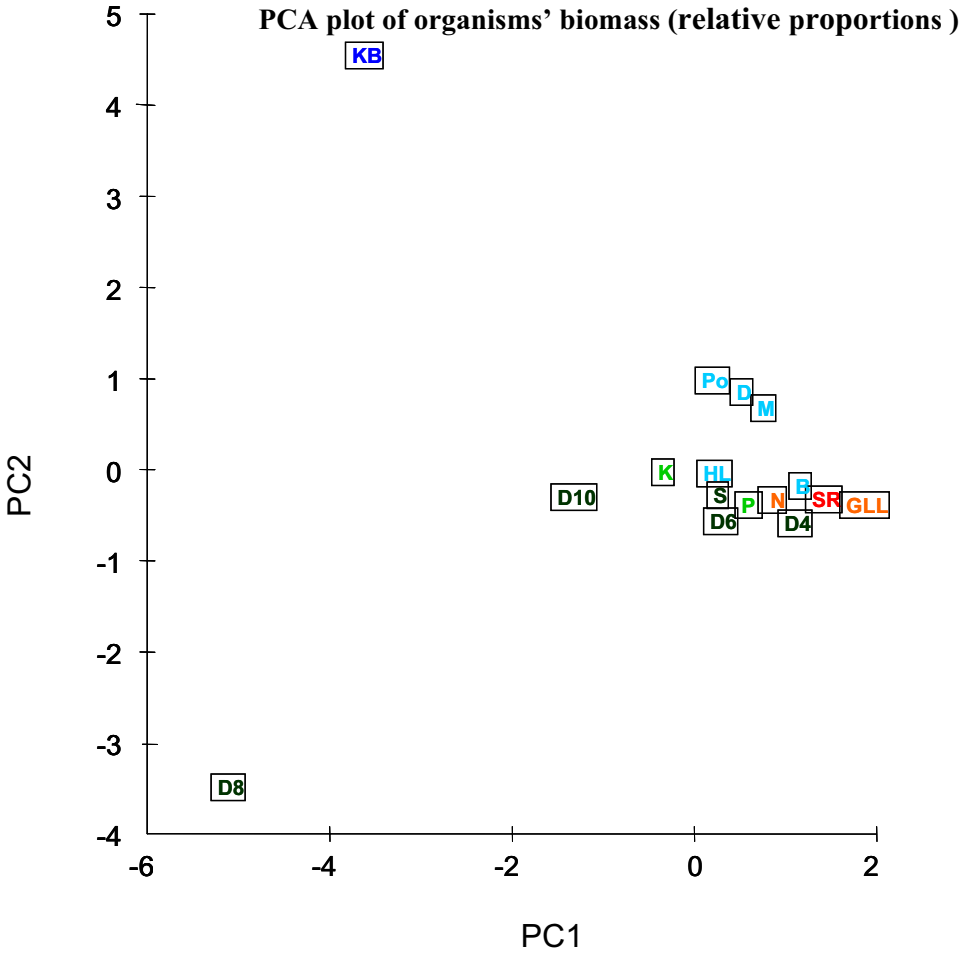


Fig. 13: PCA plot of organisms' biomass in relative proportions on the total small bentic biomass. Calculations included biomass of: diatoms, cyanobacteria, small, medium and large PNF, bacteria and small, medium and large HNF, ciliates, other protozoa and meiofauna. Eigenvalues, and variation in % of PC1 11,83 and 73.9%; PC2 1.47 and 16.4%.

Relationship between environmental parameters and abundance

The correlations between the total biomass and the abundance of organism groups with environmental parameters were calculated. No significant correlation between the total biomass and any of the measured environmental parameters was found. Only two significant correlations were identified: medium HNF correlated significantly positive with temperature and small HNF correlated significantly negative with PSU (Table 3). The concentration of chlorophyll-*a* showed significant positive correlations with small PNF as well as with bacteria and medium HNF (Table 2).

Table 3: Correlation coefficients (R) with significance level (p) of Spearman rank correlations between environmental parameters and organisms abundance.

	R	p
temperature : medium HNF	0.534	0.033
PSU : small PNF	-0.507	0.044
chlorophyll-*a* : small PNF	0.506	0.045
chlorophyll-*a* : Bacteria	0.562	0.023
chlorophyll-*a* : small PNF	0.64	0.0067

A redundance analysis (RDS) was applied to identify causal connections between the sets of environmental parameters and of organisms. No sound relationships between any of the environmental parameters and the biological data could be identified. Only a weak but not convincing influence of chlorophyll-*a* on the distribution of organisms showed up (plot not shown).

Contributions to community carbon and energy flow

The yield of heterotrophic organisms' biomass within each community was calculated and presented as percentages of the total yield of the site (Table 4; Fig. 14). Data of meiofauna and protozoa abundance and biomass were taken from study 3.

Table 4: Component's proportions of heterotrophic "yield" as percentages. Values were calculated according to Fenchel (1974). Bacteria, small HNF (2-5 µm), medium HNF (5-10 µm) and large HNF (>10 µm), ciliates, other protozoa (comprise amoeba and foraminifera) and meiofauna. Shark River Bay (SR), Green Lane Lake (GLL), Nockamixon Lake (N), Fühlinger Lake (K), Dorum June 2002 (D6), Schöhsee (P), Königshafen (S), Basalt Lake (B), Potsdam Lake (PO), Lake near Hochstetter Fjord (HL), Koldewey beach (KB), Melles Lake (M), Duck Lake (D).

	Ciliates	other Protozoa	Meiofauna	HNF 2-5	HNF 5-10	HNF>10	Bacteria
SR	0.27	0.03	0.33	34.83	2.69	3.09	58.76
GLL	0.45	0.05	0.20	26.89	0.75	6.98	64.68
N	0.50	0.08	0.29	20.98	1.94	10.11	66.10
D8	2.10	0.01	1.07	1.26	1.00	0.00	94.56
D10	0.41	0.17	0.35	9.26	5.80	2.17	81.84
D4	1.54	0.13	0.86	6.66	7.37	7.00	76.45
D6	14.59	5.64	17.05	10.12	4.66	5.73	42.21
S	2.01	3.84	17.86	4.43	4.44	2.87	64.55
K	16.78	0.43	1.68	14.41	8.27	0.00	58.44
P	2.43	0.04	1.34	8.53	3.37	3.96	80.34
KB	5.53	1.01	0.24	39.27	31.47	0.00	22.48
B	1.05	0.03	0.73	32.33	2.86	12.59	50.43
PO	1.04	0.76	1.55	31.14	10.96	4.16	50.39
HL	0.36	0.16	4.22	25.39	0.66	0.00	69.20
M	0.14	0.17	0.31	41.03	3.50	0.00	54.84
D	0.24	0.14	1.26	35.45	4.92	8.09	49.90

Regardless of differences in the heterotrophic biomass partitioning, including bacteria, HNF, ciliates, other protozoa and meiofauna, bacteria accounted for a mean of 61% ±17% of the heterotrophic yield at all sites. Bacteria and HNF therewith represented a mean contribution of 93% ± 10%.

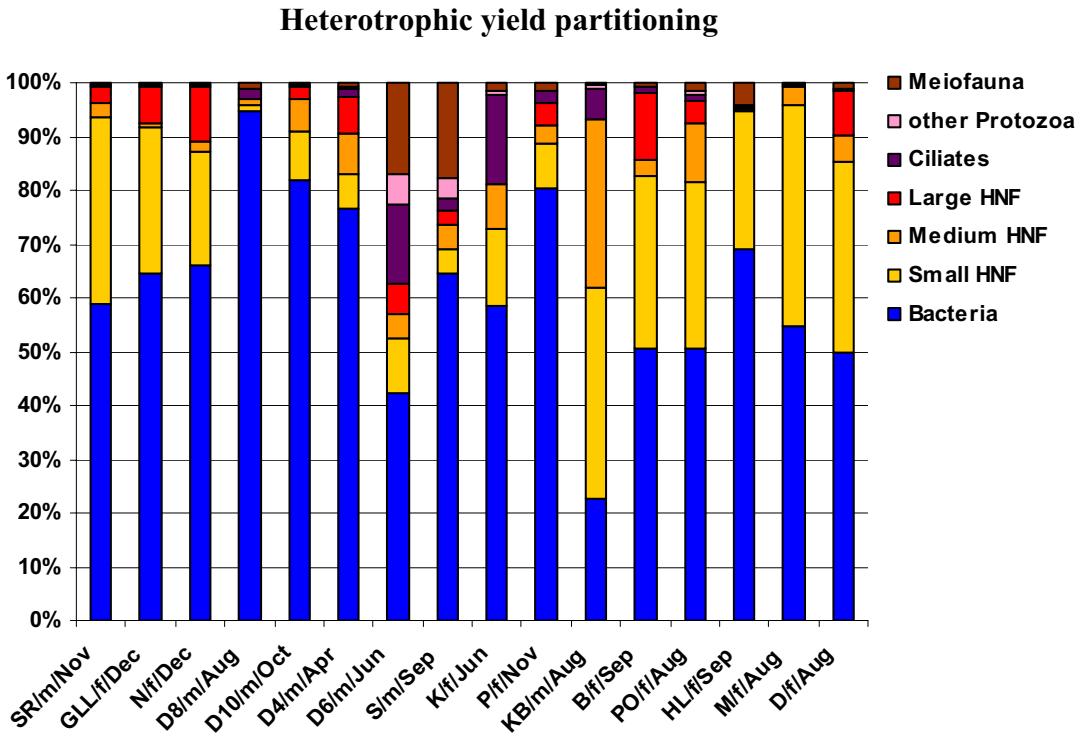


Fig. 14: Percentages of heterotrophic components of the community heterotrophic yield, with sampling month and salinity (m = marine, f = freshwater): Shark River Bay (SR), Green Lane Lake (GLL), Nockamixon Lake (N), Fühlinger Lake (K), Dorum August 2001 (D8), Dorum October 2001 (D10), Dorum April 2002 (D4), Dorum June 2002 (D6), Schöhsee (P), Königshafen (S), Basalt Lake (B), Potsdam Lake (PO), Lake near Hochstetter Fjord (HL), beach on Store Koldewey (KB), Melles Lake (M), Duck Lake (D).

4. Discussion

The investigated sites represented temperate and arctic climates, temperate regions located in western Europe (Germany), at the East coast of North America (USA; Pennsylvania, New Jersey), and the arctic region in the North East of Greenland. Most sites were investigated only once. Thus the study period comprised different seasons. Only Dorum was investigated on a monthly basis over one seasonal cycle, therefore we could use data from Dorum to cover characteristic periods (late summer to early autumn D8, winter D10, spring D4 and summer D6) as references (study 1).

The vegetative period in the arctic region is much shorter than in temperate regions and lower amounts of phototrophic and correlated heterotrophic biomass were expected. The surrounding vegetation, known to be an important source for organic matter in shallow regions of aquatic ecosystems, was clearly different between the investigated sites, on the one hand between the temperate and arctic regions, on the other hand between the marine and freshwater systems in the same region (Wetzel 2001). Therefore basic differences with regard to microbial community structures were expected between the different climate areas, continents and freshwater and marine sites.

Relationships between environmental parameters and abundance

The correlations between environmental parameters and organisms abundance were calculated in order to discover the main influencing factors. A significant positive correlation was found between temperature and medium HNF. Medium HNF are known to feed on bacteria (study 1 and references therein). But no correlation was found between medium HNF and bacteria here. We registered only the abundance of organisms, while the production could not be measured. However it is known that bacterial production rises with increasing temperatures or availability of substratum (Van Duyl & Kop 1994). Hence an increase of bacterial production because of increasing medium HNF abundance due to a higher food supply could be a possible explanation for the correlation of medium HNF with temperature. The simultaneous rise of the grazing pressure by medium HNF on bacteria in turn might have limited the bacterial standing stock. A significant negative correlation was found between salinity and small HNF, no explanation was found for this result.

Community biomass

Correlations between the environmental parameters (grain size, water content, temperature, salinity, organic carbon and nitrogen content) and the total microbial biomass at all

investigated sites were calculated in order to display eventual dependencies. However, no significant correlations and this way no relationships between the measured environmental parameters and total biomass could be identified.

In order to deepen the understanding of the differences between the observed total biomass, sites with an extremely small and sites with large total biomass were looked at in particular. The smallest biomass was found in Store Koldewey beach (KB; 0.7 µg C ml^{-1}) in Greenland, whereas the largest ones were found in Shark River Bay (SR, 257.6 µg C ml^{-1}) and Green Lane Lake (GLL, 169.2 µg C ml^{-1}) in USA. The total biomass in the other locations ranged between 20 µg C ml^{-1} and 60 µg C ml^{-1}.

KB is located on the eastern side of the island of Store Koldewey. The position of the open ocean shore line, a steep mountain slope close to the shore as well as the low amount of silt and clay indicated that the tidal change did not play an important role at this position. The wave shock however appeared to be strong ("high energy beach"; Knox 2000). These environmental attributes form an extremely unstable habitat with permanently moving sand grains, presumably hard to be colonized by microbial and interstitial organisms. The sites with the highest biomass, SR and GLL, were both investigated during winter (even under ice in GLL), when due to low temperatures and little light the biomass was expected to be relatively low (Epstein 1997; Lee & Patterson 2002; study 1). Biomass and abundance of at least phototrophic organisms will probably rise later to higher values during spring. The human activity and sewage supply at both sites provided high organic input. Even if the measured amounts of organic carbon and nitrogen were not conspicuously higher than in other investigated sediments, the composition of supplied organic matter might be of easy use for microbial organisms. The total biomass at Nockamixon Lake (N) however, suffering also from a high load of sewage and fertilizers from vicinal towns and farms and high human activity (shipping, fishing), was considerably lower (17.4 µgC ml^{-1}) compared to SR and GLL. The water of N was of a brownish to yellowish color, which pointed to a high amount of humid substances from plants. The expected high humic fraction of organic matter, difficult to use for most microbes, might have kept the organisms abundance low.

These four discussed examples revealed that others than the usually measured environmental parameters had a deciding influence on biomass: in the case of KB mechanical disturbances, in SR and GLL probably the availability of organic carbon (not measured) and in N humic substances. According to Manini et al. (2003) the source and quality of the organic matter input (organic carbon availability) are indeed the main factors influencing the total microbial biomass and community structure in a given environment.

The highest values of organic carbon were found in Potsdam Lake (PO) and the lake near Hochstetter Fjord (HL). In spite of that, total biomass in both arctic lakes during early autumn were not extraordinary high compared to the other arctic and even ultraoligotrophic Melles Lake (M) or Duck Lake (D). In HL a fluffy layer, probably of crushed plant material, was found on top of the sediment (personal observation). Humic substances of plant material cause high organic carbon and nitrogen contents but due to their complex structure they are difficult to be used by many microbial organisms. At PO signs of a former visit of geese (feathers and faeces) were observed, probably responsible for a high temporary nutrient content there. In contrast to plant material, animal faeces are in general easily used by microbes.

At Dorum (D8, D10, D4, D6) total biomass ranged from 9.7 µgC ml^{-1} to 60.1 µgC ml^{-1}. The main changes of biomass were less directly caused by the measured environmental attributes, than controlled by the seasonal development of the community trophic structure (shift from bottom-up to top-down control during summer; study 1).

Microphytobenthos structure

The microphytobenthic components at many of the investigated sites were dominated by small diatoms. This is also reported in literature about other shallow sediments (Agatz et al. 1999; Dietrich & Arndt 2000; Lee & Patterson 2002). However, different results were found in this study at D8, Duck Lake (D), HL, PO and Sylt (S), where PNF and cyanobacteria were dominant respectively. It is known from studies that the dominance of diatoms might shift to cyanobacteria and chlorophytes, especially during late summer (Epstein 1997; Lucas & Holligan 1999; Cook et al. 2004; Aberle-Malzahn 2004; study 1). Therefore the high percentages of large PNF in D8 could be attributed to the seasonal development of the heterotrophic components during summer (study 1). The same mechanisms were assumed to be responsible for the high cyanobacteria percentages in S, D, PO and HL.

KB, B and M were also sampled during late summer, but diatoms dominated the microphytobenthos there. Same sampling dates (seasons) implied the same developmental status for the communities, but differences were found. The short generation times of the microbial organisms however enable them to react very quickly to changed conditions, thus seasonal phases are not linked to fixed dates neither in one region nor in different years. Anyway the “snapshot”- results of each community were attributed to the four seasonal phases in Dorum (study 1).

Community structure

The ratio of microphytobenthic biomass to bacteria biomass in per cent as well as the ratio of ciliates to HNF were calculated for all sites according to Dietrich & Arndt (2000). In contrast to Dietrich & Arndt (2000) no obvious shift of the percentages of microphytobenthos and bacteria according to temperate and arctic climate were identified. The highest portions (>10%) of ciliates compared to HNF were found in S, P, D6 and K. This might lead to the assumption that ciliates were always more abundant in temperate than in arctic regions but only during summer, if compared with the other temperate sites N, GLL and SR. Dietrich & Arndt (2000) suggest higher portions of microphytobenthos and ciliates respectively in Antarctic sediments than in temperate ones thus the results of this study contradict to those of Dietrich & Arndt (2000).

Heterotrophic to phototrophic biomass

The mean percentage of total heterotrophic biomass (bacteria, HNF, ciliates and meiofauna) of total biomass was found to be smaller in freshwater sites than in marine sites (27.3% in freshwater and 55,2% in marine water; Fig. 15). According to literature the organic matter of marine and freshwater sediments is qualitatively different (Capone & Kiene 1988). In freshwater systems complex structural polysaccharides and phenolic polymers (i.e. ligno-cellulose) represent a greater fraction of the organic input than in marine systems. This higher fraction of humic substances, difficult to be used by detritus feeding heterotrophs, might have kept the heterotrophic biomass at a low level.

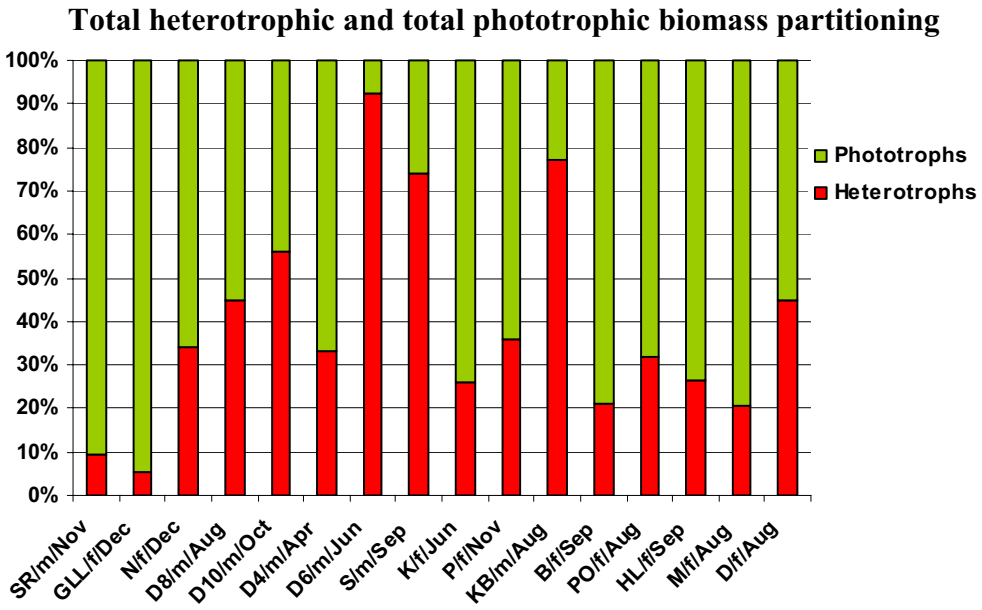


Fig. 15: Total heterotrophic and phototrophic biomass partitioning

The community exhibited a trend to lower portions of meiofaunal biomass in arctic sites (except HL) as well as a trend to lower portions during winter in temperate sites (SR, GLL, N, D10, D4) (Fig. 12). This could be explained by the difference of generation times of meiofauna (7 d to 365 d) compared to protozoa (2.8 h to 1200 h) (Wetzel 2001; Stead, et al. 2005). The vegetation period in arctic regions might be too short for meiofauna to reproduce effectively. In contrast to this protozoa can react quickly to favourable conditions with a rapid increase of abundance, also in predominantly inhospitable environments as a result of short generation times and robust resting stages (as cyst, germ, dormant egg) (Fenchel 1987; Patterson et al. 1989). The low portions of meiofauna during winter in temperate regions could be simply caused by the low temperature. Another possible explanation could be found in the fact that in temperate regions the primary production during summer was high because of high temperatures, whereas in arctic regions the temperature also during summer is normally not higher than 10°C and consequently primary production was comparably low. Hence the food supply in temperate regions was larger during summer than during winter and in arctic regions during all seasons, and accordingly meiofauna increased abundance due to high prey availability. However these mechanisms could have held true simultaneously rather than alternatively.

The high percentage of HNF of the community biomass in KB compared to all other sites attracted attention. We assumed that a successful settlement of small macrozoobenthos, meiofauna and even ciliates was impossible in such an extremely unstable sediment. It is known that in high energy beaches the numbers of meiofauna and macrofauna organisms are much lower than in intertidal habitats (Menn 2002). In contrast to meiofauna and larger protists HNF might balance a loss because of disturbance by high growth rates and might therefore become the main predator in highly unstable sediments

Potential yield

Bacteria contributed with a mean value of 61% to the total heterotrophic yield, with HNF up to 93% in most investigated sites (Fig. 14). Even in quite different compositions of the communities' biomass in the different sites, the contribution of bacteria and HNF (nanobenthos) to the heterotrophic yield was quite similar. The calculation of the yield according to the equation of Fenchel (1974) however is valid only for communities in an equilibrium and in an habitat without limiting factors. These preconditions were at the best approximately fulfilled at the investigated sites. Anyhow the calculation allowed a rough estimation of components' contribution to the carbon and energy flow.

A yield to body size relationship similar to heterotrophic organisms exists also in phototrophic organisms (Odum 1971). Consequently the contributions of the tiny cyanobacteria and small PNF to the total microphytobenthic yield (total primary production) could considered to be much higher than their biomass let assume.

The redundance analysis (RDS) revealed that none of the environmental factors could really explain the distribution of organisms in the different sites. The amount of chlorophyll-*a* was the only factor slightly influencing the microbial organism abundance (cyanobacteria, diatoms, PNF, bacteria, HNF). This seemed to correspond with the results derived in study 3, where also the amount of chlorophyll-*a* slightly influenced the abundance and biomass of meiofauna and ciliates. Surprisingly the only significant correlation between chlorophyll-*a* concentration and primary producers was negative with small PNF, whereas no significant correlations existed with diatoms, cyanobacteria or total phototrophic biomass.
Thus it is important to determine the main sources of chlorophyll-*a*. A common method to convert the measured chlorophyll-*a* values into the biomass of primary producers is to multiply the chlorophyll-*a* value with a conversion factor (e.g. 40; Manini et al. 2003 and references therein). The this way calculated microphytobenthic biomass exceeded the values originating from the counted microbenthic organisms by a factor up to 14. This excess biomass suggested important other sources of chlorophyll-*a*. Indeed due to the methods used we neither counted large (>50 µm) phototrophic flagellates such as large Eugelnides, Dinoflagellates and diatoms (>200 µm), nor pieces of undegraded macrophytes, shreddered plant material and sedimented phytoplankton nor undigested primary producer residuals in fecal pellets. The contribution of these groups to chlorophyll-*a* and phaeopigment seemed to play an important role in structuring the benthic microbial community.

Conclusion

The total biomass exhibited large differences in-between the investigated sites, up to a factor 370. No obvious trend in values of total biomass related to latitude, salinity, the amount of total organic carbon and the amount of total nitrogen was found. The results of this study revealed that next to seasonal influences, the availability of organic carbon and chlorophyll-*a* controlled biomass as well as high disturbance resulting from physical circumstances as found at the “high energy beach” of KB.

The community biomass was dominated by microphytobenthos in most sites. The portion of diatoms, cyanobacteria and PNF was supposed to change seasonally.

The heterotropic biomass portion showed a trend to higher percentages in marine compared to freshwater sites. The qualitative differences of organic matter in freshwater compared to marine systems were a possible explanation. The higher fraction of humic substances in freshwater systems therefore might have limited the heterotrophic detritus feeders.

Meiofauna biomass showed a trend to lower values in arctic regions compared to temperate ones, at least during summer. This was suggested to be caused by the longer generation times of meiofauna compared to protozoa and by the shorter vegetation period in arctic regions in combination with low temperature.

The structure of the heterotrophic biomass (bacteria, HNF, ciliates and meiofauna) was different between the sites and no pattern could be identified, which could allow to allocate these differences to environmental parameters. However, abundance and the calculated potential yield were clearly dominated by bacteria and HNF in all sites, regardless of their mostly low portion in total biomass.

Acknowledgments

The authors thank the following people for their help: Dr. J. Matthiessen for assistance in measuring environmental parameters; Dr. W. Wosniock in statistical questions; Dr. W. Stumm for reading the proofs. Dr. T. Brey; Dr. S. Schultes and Dr. U. Schneider for comments on previous versions of the manuscript. The present work has been financed by the DFG (Deutsche Forschungs Gemeinschaft) grant number BE 2279/3-1, by the Alfred-Wegener-Institute for Polar and Marine Research and by the German Social Security System.

References

Aberle-Malzahn, N., 2004. The microphytobenthos and its role in aquatic food webs. Ph.D. Thesis, Christian-Albrechts-Universität Kiel, Germany, unpublished.

Agatz, M., Asmus, R. M., Deventer, B., 1999. Structural changes in the benthic diatom community along a eutrophication gradient on a tidal flat . Helgol. Mar. Res. 53, 92-101.

Alongi, D.M., 1991. Flagellates of benthic communities: characteristics and methods of study. In: The biology of free-living heterotrophic flagellates. Eds: Patterson DJ, Larsen J. Systematics association by Clarendon press, Oxford, pp 57-75.

Alongi, D. M., 1994. The role of bacteria in nutrient recycling in tropical mangrove and other coastal benthic ecosystems. Hydrobiologia 286, 19-32.

Auer, B., Kiertucki, E., Arndt, H., 2003. Distribution of heterotrophic nano- and microflagellates in sediments of two small mesotrophic lakes . Archiv für Hydrobiologie 158 (1), 127-144.

Azam, F. T., Field, J. G., Gray, J. S., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 10, 257-263.

Begon, M. E., Harper, J. L., Townsend, C. R. , 1996. Ecology. Oxford, Blackwell Science, pp738.

Berninger, U.-G., Epstein, S. S., 1995. Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea sediment. Aquat. Microb. Ecol. 9, 229-236.

Berninger, U.-G., Huettel, M., 1997. The impact of flow on the oxygen dynamics in photosynthetically active sediments. Aquat. Microb. Ecol.12, 291-302.

Borsheim, K. Y., Bratbak, G., 1987. Cell volume to cell carbon conversion factors for a bacterivorous Monas sp. enriched from seawater. Mar. Ecol. Prog. Ser. 36, 171-175.

Bratbak, G., 1985. Bacterial biovolume and biomass estimations. Appl. Environ. Microbiol. 49, 1488-1493.

Buchanan, J. B., 1984. Sediment analysis. In 'Methods for study on marine benthos'. Eds. N. A. Holme and A. D. McIntyre. pp. 41-65. Blackwell science.

Caron, D. A., 1991. Evolving role of protozoa in aquatic nutrient cycles. Protozoa and their role in marine processes G 25, 387-415.

Capone, D. G., Kiene, R. P., 1988. Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon metabolism. Limnol. Oceanogr. 33 (4/2), 725-749.

Cook, P. L. M., Revill, A. T., Butler, E. C. V., Eyre, B. D., 2004. Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary. II. Nitrogen cycling. Mar. Ecol. Prog. Ser. 280, 39-54.

Daemen, E. A. M. J., 1986. Comparison of methods for the determination of Chlorophyll in estuarine sediments. Neth. J. Sea Res. 20, 21-28.

Dell'Anno, A., Mei, M. L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Mar. Pollut. Bull. 44, 611-622.

Dietrich, D., Arndt, H., 2000. Biomass partioning of benthic microbes in a Baltic inlet: relationships between bacteria, algae, heterotrophic flagellates an ciliates. Mar. Biol. 136, 309-322.

Edler, L., 1979. Recommendations for marine biological studies in the Baltic Sea: Phytoplankton and Chlorophyll. The Baltic Marine Biologists 5, 1-37.

Ekebom, J., 1999. Heterotrophic nanoflagellates and bacteria in sediment of a brackish water sill basin in the Baltic Sea. Hydrobiologia 393, 151-161.

Epstein, S. S., 1997. Microbial food webs in marine sediments. II Seasonal changes in trophic interactions in a sandy tidal flat community. Microbiol. Ecol. 34, 199-209.

Epstein, S. S., Rossel, J., 1995. Enumeration of sandy sediment bacteria: search for optimal protocol. Mar. Ecol. Prog. Ser. 117, 289-298.

Fenchel, T., 1969. The ecology of marine Microbenthos IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated Protozoa. Ophelia 6, 1-182.

Fenchel, T., 1974. Intrinsic rate of natural increase: the relationship with body size. Oecologia 14, 317-326.

Fenchel, T., 1987. Ecology of Protozoa. The biology of free-living phagotrophic Protists. Science Tech, Wisconsin, pp197.

Fogg, G. E., 1998. The biology of polar habitats. Oxford University Press, pp263.

Fry, J. C., 1990. Direct methods and biomass estimation. In: 'Methods in microbiology'. Eds: Grigorova, R., Norris, J.R. Academic Press: London, pp 41-85.

Gray, J.S., 1981. The ecology of marine sediments. Cambridge University Press, Cambridge, pp185.

Hondeveld, B. J. M., Nieuwland, G., Van Duyl, F. C., Bak, R. P. M., 1994. Temporal and spatial variations in heterotrophic nanoflagellate abundance in North Sea sediments. Mar. Ecol. Prog. Ser. 109, 235-243.

Hubberten, H.-W., 1994. Reports on Polar Research No.174 The Expedition ARKTIS X/2 of the research vessel POLSARSTERN in 1994. Alfred-Wegener-Institute for Polar and Marine Research, Germany.

Jokat, W., 2004. Reports on Polar Research No.475 The Expedition ARKTIS XIX/4 of the research vessel POLSARSTERN in 2003. Alfred-Wegener-Institute for Polar and Marine Research, Germany.

Knox, G.A., 200. The ecology of seashores. CRC Press, London, pp 557.

Lee, W. J., Patterson, D. J., 2002. Abundance and Biomass of Heterotrophic Flagellates, and Factors Controlling Their Abundance and Distribution in Sediments of Botany Bay. Microb. Ecol. 43, 467-481.

Lorenzen, C. J., 1976. Determination of Chlorophyll and Pheo-Pigments: Spectrophotometric equations. Limnol. Oceanogr. 12, 343-346.

Lucas, C. H., Holligan, P. M., 1999. Nature and ecological implications of algal pigment diversity on the Molenplaat tidal flat (Westerschelde Estuary, SW Netherlands). Mar. Ecol. Prog. Ser. 180, 51-64.

Maclassac, E. A., Stoeckner, J. G., 1993. Enumeration of phototrophic picoplancton by autofluorescence microscopy. In: Handbook of Methods in Aquatic Microbial Ecology. Eds: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J., Lewis, Boca Raton, FL, USA, pp 187-97.

Manini, E., Fiordelmondo, C., Gambi, C., Pusceddu, A., Danovaro, R., 2003. Benthic microbial loop functioning in coastal lagoons: a comparative approach. Oceanologica Acta 26, 27-38.

Menden-Deuer, S., Lessard, E. J., 2000. Carbon to volume relationship for dinoflagellates , diatoms, and other protist plankton. Limnol. Oceanogr. 45, 569-579.

Menn, I., 2002. Ecological comparison of two sandy shores with different wave energy and morphodynamics in the North Sea- Ph.D. Thesis, University of Hamburg, Germany, unpublished.

Odum, E. P., 1971. Fundamentals of ecology. Saunders Company, London, pp574.

Patterson, D. J., Larsen, J., 1991. The biology of free-living heterotropic flagellates. The systematics association Special volume 45, 1-505.

Patterson, D. J., Larsen, J., Corliss, J. O., 1989. The ecology of heterotrophic flagellates and ciliates living in

marine sediments. Progress in Protistology 185-277.

Porter, K. G., Sherr, E. B., Sherr, B. F., Pace, M., Sanders, R. W., 1985. Protozoa in planktonic food-webs. J. Protozool. 32, 409-415.

Reise, K., 1985. Tidal flat ecology. Springer-Verlag, Heidelberg, pp191.

Sherr, E. B., Caron, D. A., Sherr, B. F., 1993. Staining of heterotrophic Protists for visualization via Epiflourescence Microscopy. In: Handbook of Methods in Aquatic Microbial Ecology. Eds: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J., Lewis, Boca Raton, FL, USA, pp213-27.

Stead T. K., Schmid-Araya, J.M., Hildrew, A.G., 2005. Secondary production of a stream metazoan community: Does the meiofauna make a difference? Limnol. Oceanogr. 50:398–403.

Stonehouse, B., 1989. 'Polar ecology.' Blackie and Son, Glasgow, pp222.

Van Duyl, F. C, Kop, A. J., 1994. Bacterial production in North Sea sediments: Clues to seasonal and spatial variations. Mar. Biol. 120, 323-337.

Velji, M. I., Albright, L. J., 1993. Improved sample preparation for enumeration of aggregated aquatic substrate Bacteria. In: Handbook of Methods in Aquatic Microbial Ecology. Eds: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J., Lewis, Boca Raton, FL, USA, pp139-42.

Wagner, B., 2000. Reports on Polar Research Holovcene encvironmental history of East Greenland - evidence from lake sediments. Germany, Alfred-Wegener-Institute for Polar and Marine Research.

Waterbury, J. B., Watson, S. W., Valois, F. W., Franks, D. G., 1986. Biological and ecological characterization of the marine unicellular cyanobacterium Synechococcus. Can. Bull. Fish. Aquat. Sci. 214, 71-120.

Wetzel, R.G., 2001. Limnology. Academic Press, London, pp1006.

Study 3

Temporal dynamics and spatial distributions of ciliate community structure and meiofauna in sediments

Yanli Lei[1, 3], Karen Stumm[2], Stephen A. Wickham[3] and Ulrike-G. Berninger[3]

[1] Institute of Oceanology, Chinese Academy of Science, 266071 Qingdao, PR China

[2] Alfred - Wegener - Institute for Polar and Marine Research. Am Handelshafen 12, 27570 Bremerhaven, Germany

[3] Universität Salzburg, FB Organismische Biologie, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria

Abstract

Ciliate community structure, and abundance and biomass of micro- and meiofauna were temporally investigated over a year in a brackish sediment, and spatially studied in different types (marine, brackish and freshwater) of sediments. The temporal study revealed that ciliate diversity and species richness did not show seasonal patterns, though there were significant monthly shifts in species composition. In contrast, the abundance and biomass of micro- and meiofauna showed a seasonal cycle with two distinguishable stages. During the first stage (spring), the feeding type of dominant ciliates and their size spectra suggested that ciliates and meiofauna might be the main diatom consumers, and predation pressure from meiofauna on ciliates was low. During the second stage (summer), there was a shift from meiofauna and foraminifer dominance in June to ciliate dominance in July, coinciding with a shift from high to low potential predation pressures from meiofauna on ciliates.

The spatial study revealed great differences in ciliate species composition between sites. The occurrence of dominant ciliates and their allocation to feeding types indicates that herbivory was the most common and important feeding strategy, while the importance of bacterivory varied significantly among sediments investigated. Statistical analyses showed that ciliate diversity and species richness, and biomass of ciliates and meiofauna were largely controlled by chlorophyll-*a* concentrations, and the potential biotic interactions among organism groups. In contrast, abiotic factors including temperature, salinity, nitrogen and carbon contents were only sometimes important for certain ciliate or meiofaunal groups. Abundance and biomass of the entire micro- and meiofauna community varied greatly among sites. In general, ciliates dominated in terms of abundance, while meiofauna contributed most of the biomass. Based on biomass ratios and estimated weight-specific metabolic rates, ciliates accounted on average for 34% of estimated metabolic rate of the micro- and meiofauna consumers in the respective sediment investigated. These data suggest that ciliates are important components of benthic food webs. Furthermore, numerous cysts were found in sediments, and their potential ecological significance is discussed.

1. Introduction

Numerous studies have been carried out on the pelagic microbial food web and demonstrated the significant role of its components in the cycling of matter and the transfer to higher trophic levels (e.g. Azam et al. 1983; Porter et al. 1985; Berninger et al. 1991; Sherr & Sherr 1994). In contrast, the quantitative importance, community structure, and ecological functions of protozoa in benthic environments are less known (Epstein & Gallagher 1992; Santangelo & Lucchesi 1992; Berninger & Epstein 1995; Dietrich &Arndt 2000, McCormick & Cairns 1991; Wickham et al. 2004). This can partly be explained by methodological difficulties in experimental approaches, especially in the extraction of these comparatively fragile organisms from sediments (Alongi 1986; Tso & Taghon 1997; Wickham et al. 2000; Wieltschnig et al. 2003). Sediments harbour very diverse microbial organisms (Hartwig 1973; Fenchel 1967; 1968; 1969; Patterson et al. 1989; Böttcher et al. 2000). Moreover, cysts in sediments constitute a potential seed bank that can be important in influencing the dynamics of the pelagic microbial food web (Rubino et al. 2000; Müller et al 2002; Godhe & McQuoid 2003; Tommasa et al. 2004). Recent molecular studies further demonstrated that biodiversity and ecological importance of unicellular eukaryotes in some special benthic habitats still await discovery (e.g. Stoeck & Epstein 2003; Stoeck et al. 2003).

Both protozoa and meiofauna can be allocated to a variety of nutrition types. For instance, ciliates feed on various trophic levels including bacteria, algae, flagellates, other ciliates, and some even prey on metazoans (Sanders & Wickham 1993; author's personal observations). These diverse feeding types may complicate the proper assignment of a trophic position. However, such diversity and complexity are often overlooked in ecological researches when only quantitative aspects are considered (Epstein & Gallagher 1992; Hausmann et al. 2002). Due to their high functional diversity and high growth rates, and especially the fact that the number of both microbial prey and micrograzers present in sediments are higher by one to several orders of magnitude than those in the water column, protozoa have the potential to fulfil an important ecological role in benthic ecosystems (Fenchel 1969; 1987; Arndt et al. 2000; Garstecki et al. 2000; Wickham et al. 2000; Hamels et al. 2003).

So far, only few studies have quantitatively addressed the structure and dynamics of protozoa and meiofauna from sediments (Bak & Nieuwland 1989; Epstein 1997a; b; Lee & Patterson 2002; Hamels et al. 2003). Even less information is available on qualitative studies of community structure of protozoa in sediments (Wickham et al. 2004). Kemp (1988; 1990) studied ciliate grazing rates on bacteria in benthic habitats and found that benthic ciliate grazing could not control the growth of sediment bacteria. Later, limited information on

protozoan numbers and activities in sediments provided increasing evidence of a comparatively smaller role of protozoa than that in the water column (Epstein & Shiaris 1992; Arndt et al. 1990). However, Finlay & Esteban (1998) reviewed the biodiversity and ecological function of freshwater protozoa and suggested that though benthic ciliate biomass accounts for slightly less than 10% of total benthic invertebrate biomass, ciliate production may equal or even exceed invertebrate production and thus play an important role in benthic microbial food web.

The main goals of this study are: 1) to investigate the seasonal dynamics in ciliate community structures (abundance, biomass, species composition and diversity) and abundance and biomass of micro- and meiofauna in a selected brackish sediment; 2) to study the spatial distribution of micro- and meiofauna in marine, brackish and freshwater sediments; 3) to examine which environmental factors (temperature, salinity, silt and clay, carbon, nitrogen, and chlorophyll *a*) influence their biomass distribution; and 4) to evaluate the potential importance of protozoa in these environments.

2. Material and Methods

Study sites and sampling

The study was conducted at 15 sampling sites from different benthic habitats, including marine, brackish and freshwater sediments. Among the 15 sites, 3 were from the USA, 4 from northern Germany and 8 from Arctic regions (Greenland) (Table 1). The temporal study was carried out in a brackish mudflat of Dorum, Germany, including ten samplings from August 28, 2001 to October 22, 2002 (Table 2). The spatial distribution study was based on one sample from each of the 15 sites (Table 1). All samples were taken from the surface layer of the respective sediments. At each sampling, environmental factors (temperature, salinity, silt and clay, carbon, nitrogen, and chlorophyll-*a*) were measured (study 1 and 2).

The marine and brackish sediment samples were taken from the intertidal flats at low tide about 500 m from the seashore within a time period of 30 min before or after low tide. The freshwater sediments were collected from the littoral zone, e.g. the shore or beach of the respective lakes. The sediments were sampled by using round plexiglass cores (36 mm inner diameter) or by carefully removing an undisturbed section of the sediment with a shovel. The samples were stored in a dark, cooling box and transported to the laboratory immediately. In the laboratory, the upper 3 mm (within the oxic layer) of each sediment (about 2 ml) was sliced off and transferred a into tube and diluted with 2 ml filtered (0.2 µm) seawater, and immediately fixed with ice cold glutaraldehyde solution (final concentration 2%).

Extraction and staining

The protozoa and meiofauna were separated from the sediments by using centrifugation in silicagel (Percoll®) gradients (Epstein 1995, Starink et al. 1994). The Percoll gradients were prepared after Alongi (1986), Epstein (1995) and Burgess (2001) with minor modifications. Briefly, 6 ml of 50% Percoll solution in twice concentrated artificial seawater were placed into centrifuge tubes, 2 ml of 100% Percoll solution were gently injected into the 50% Percoll solution from the bottom of the tubes, and then the stacked Percoll gradient was obtained. 1 ml to 3 ml of the sediment sample was placed on the top of the gradient and centrifuged at 4°C for 15 min at 3026 g in an swing out rotor. The samples were gently (< 5 mm Hg) filtered onto 1.2 µm pore-size cellulose nitrate filters and impregnated by the Quantitative Protargol Stain (QPS) method (Montagnes & Lynn 1993, Skibbe 1994). Using this method, ciliate's infraciliature and nuclear apparatus were revealed. Combining live observation and QPS, ciliates could be identified generally to genus level, and almost half of them to species level.

Table 1: Basic information of the 15 sampling sites. The samples were taken from the 3 mm upper layers of sediments. Temperature was measured inside the sediments.

Sampling sites		Habitats	Date	Temperature (°C)	Salinity (psu)	Location
GLL	Greenlane Lake, USA	Lake	Dec 3, 2003	0	0	40º 20' N; 75º 27' E
N	Nockamixon Lake, USA	Lake	Dec 3, 2003	7	0	40º 28' N; 75º 13' W
SR	Shark River Bay, USA	Estuary	Nov 20, 2003	12	26	40º 10' N; 74º 01' E
B	Basalt Lake, Arctic	Lake	Sep 8, 2003	9	5	72º 43' N; 22º 28' W
D	Duck Lake, Arctic	Lake	Aug 22, 2003	10	0	76° 25' N; 18° 45' W
HL	Hochstätter, Arctic	Pond	Sep 6, 2003	6	4	75° 37' N; 19° 44' W
IF	Ice floe, Arctic	Pond	Aug 20, 2003	1	5	77º 09' N; 01º 12' W
KB	Store Koldewey Beach, Arctic	Marine beach	Aug 13, 2003	2	36	76º 07' N; 18º 31' W
M	Melles Lake, Arctic	Lake	Aug 13, 2003	9	3	76º 08' N; 18º 36' W
SB	Shannon Beach, Arctic	Marine beach	Aug 26, 2003	5	36	76º 07' N; 18º 31' W
PO	Potsdam Lake, Arctic	Lake	Aug 26, 03	7	3	75º 03' N; 18º 46' W
D6	Dorum, Germany	Tidal flat	Jun 13, 2002	16	25	53º 42' N; 08º 29' E
K	Fühlionger See, Germany	Lake	Jun 2, 2003	24	0	50º 58' N; 74º 01' E
P	Schöhsee, Germany	Lake	Nov 13, 2001	4	0	54º 09' N; 10º 26' E
S	Königshafen, Sylt, Germany	Tidal flat	Sep 12, 2002	21	31	55º 02' N; 08º 25' E

Table 2: Sediment temperature (Tem) and salinity (Sal), and ciliate dominant species, feeding types, and biomass and abundance contributions at Dorum from Aug 28, 2001 to Oct 22, 2002. Note that the samples were taken from 3 mm upper layers of sediments. Bolds emphasize remarkable contributions.

Date	Tem (°C)	Sal (psu)	Dominant species	Body length (µm)	Main feeding types [a]	Biomass (%)	Abundance (%)
2001							
Aug 28	18	31	*Pleuronema marinum*	100	Herbivory (diatoms, flagellates)	**30**	20
			Prorodon discolor	120	Herbivory (flagellates, diatoms)	16	4
Sep 25	17	nd	*Pleuronema marinum*	100	Herbivory (diatoms, flagellates)	**51**	**38**
			Frontonia marina	125	Herbivory (cynobacteria, diatoms, flagellates)	**37**	10
Oct 22	11	18	*Chlamydodon triquetrus*	140	Herbivory (diatoms)	**47**	18
			Condylostoma arenarium	600	Carnivory (ciliates, metazoans)	**42**	8
			Aspidisca lyncaster	30	Bacterivory	1	**32**
2002							
Mar 5	7	17	*Condylostoma arenarium*	600	Carnivory (ciliates, metazoans)	**92**	**36**
Apr 18	18	26	*Chlamydodon triquetrus*	140	Herbivory (diatoms)	**71**	28
			Chlamydodon cyclops	120	Herbivory (diatoms)	20	14
			Uronema marinum	40	Bacterivory	2	18
May 14	16	21	*Prorodon vermiforme*	150	Herbivory (flagellates, diatoms)	**33**	12
			Frontonia marina	120	Herbivory (cynobacteria, diatoms, flagellates)	21	7
Jun 13	16	25	*Trachelocerca* sp.	500	Omnivory (flagellates, diatoms, cynobacteria, small ciliates)	**37**	16
			Aspidisca fusca	60	Omnivory (bacteria, flagellates)	19	**36**
Jul 18	17	10	*Condylostoma arenarium*	600	Carnivory (ciliates, metazoans)	24	2
			Aspidisca fusca	60	Omnivory (bacteria, flagellates)	20	28
			Trachelocerca sp.	500	Omnivory (flagellates, diatoms, cynobacteria, small ciliates)	14	4
			Cyclidium sp.	40	Bacterivory	6	24
Aug 12	21	10	*Chlamydodon cyclops*	120	Herbivory (diatoms)	**61**	13
			Pleuronema marinum	100	Herbivory (diatoms, flagellates)	13	5
			Dysteria pusilla	25	Herbivory (diatoms, cynobacteria)	2	**34**
Oct 22	10	15	*Chlamydodon cyclops*	120	Herbivory (diatoms)	**65**	22
			Frontonia marina	125	Herbivory (cynobacteria, diatoms, flagellates)	11	2

[a] Feeding types of dominant species were mainly determined by our observations, that is, food vacuoles contents in protargol impregnated specimens and/or live cells. nd, data were not determined.

Analysis

Investigation was conducted under a compound microscope (Nikon Eclipse E800) equipped with a high-power oil immersion objective as well as with bright-field and interference contrast optics. Micro- and meiofauna were restricted to ciliates, amoebae, foraminifera, nematodes, copepods, rotifers, ostracods, tardigrades, cladocerans and oligochaetes. Each filter was counted in its entirety at ×200 or ×400 magnification, and identification was conducted at ×1000 magnification. Ciliates were identified using related literature, including

Kahl (1930-1935), Carey (1992), Foissner (1995/96, 1997), Foissner et al. (1999) and Lynn & Small (2002), and some original species descriptions. Other microfauna (amoebae, foraminifera) and meiofauna (nematodes, copepods, rotifers, ostracods, tardigrades, cladocerans and oligochaetes) were identified to group level using Page (1988) and Giere (1993). In addition, numerous cysts were found on the QPS slides. Ciliate cysts could be identified from their series of encyst/excyst stages impregnated by protargol, or by possession of macronucleus and micronuclei, and somatic kineties; other cyst identifications were mainly based on relevant references (e.g. Reid & Boalch 1987; Rubino et al. 2000; Müller et al. 2002; Tommasa et al. 2000; 2004). Due to limited knowledge and available information on cysts, many individuals, which could not unambiguously be attributed to cysts or other organisms, were excluded from the statistical analyses.

Measurements were performed during counting. In each sample, abundance and biomass of all micro- and meiofauna were analysed. For ciliates, species composition, community similarity, species richness (number of species for each sample) and diversity were determined. Diversity was measured using the Shannon-Wiener index (H'). Similarity in community composition between two samplings was measured using the Jaccard measurement of similarity. As $C_j = j / (a + b - j)$, where a and b are the number of morphotypes in samples A and B, and j is the number of morphotypes found in both A and B (Magurran 1988). C_j ranges from 0 to 1, and is the proportion of shared morphotypes.

Biovolumes of ciliates (species-specific) and other protozoa (amoebae, foraminifera) were calculated using the cell size measured from protargol preparations and common geometric equations. Protozoan biovolumes were converted to carbon content, allowing for shrinking due to fixation, using individual conversion factors (Table 3). Biovolumes of meiofaunal groups were calculated from length and width measurements and were converted to wet weight assuming specific gravity of their tissue to be 1.0 g cm^{-3} (Finlay 1982). The wet weight was converted to dry weight and carbon content, using conversion factors corresponding to each taxonomic group (Table 3).

Table 3: Individual biomass estimates for protozoa and meiofauna.

Micro- and meiofauna	Volume	Conversion factor (C)	Dry matter	Carbon/dry matter	References
Ciliates	Biovolume [a]	0.14 pgC μm^{-3}	-	-	Putt & Stoecker (1989)
Amoebae	Biovolume [a]	0.2 pgC μm^{-3}	-	-	Borsheim & Bratbak (1987); Ohman & Snyder
Foraminifers	Biovolume [a]	0.2 pgC μm^{-3}			(1991); Menden-Deuer & Lessard (2000)
Copepods	$V=W^2\times L\times C$ [b]	440	20%	0.45	Finlay (1982); Feller & Warwick (1988); Baguley et al. (2004)
Nematodes	$V=W^2\times L\times C$ [b]	530	20%	0.45	Finlay (1982); Feller & Warwick (1988)
Tardigrades	$V=W^2\times L\times C$ [b]	614	20%	0.45	Finlay (1982); Feller & Warwick (1988)
Ostracods	$V=W^2\times L\times C$ [b]	450	20%	0.45	Finlay (1982); Feller & Warwick (1988)
Oligochaetes	$V=W^2\times L\times C$ [b]	530	20%	0.45	Finlay (1982); Feller & Warwick (1988)
Cladocerans	Biovolume [a]	0.05 gC cm^{-3}	16%	0.31	Wiebe (1988); Finenko et al. (2003)
Rotifers	$V=0.26\times W^2\times L$	C_{org} = 8% wet weight			Gradinger et al. (1999); Meiners et al. (2002)

[a] Calculations were based on Edler (1979).
[b] W = max. width (mm); L = length (mm); C = conversion factor; V is wet weight, in ml (µg).

Feeding types of dominant species of ciliates were mainly determined by the intensive observations for their food vacuoles contents in protargol impregnated specimens and/or live cells. For example, the ciliates, whose food vacuoles always contained diatoms and/or flagellates, were attributed to herbivorous species; contained only remnants of bacteria were bacterivorous; contained both algae and bacteria were omnivorous; and contained other protozoa or metazoa were carnivorous. For each feeding type determined, at least 20 specimens were investigated. Occasionally, no information was available for the food contents on a few less abundant species, whose feeding types were determined according to the literatures such as Fenchel (1969) and some original species descriptions.

Statistics

Principal components analyses (PCA, rotation method) were conducted based on the biomass of individual groups of micro- and meiofauna in order to determine the relationships between each group and between organisms and environmental parameters (temperature, salinity, silt and clay, carbon, nitrogen, and chlorophyll-*a*). Ciliates were divided into 18 taxonomic groups, mostly at class or order levels, occasionally at genus level. Foraminifera, oligochaetes, and cladocerans were excluded from the PCA analysis because they occurred only in 1 or 2 of the 15 stations investigated. Spearman's rank correlation analyses were carried out to test for possible relationships between ciliates and meiofauna, and between the first two factors of the PCA and environmental factors. To account for the multiple uses of data in the correlation analyses, we used Bonferroni adjustments of the significance level. All data analyses were carried out using SAS, 6.12 (SAS Institute 1989).

3. Results

Temporal dynamics of micro- and meiofauna at Dorum

Ciliate community structure

A total of 78 ciliate morphospecies were found on a total of 10 sampling occasions at Dorum (see Appendix). Mean species richness (number of species for each sample) was 13, and Shannon-Wiener diversity was 1.9, respectively, across all samplings (Fig. 1a). Both species richness and diversity were lowest in September 2001, and increased through October; the highest values occurred in July and October 2002. There were no seasonal patterns of species richness over the year, but there was a tendency towards higher diversity from spring to summer and autumn (Fig. 1a).

Temporal changes in ciliate abundance and biomass both showed a bimodal pattern, and peaks in abundance coincided with peaks in biomass (Fig. 1b). Ciliate abundance varied between 20 and 729 ind. ml^{-1}, and biomass between 0.14 and 5.28 µg C ml^{-1} across all the samplings. The first abundance and biomass peak was observed in April 2002 (Fig. 1b), due to the increase of two herbivorous species, *Chlamydodon triquetrus* and *C. cyclops* (Table 2). Total ciliate numbers decreased in May, increased again in June and reached the year's second maximum in July, thereafter declined from August through October (Fig. 1b). During the second peak (July), a medium-sized hypotrich, *Aspidisca fusca* and a small scuticociliate, *Cyclidium* sp. contributed most of the abundance, while a large heterotrich *Condylostoma arenarium*, contributed most of the ciliate biomass (Table 2).

The individual species showed various specific patterns of dynamics (Fig. 1c, d). Some species were observed throughout the year, but most occurred only seasonally, whereas some were only present on rare occasions (Fig. 1c, d, Appendix). The most frequently occurring dominant group were chlamydodontids, which mainly feed on diatoms, occupying more than 30% of total ciliate biomass across the year (Table 2). The large herbivorous species, *Chlamydodon triquetrus* reached a density of 118 ind. ml^{-1} in April 2002. Besides, *C. cyclops* occurred year-round and sometimes in high densities, while the other chlamydodontids (*Chilodonella*, *Atopochilodon*, *Chlamydonella*, *Chlamydonellopsis*) were only occasionally observed and with low abundance (Appendix). However, the most frequent group were scuticociliates, among which bacteriovorous species (e.g. *Uronema marinum* and *Cyclidium* sp.) were sometimes dominating in terms of abundance, while herbivorous species (e.g. *Pleuronema marinum*) dominated the ciliate biomass (Table 2).

The second most frequent group was *Aspidisca* (several species, see Appendix). In addition, the large carnivorous heterotrich, *Condylostoma arenarium*, was sometimes dominant in terms of biomass (October 2001, March and July 2002). Especially in March, this species occupied up to 92% of total ciliate biomass (Table 2). Moreover, large karyorelictids were commonly observed but with low abundance, but occasionally dominated in terms biomass (Table 2). Further, the herbivorous *Prorodon* and *Frontonia* sometimes dominated the community when the community total biomass was low. The carnivorous litostomes (such as *Lacrymaria* and *Loxophyllum*) and some stichotrichs and oligotrichs were never dominant (see Appendix). Ciliate community similarity indices between samplings were low (Table 4), no more than 40% of the taxa were shared by any two samples, implying dramatic monthly shifts in species composition. The highest species similarity occurred in June and July samples, where both communities were dominated by similar species (Fig. 1c, d). Further, the June (2002) sample shared more taxa with other samples, mainly because it contained many of the common species (Table 4). The September (2001) sample shared the least taxa with other sampling occasions due to containing least number of species.

Table 4: Jaccard similarity index, a measure of proportion of shared species of ciliate communities between samplings in Dorum. The index ranges from 0, where no species are shared, to 1, where all species are present in both samples. Bolds indicate more species were shared by samplings.

	28.08.01	25.09.01	22.10.01	05.03.02	18.04.02	14.05.02	13.06.02	18.07.02	12.08.02	22.10.02
28.08.01	-	0.077	**0.233**	**0.273**	0.118	**0.233**	**0.206**	**0.262**	0.143	**0.217**
25.09.01		-	0.133	0.100	0.059	**0.214**	0.105	0.065	0.111	0.125
22.10.01			-	**0.292**	**0.227**	**0.238**	0.154	0.167	0.074	0.184
05.03.02				-	0.185	0.148	**0.207**	0.146	0.172	**0.282**
18.04.02					-	0.174	**0.240**	0.132	0.111	0.179
14.05.02						-	**0.250**	0.135	0.115	0.125
13.06.02							-	**0.353**	**0.269**	**0.225**
18.07.02								-	**0.216**	**0.220**
12.08.02									-	**0.263**

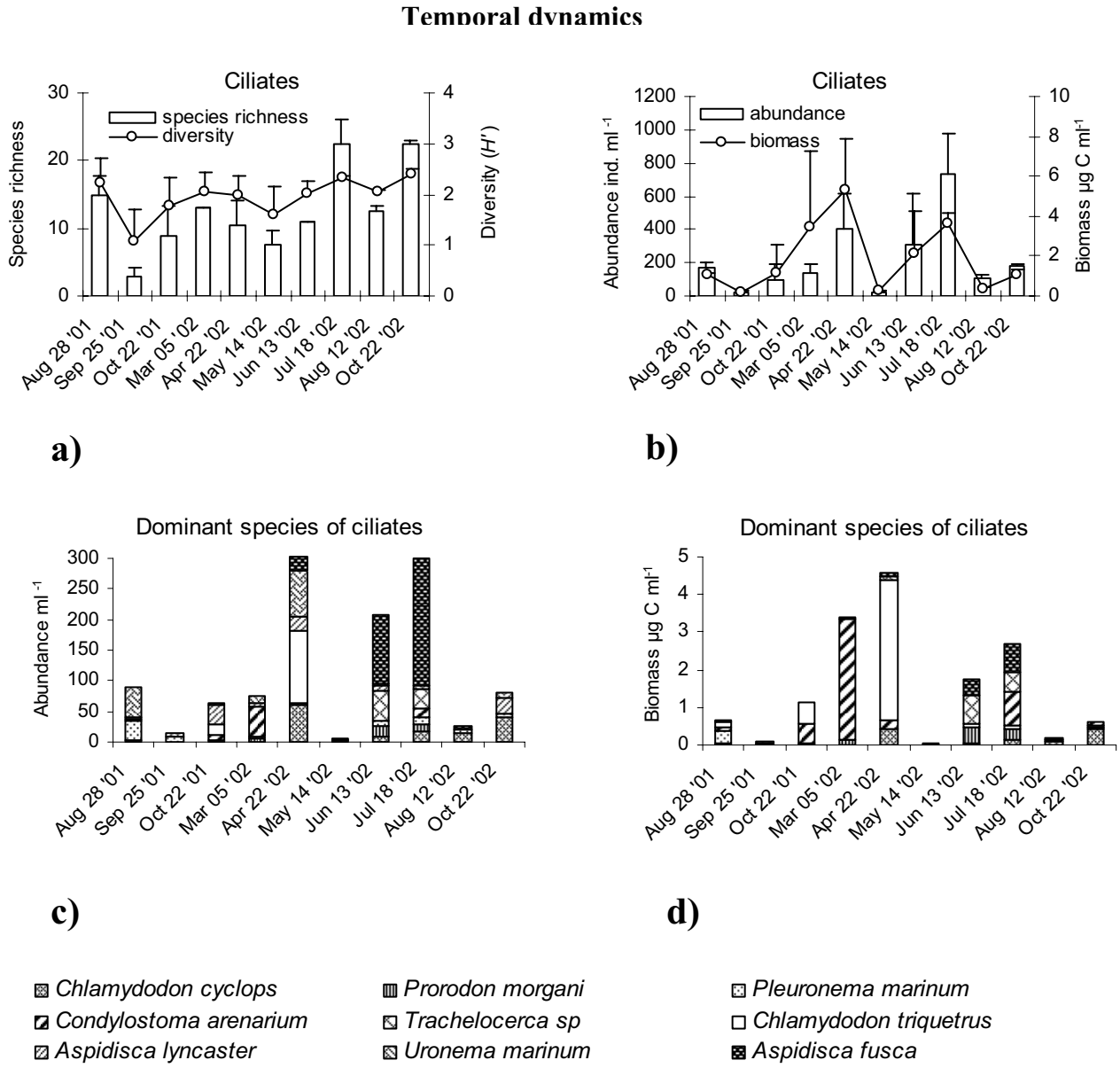


Fig. 1: Seasonal variations of ciliate species richness and Shannon-Wiener diversity (a), abundance and biomass (b), and dominant species (c, d) at Dorum from August 28, 2001 to October 22, 2002. Error bars are 1 SD.

Meiofauna

The abundance of the entire meiofauna varied between 8 and 291 ind. ml^{-1}, and biomass between 0.66 and 11.5 µg C ml^{-1} across all the samplings (Fig. 2a, b). On average, copepods contributed most (49%) of the biomass of meiofauna, while nematodes occupied most (55%) of the abundance. Additionally, abundance and biomass of nematodes were significantly positively correlated with those of ciliates ($p < 0.001$), while the other meiofaunal groups were not, except for ostracods, whose biomass was marginally correlated with that of ciliates (Table 5). Dynamics of the entire meiofauna community showed a seasonal pattern (Fig. 2a, b). The first abundance peak occurred in April due to increase of nematodes (66% biomass contribution) and tardigrades. The entire meiofauna decreased in May, but peaked again and

reached the year's maximum in June both in terms of abundance and biomass, due to the increase of all meiofaunal groups, among which copepods contributed most (63%) of the biomass. Meiofauna decreased during mid-summer, but the biomass peaked again in October due to an increase of large copepods (85% biomass contribution).

Total micro- and meiofauna community

Total micro- and meiofauna (ciliates, amoebae, foraminifera and meiofauna) abundance varied between 54 and 845 ind. ml^{-1}, and biomass between 1.38 and 29.82 µg C ml^{-1} across all samplings (Fig. 2c, d). Total micro- and meiofauna had a small peak in April (Fig. 2c, d), when ciliates occupied most of abundance (70%) and biomass (58%). Soon after total micro- and meiofauna declined in May, while they increased and reached the year's maximum in terms of biomass in June, when foraminifera and meiofauna contributed most of the biomass. In July, total micro- and meiofauna decreased in terms of biomass due to decreasing of meiofauna and other microfauna (foraminifera and amoebae), while increased in terms of abundance due to increasing of ciliates (86% abundance and 60% biomass contribution). Generally, ciliates occupied most of the total abundance of micro- and meiofauna (on average 57%), but only contributed on average 31% to the biomass across all samplings (Table 7). In contrast, meiofauna contributed most (on average 49%) of biomass, except in April, June and July (Fig. 2c, d, Table 7).

Cysts

Many cysts were observed during the microscopical analysing the samples. Some of them could be attributed to ciliate or flagellate cysts, however, most were not known. Only one type of ciliate cyst could be identified to the species level of *Aspidica lynceus* by a series of stages of encystment or excystment. These cysts were covered with a mucous layer colonized by numerous bacteria. The density of cysts was lowest in March (3 ind. ml^{-1}), it dramatically increased in April and reached the highest value in May (49 ind. ml^{-1}). From June onwards numbers of cysts gradually decreased through October (Fig. 2e).

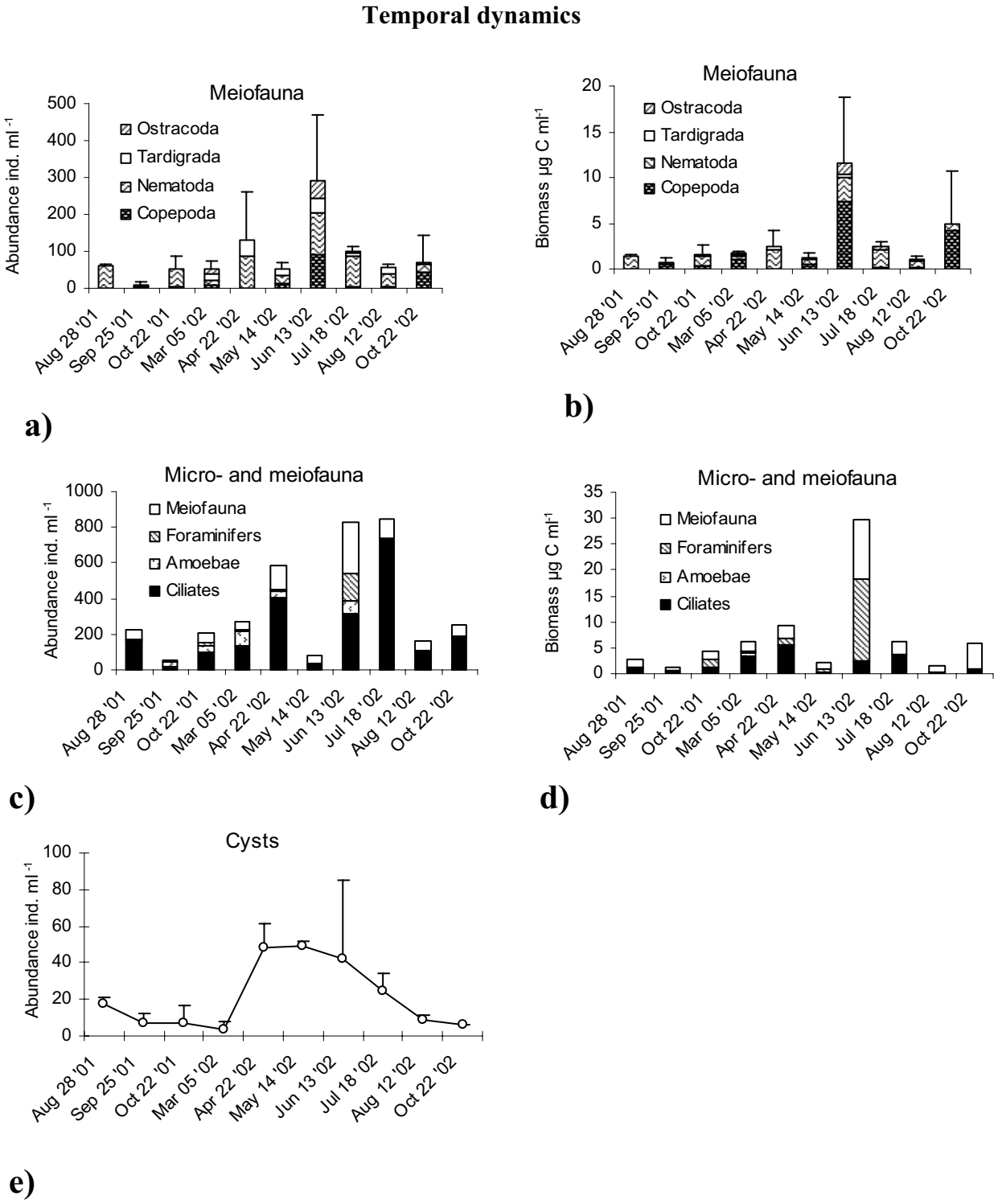


Fig. 2: Seasonal variations of abundance and biomass of meiofauna (a, b), and micro- and meiofauna (c, d), and density of cysts (e) at Dorum with standard deviation.

Table 5: Correlations (Spearman's rank) between abundance of ciliates and meiofauna at Dorum. Because the same data on ciliates were used in 2 different analyses (abundance and biomass), the α-level was reduced from 0.05 to 0.025. The p-values (in the lower line parentheses) beneath 0.025 are marked in bold. Effects with a p-level between 0.025 and 0.05 were considered as marginally significant trends, and are marked in italics.

		Copepods	Nematodes	Tardigrades	Ostracods
Ciliates	Abundance	-0.1524 (0.5095)	0.7061 **(0.0003)**	0.1298 (0.5748)	0.2447 (0.2851)
	Biomass	-0.0782 (0.7363)	0.6853 **(0.0006)**	0.1893 (0.4111)	0.4447 (*0.0434*)

Spatial distributions of micro- and meiofauna at multiple sites

Ciliate community structure

A total of 125 ciliate morphospecies were found at the 15 sampling sites (Table 1, Appendix). Among those species, 114 were recorded from Germany, 42 from Arctic, and 32 from USA. The maximum number of species (78 species) was recorded at Dorum (D6) from 16 separate samples; the second maximum number of species (72 species) was recorded at Sylt (S) from 14 separate samples. All other stations were sampled only once, and a total of 6-30 morphospecies were identified at each site (Appendix). There were tendencies of more species observed with increasing sampling frequency, sample numbers and sample volumes across all sampling sites.

Ciliate species richness ranged from 3 to 30, and Shannon-Wiener diversity varied between 0.87 and 2.78 across all sites (Fig. 3a, b). Ciliate abundance varied between 9 ind. ml^{-1} and 562 ind. ml^{-1}, and biomass between 0.004 and 2.34 µg C ml^{-1} (Fig. 3a, b). There was a tendency for higher ciliate diversity, species richness, abundance and biomass at German stations (D6, K, PO, S) compared to the other sites (Fig. 3a, b). Statistical analysis showed that ciliate diversity and species richness were both significantly positively correlated with ciliate abundance and chlorophyll-*a* concentration; additionally, they were also associated with ciliate biomass, and meiofauna abundance and biomass to different extents (Table 6).

The most frequent dominant ciliate groups were chlamydodontids, scuticociliates and prorodontids across the 15 sites, contributing on average 22%, 20% and 12% of total abundance, respectively, and 13% 14% and 16% of total biomass, respectively (Fig. 4a, b, Appendix). About 80% of the scuticociliate biomass was made up by bacterivorous species (e.g. *Cyclidium* sp. and *Uronema marinum*), while 20% was occupied by herbivorous taxa (e.g. *Pleuronema marinum*). Especially in Store Koldewey beach (KB), where the bacterivorous *Cyclidium* sp. occupied 65% of total ciliate biomass, and in Fühlinger See, Cologne (K), where a bacterivorous scuticociliate reached to a high density of 193 ind. ml^{-1} (27% biomass contribution) (Fig. 4b). *Frontonia* was frequently dominant in terms of biomass

(10% contribution), while euplotids (mainly *Aspidisca*) were sometimes dominant in terms of abundance (13% contribution) (Fig. 4a, b). Some groups were occasionally dominant either in terms of abundance and/or biomass, e.g. peritrichs, stichotrichs and heterotrichs. In contrast, several groups were always of minor quantitative importance, e.g. strombidiids, plagiophylids, and synhymeniids (Fig 4 a, b).

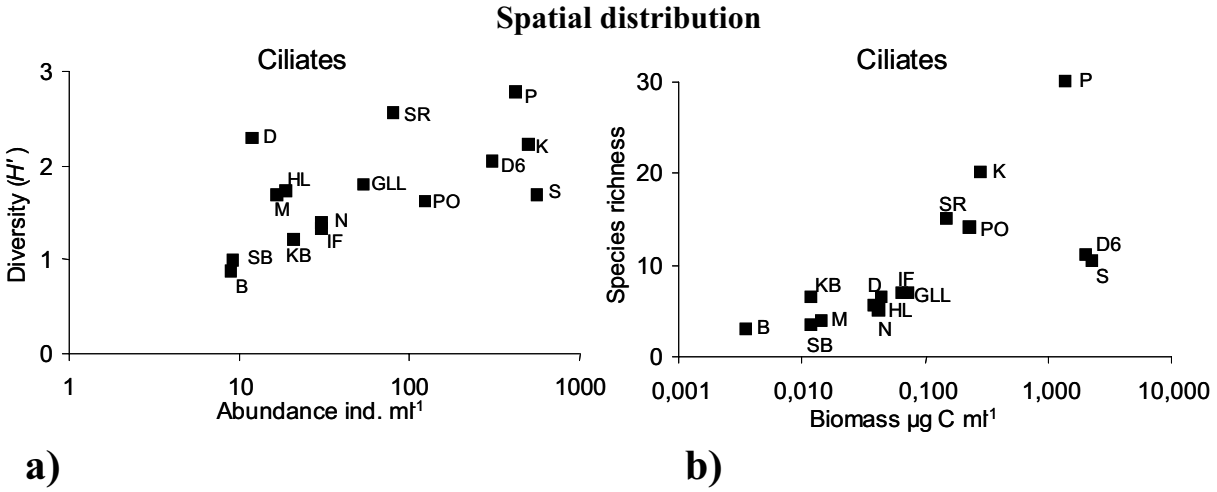


Fig. 3: Relationships between Shannon-Wiener diversity and abundance (a) and between species richness and biomass (b) for ciliate communities at 15 sampling sites. Shark River Bay (SR), Green Lane Lake (GLL), Nockamixon Lake (N), Fühlinger See (K), Dorum June 2002 (D6), Schöhsee (P), Königshafen (S), Basalt Lake (B), Potsdam Lake (PO), Lake near Hochstetter Fjord (HL), beach on Store Koldewey (KB), Beach on Shannon Island (SB), Melles Lake (M), Duck Lake (D), Ice floe (IF). Note that low species richness at station D6 and S was experimental artefact, which was caused by smaller sample volumes used than those used for the other sites (b).

Table 6: Correlations (Spearman's *r* values) between ciliate diversity and species richness and environmental factors. Data were based on the 15 sampling sites. Because the same data for ciliates were used in 2 different analyses (diversity and species richness), the α-level was reduced from 0.05 to 0.025. p-values (in the lower line parentheses) beneath 0.025 are marked in bold. Effects with a p-level between 0.025 and 0.05 were considered as marginally significant trends, and are marked in italics.

Ciliate	Ciliate abundance	Ciliate biomass	Meiofauna abundance	Meiofauna biomass	Chl-*a*	Salinity	Temperature	Silt & Clay	Carbon	Nitrogen
Diversity	0.6500	0.5107	0.6500	0.6643	0.7177	-0.3822	0.4286	0.1251	0.3643	0.2821
	(0.0087)	(0.0517)	**(0.0087)**	**(0.0069)**	**(0.0026)**	(0.1597)	(0.1110)	(0.6568)	(0.1819)	(0.3083)
Species	0.8784	0.8712	0.5886	0.6172	0.7209	-0.1541	0.3274	-0.0081	0.2576	0.1270
richness	**(0.0001)**	**(0.0001)**	(*0.0210*)	(*0.0142*)	**(0.0024)**	(0.5835)	(0.2336)	(0.9773)	(0.3640)	(0.6519)

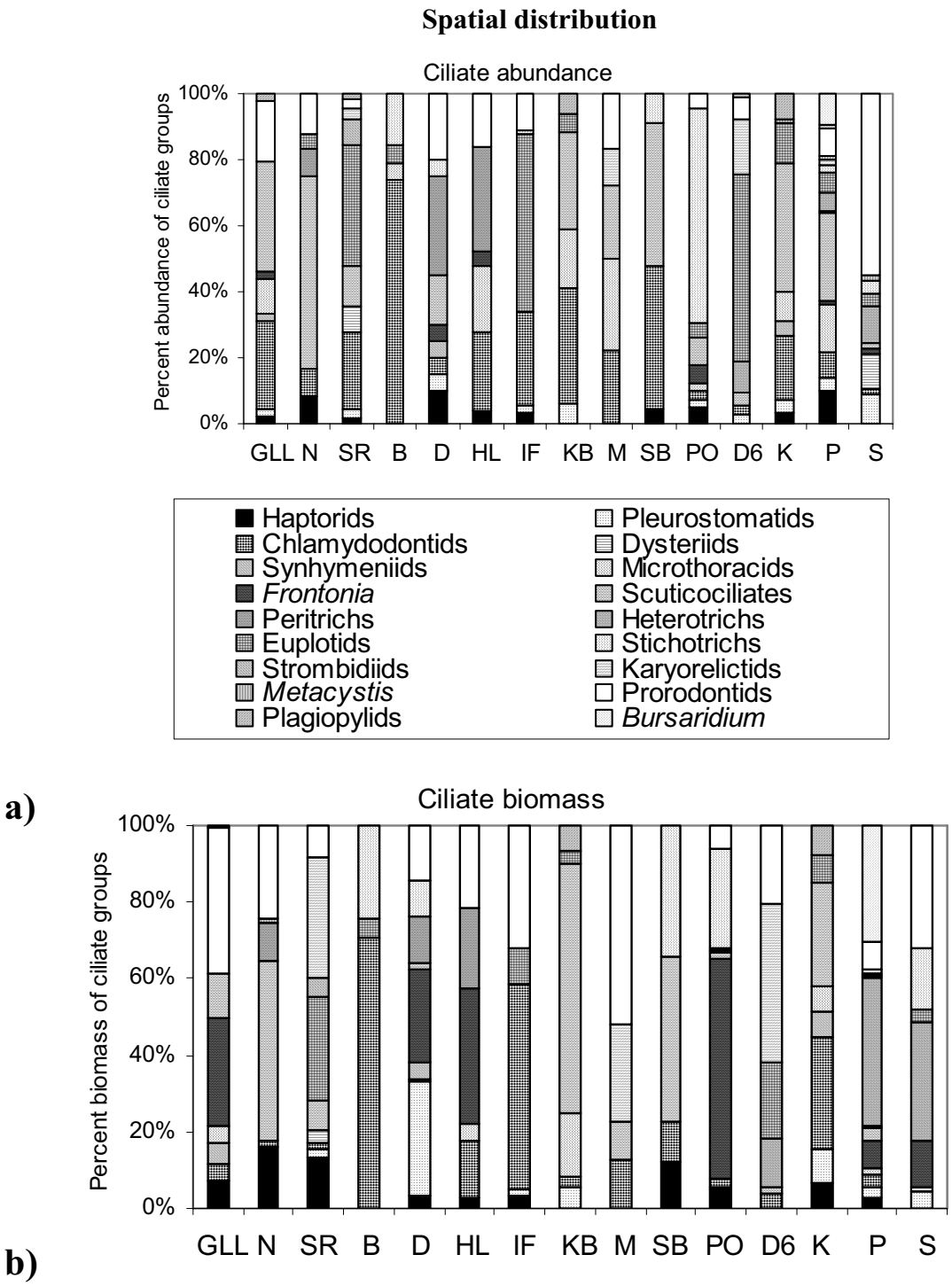


Fig. 4: Percent composition of abundance (a) and biomass (b) of the ciliate communities at the 15 stations. Total ciliate abundance and biomass were partitioned among 18 groups, mostly at the class or order levels, occasionally at family levels.

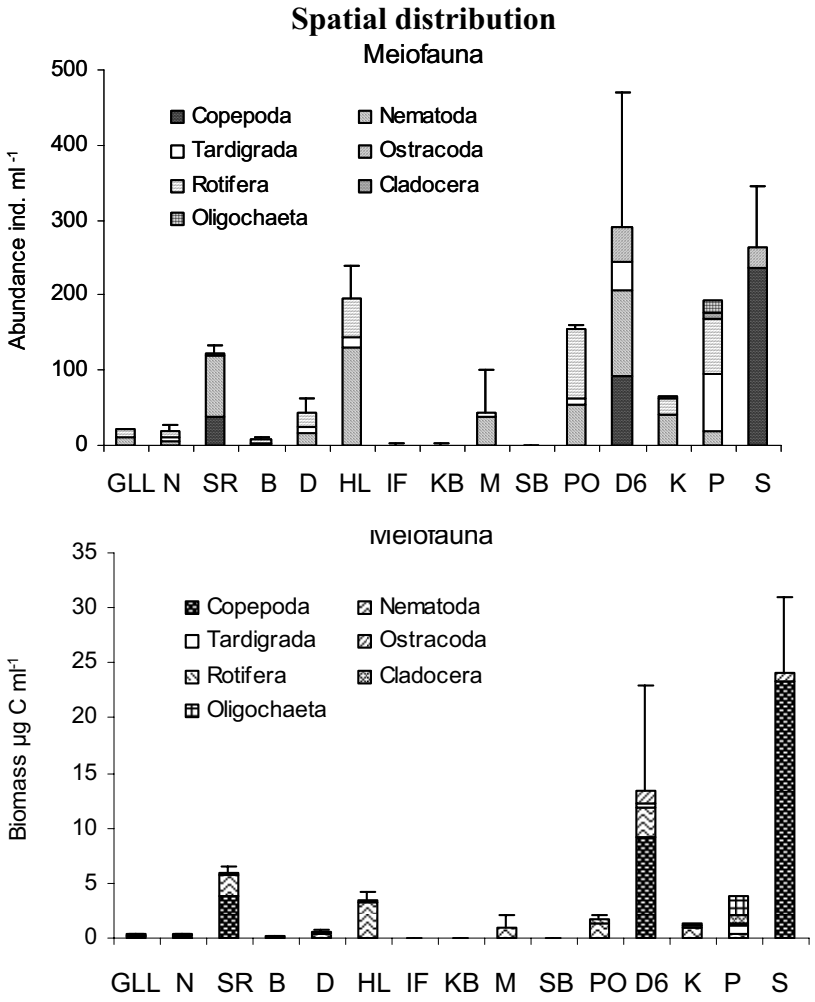


Fig. 5: Abundance (a) and biomass (b) proportions for each group of meiofauna at the 15 stations. Error bars are 1 SD.

Meiofauna

The entire meiofauna abundance varied between 0.4 and 291 ind. ml^{-1}, and biomass between 0.001 and 24.02 µg C ml^{-1}, at the 15 stations (Fig. 5a, b). Nematodes were the most frequent dominant meiofaunal group, occupying on average 56% of biomass of meiofauna, and 47% of abundance. In addition, copepods and rotifers were sometimes dominant, contributing on average 22% and 13%, respectively, of meiofauna biomass. Tardigrades was frequently occurred but with relatively lower abundance and biomass contributions, while ostracods, cladocerans and oligocheates were only occasionally observed with low density (Fig. 5a, b).

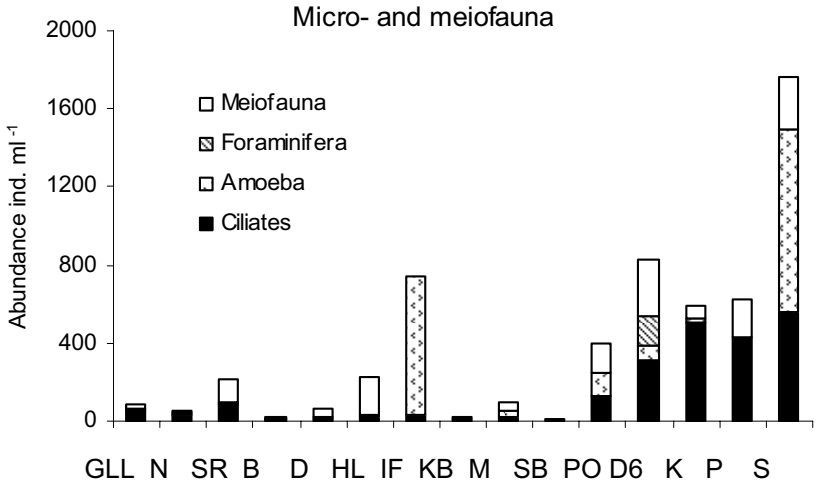
Total micro- and meiofauna community

The abundance of total micro- and meiofauna varied between 10 and 1763 ind. ml^{-1}, and biomass between 0.01 and 31.08 µg C ml^{-1}, at the 15 sites investigated (Fig 6a, b). Ciliates were the most frequent group, usually representing most (on average 46 %) of the total

abundance, but only approximate 15% of the biomass (Fig. 6a, Table 7). Amoeba and foraminifera together contributed about 18% of the total micro- and meiofauna biomass. In contrast, meiofauna frequently occupied most (on average 67%) of the biomass across all stations, except for station 7 (amoeba dominance), station 9 (ciliate dominance) and station 12 (foraminifer dominance) (Fig. 6b, Table 7).

Spatial distribution

a)



b)

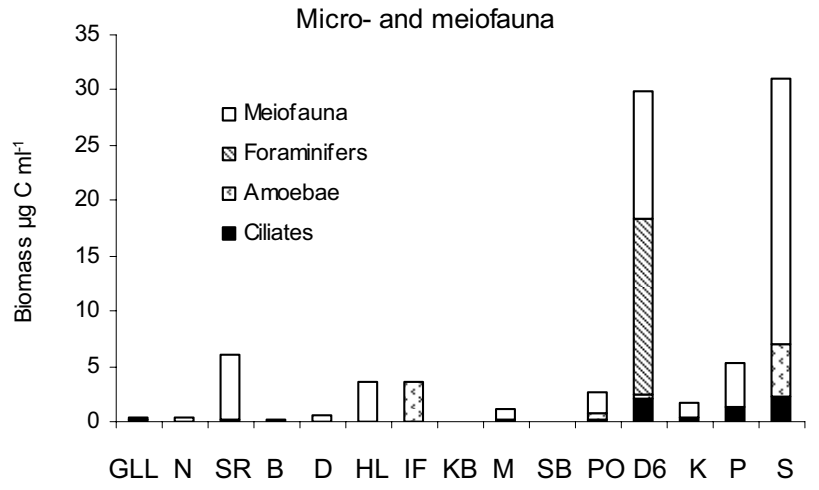


Fig. 6: Proportions of abundance (a) and biomass (b) of micro- and meiofauna at the 15 stations.

Table 7: Average and range of relative abundance (%), biomass (%) and relative metabolic rate (%) of ciliates, amoebae and foraminifers, and meiofauna in sediments investigated.

Stations		Ciliates	Amoebae and foraminifers	Meiofauna
Temporal study [a]	Abundance (%)	57 (28-86)	15 (0-48)	28 (12-61)
	Biomass (%)	31 (7-60)	20 (0-54)	49 (26-82)
	Metabolism (%)	32 (24-43)	22 (0-33)	46 (35-57)
Spatial study [b]	Abundance (%)	46 (8-96)	20 (0-96)	34 (0.1-87)
	Biomass (%)	15 (1-89)	18 (0-98)	67 (0.4-98)
	Metabolism (%)	34 (12-41)	21 (0-28)	46 (40-78)

[a] data based on ten samplings at Dorum from August 2001 to October 2002;
[b] data based on 15 sampling sites (see Table 2).

Cysts

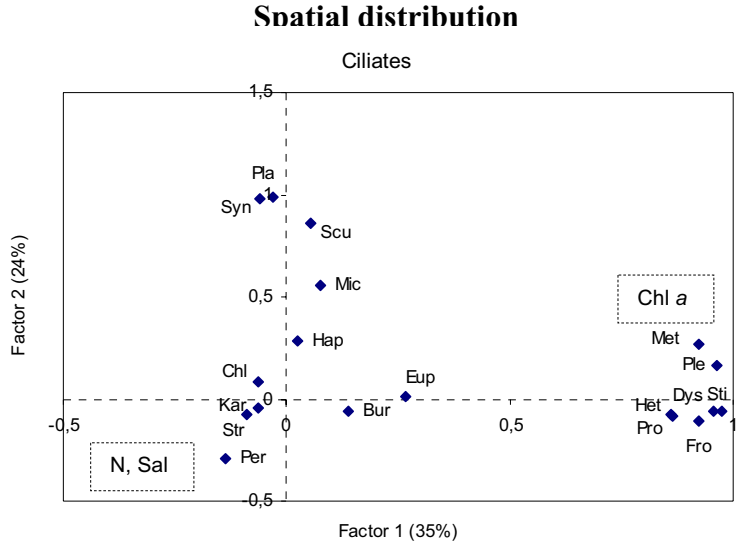
Numerous cysts were observed at the 15 stations, and their densities reached up to 113 ml^{-1} (D). At this station the abundance of micro- and meiofauna were very low (64 ind. ml^{-1}). Ciliate cysts were attributed to the following species or genera: *Aspidisca lynceus*, *Peritromus californicus*, *Fontonia*, *Loxophyllum* and *Chilodonella*. Some ciliate cysts were recognizable by possessing the macronucleus and micronuclei, and somatic kineties. In addition, some diatom and flagellate cysts were also observed. However, most cysts were unknown or uncertain. Remarkably, some cysts (including ciliate cysts) were in the stage of division.

Factors influencing micro- and meiofauna biomass distribution

Fig. 7a shows a diagram of the 18 ciliate groups plotted by PCA. The first two factors of the PCA accounted for 59% of the total variability of the ciliate community, however 4 factors of the PCA with eigenvalues exceeding 2, explained 88% of the total variability. Statistical analyses showed that the Factor 1 of the PCA was significantly positively correlated with chlorophyll-*a*, while the Factor 2 of the PCA was negatively correlated with nitrogen and salinity. Most of ciliate groups were positively correlated with the Factor 1 (e.g. pleurostomatids, *Frontonia*, heterotrichs, prorodontids, and dysteriids). Two groups, viz. plagiophylids and synhymeniids, showed strong positive correlations with the Factor 2. Most of these are freshwater species. In addition, sessile peritrichs had high negative correlation with the Factor 2, and were found abundantly in the stations (D and HL), characterized by relatively higher nitrogen contents (Fig. 7a). Fig. 7b displays distributions of micro- and meiofauna plotted by PCA. The first two factors of the PCA accounted for 69% of the total variability of the micro- and meiofauna community, however 3 factors of the PCA with eigenvalues exceeding 1, explained 85% of total variability. Spearman's correlation analyses showed that the Factor 1 of the PCA was significantly positively correlated with chlorophyll-*a*, and marginally significantly correlated with temperature, nitrogen and carbon, while the Factor 2 of the PCA was only significantly positively correlated with salinity. Ciliates and all the meiofaunal groups were positively correlated with the Factor 1. Statistical analyses showed that total ciliate biomass were correlated with ostracod biomass, and both the groups were significantly correlated with chlorophyll-*a*; tardigrades were correlated with nematodes, and they were associated with nitrogen, respectively, and carbon. Further, copepods and amoebae had high positive correlations with the Factor 2, which was, however, negatively associated with rotifers. Moreover, only amoebae were separated from other

organisms by the Factor 1, and were not correlated with any other groups and environmental factors.

a)



b)

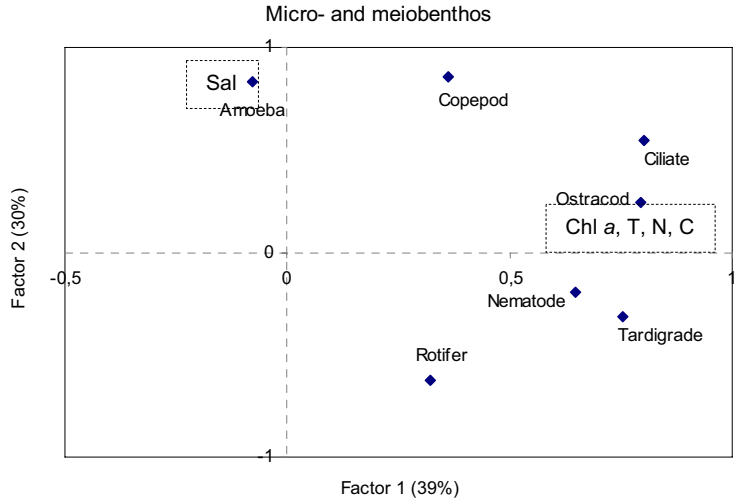


Fig. 7: Principal component analysis (PCA) ordination of ciliate groups (a) and micro- and meiofaunal groups (**b**) based on biomass of the organisms from the 15 sampling sites. Correlation arrows are removed to improve clarity. Values in parentheses are the amount of variation explained by the principal component. The environmental factors influencing the ciliate and meiofauna biomass distributions are showed in boxes. **a)** The Factor 1 was only significantly positively correlated with chlorophyll-*a* (Chl *a*); the Factor 2 was negatively correlated with nitrogen (N) and salinity (Sal). **b)** The Factor 1 was significantly positively correlated with chlorophyll-*a* (Chl *a*), and marginally significantly correlated with temperature (T), nitrogen (N) and carbon (C); the Factor 2 was only significantly positively correlated with salinity (Sal). The ciliates were grouped mainly at class or order levels, occasionally at genus levels: Bur, *Bursaridium*; Chl, chlamydodontids; Dys, dysteriids; Eup, euplotids; Fro, *Frontonia*; Hap, haptorids; Het, heterotrichs; Met, *Metacystis*; Mic, microthoracids; Per, peritrichs; Pla, plagiopylids, Ple, pleurostonatids; Pro, prorodontids; scaphidiodontids; Scu, scuticociliates; Kar, karyorelictids; Str. strombidiids; Sti, stichotrichs; Syn, synhymeniids.

4. Discussion

Seasonal dynamics of micro- and meiofauna and potential trophic relationships

The dynamics of micro- and meiofauna show a seasonal pattern in the brackish intertidal flat of Dorum, and two stages could be distinguished. In the first stage (spring), ciliates peaked with the spring bloom of diatoms in April, and the community was predominated by two herbivorous chlamydodontids, whose food vacuoles were filled with diatoms up to 100 µm long; simultaneously, entire meiofauna had a small abundance peak (Fig. 1b, c, d, Fig. 2a, c, Table 2). During this period ciliates seemed not be effectively consumed by meiofauna because the size of the dominant ciliates might be too large (about 140 × 65 µm) for the main constitutes of meiofauna: nematodes (about 800 × 35 µm) and tardigrades (about 150 × 35 µm). On the other hand, 58% of micro- and meiofauna biomass was ciliates. Thus, ciliates and meiofauna might be main diatom consumers and the predation pressure from meiofauna on ciliates was presumably low in spring.

In the second stage (summer), total micro- and meiofauna reached the year's maximum in terms of biomass in June, and the entire community was dominated by meiofauna and foraminifera. Total ciliates occupied only 7% of the total micro- and meiofauna biomass. According to study 1 and 2, the abundance of bacteria, diatoms and flagellates were all very low in June, thus the food supply for the total micro- and meiofauna including ciliates was rather tight. Therefore, the ciliates were probably suffered from the pressures of both predations from meiofauna and food limitation at this time. Soon after in July, with the decrease of meiofauna and other protozoa, ciliates increased in numbers and dominated the micro- and meiofauna community. Since 60% of micro- and meiofauna biomass was occupied by ciliates at this time, the predation pressure from meiofauna on ciliates was presumably very low. During the second stage, the ciliate communities showed more diverse size spectra (60 to 600 µm) and feeding strategies (e.g. carnivory and omnivory) (Table 2). The food inclusions of the large dominant ciliates, *Condylostoma arenarium* and *Trachelocerca* sp. sometimes contained small ciliates such as *Aspidisca fusca* and *Cyclidium* sp. Thus, the smaller ciliates were probably affected by predation from both meiofauna and the larger ciliates at this time.

Although statistics suggest nonsignificant negative relationships between ciliates and copepods, occasionally there were significant positive relationships between ciliates and other meiofaunal groups in the temporal study (Table 5). The biological meanings are therefore ambiguous. On one hand, certain carnivorous ciliates (e.g. *Condylostoma arenarium*) can

prey on both small ciliates and metazoa (Fenchel 1969). Thus, in March, when *C. arenarium* occupied up to 92% of ciliate biomass, and 35% of the entire micro- and meiofauna biomass, ciliates could potentially act on several trophic levels in the microbial food web; and the same was true in October (2001) and July. On the other hand, some protozoa such as foraminifera, whose food vacuoles contained many diatoms and flagellates (author's personal observations), can also prey on some metazoans (Sanders & Wickham 1993). Therefore in June, certain fraction of meiofauna was probably consumed by foraminifera. Thus, the species known as predators and herbivores were present simultaneously, making it difficult to evaluate the overall predation effect from meiofauna on ciliates (Johansson et al. 2004).

Ciliate herbivory and bacterivory

Kemp (1988; 1990) suggested that benthic ciliate grazing could not control the growth of sediment bacteria. Further, Lee & Patterson (2002) estimated that ciliates might consume about 6% of algal standing stock, but only 1% of bacteria standing stock per day in marine intertidal sediments of Australia. However, Epstein (1997b) revealed a seasonal pattern for microbial assemblages in a marine tidal flat of the USA, with two alternating phases, viz. a herbivory phase in spring and a bacterivory phase in autumn. Ciliates acted as important diatom consumers in the first phase, while in the second phase, the ciliate community containing mainly bacterivorous scuticociliates, were important bacterial grazers. Our result of the temporal study resemble Epstein's first phase, but differ from the second. In the present study, ciliate communities were frequently dominated by herbivorous species (prey organisms including diatoms, flagellates and cyanobacteria). In addition, carnivorous and/or ominivorous species sometimes dominated the community (Table 2). Unexpectedly, there was no dominance of bacterivorous forms in terms of biomass over the year. A possible explanation is that the bacterivorous species (e.g. *Uronema marinum* and *Cyclidium*) are small and contributed usually a small portion of biomass, even though they were sometimes abundant. However, we can not exclude that bacterivorous species dominated the community at other times. The present study indicated that ciliates were important diatom consumers, but probably not important as bacteria grazers at Dorum.

On the other hand, our spatial studies showed that the herbivorous forms such as chlamydodontids, prorodontids, and *Frontonia* very frequently dominated the ciliate communities across all sites, indicating that herbivory might be the most common and important feeding types in the sediments investigated. However, the bacterivorous scuticociliates, which were never dominant in the temporal study, dominated the ciliate

communities at several other stations (Fig. 4a, b), implying great difference in the importance of ciliate bacterivory among sampling sites. Likely, the contrasting results between the present temporal and spatial studies, and between ours and other studies were caused either by different habitats or by temporal variations in the respective communities, or by both.

Factors influencing ciliate diversity and species richness, and micro- and meiofauna biomass distribution

For better understanding of the role of ciliates and their relationships with the meiofauna in benthic microbial food webs, it is necessary to know which factors may influence the ciliate diversity and species richness, and the distribution of ciliate and meiofauna biomass. Previous studies revealed that many factors can affect the distribution of protozoa and meiofauna in sediments, including temperature, salinity, silt and clay content/grain size, content of dissolved oxygen, carbon, nitrogen or chlorophyll, or interactions between biotic and abiotic factors (e.g. Fenchel 1969; Patterson et al. 1989; Carey 1992; Santangelo & Lucchesi 1992; Finlay et al. 1997; Finlay & Esteban 1998; Dietrich & Arndt 2000; Hamels et al. 2003).

Ciliate diversity and species richness did not show seasonal patterns in the temporal study, though there were significant monthly shifts in species compositions (Fig. 1a, c, d, Table 4). The tendency towards higher diversity and species richness through spring to summer and autumn might be due to the greater shifts in species composition during spring than in summer and autumn, as indicated by the community similarity index, which were lower from March through May than form June through October (Fig. 1a, Table 4).

Spearman's correlations and PCA showed that ciliate diversity and species richness, and biomass of ciliates and meiofauna were largely controlled by chlorophyll-*a* concentrations. In particular, a cluster of herbivorous ciliate groups, which were plotted closely to each other, had high correlations with chlorophyll-*a*, indicating the closer relationships among those groups and their strong chlorophyll-*a* preferences (Fig. 7a). In contrast, the abiotic factors including temperature, salinity, nitrogen and carbon contents were not important in determining ciliate diversity and species richness (Table 6), but sometimes were important for certain ciliate or meiofaunal groups. For example, tardigrades were found positively correlated with nitrogen concentration, and nematodes were positively associated with carbon contents; copepods had high correlations with salinity, which was, however, negatively associated with rotifers (Spearman's correlations). Moreover, total ciliates were plotted among meiofaunal groups in the positive direction of the axis of the PCA (Fig. 7b), indicating the close relationships between ciliates and meiofauna, and associations between these groups

and similar environmental conditions. However, several dominant ciliate groups (e.g. chlamydodontids and karyorelictids) had very low correlations with all the factors investigated (Fig. 7a), implying that conditions (e.g. diatoms or bacteria) other than the studied factors might play a more important role in regulating the biomass distributions for some particular ciliate groups.

Remarkably, there were significant correlations between ciliate diversity and meiofauna abundance and biomass, and high positive correlations between ciliate groups, and between total ciliates and meiofauna (Spearman's correlation and PCA results). These indicate that organisms were not randomly distributed in the sediments, and coexistence between organism groups was important in the sediments investigated. A possible explanation is that ciliates and meiofauna had similar responses to the environmental conditions because, obviously, sites or conditions favourable for one taxocoenosis favoured another also and vice versa (Petz 1997). Therefore, the present study suggest that besides chlorophyll-*a* concentration, potential biotic interactions among organism groups were also important in regulating ciliate diversity, and ciliate and meiofauna biomass distributions in sediments investigated. In contrast, the abiotic factors were only sometimes important for certain ciliate and meiofaunal groups.

Potential ecological significance of cysts

The temporal study revealed a high density of cysts occurring in May, possibly, the rapid increase of micro- and meiofauna in June was partially benefited from the excystments of the cysts (Fig. 2c, e). The spatial study showed that the maximum abundance of cysts was observed in an Arctic station, where the abundance of micro- and meiofauna were very low, indicating a potential population harboured. Cysts are regarded as important components of benthic-pelagic coupling and a possible fundamental biological link, via submarine canyons, in shelf-slope and shallow-deep sea coupling (Marcus & Boero 1998). It is now well established that many protozoa and metazoa may pass through a benthic resting stage during their life cycle (e.g. Tommasa et al. 2000; Kremp 2001; Müller et al. 2002). The function of such resting stages is usually to promote survival during unfavourable periods with low food abundance and/or high predation risk (Godhe & McQuoid 2003). The highest density of cysts in the Arctic sediment under study exhibited the ecological advantage for organisms/populations if they can produce resting stages to overcome the unfavourable situation and assure the persistence of the population. In this study, the density of cysts was probably largely underestimated because many uncertain individuals were excluded from the statistics due to limited knowledge and available information on cysts. The studies of resting

stage dynamics in sediments might be a further interest for better understanding the microbial food webs.

Abundance and biomass of micro- and meiofauna, and potential importance of ciliates in the benthic metabolism

Our study shows that abundance and biomass of total micro- and meiofauna communities varied significantly from month to month, and among sites. In general, ciliates dominated in terms of abundance, while meiofauna contributed most of the biomass, which even largely exceeded total protozoa biomass (Table 7). This is in accordance with earlier studies that meiofauna biomass was much higher than heterotrophic protists in sediments (Arndt et al. 1990; Garstecki et al. 2000).

Nevertheless, the contribution of protozoa to benthic energetics is disproportional to their biomass due to a higher turnover of smaller cells. Hamels et al. (2003) suggested the importance of protozoa (mainly by nanoheterotrophs) in the benthic metabolism based on the following calculations: $R = a\ W^{-0.249}$ where weight-specific metabolic rate (R) is associated to body weight (W), and a is a constant with a value of $10^{-1.94}$ for protozoa and $10^{-1.64}$ for metazoa (Fenchel 1974). An estimated mean individual body weight (roughly total biomass/total abundance) and relative metabolic rates were thus calculated for ciliates, amoebae and foraminifera, and meiofauna at each of our sampling sites (Table 7). These ratios are merely rough estimates, but distinctly suggest that, compared to meiofauna, protozoa are more important in sediment respirations. Especially for ciliates, the most important components among protozoa, constitute on average 34% of the combined metabolic rate of benthic consumers, though they only contributed about 15% of biomass at our sampling sites (Table 7). These data suggest that ciliates are important components of benthic food webs.

Conclusions

1. The temporal study revealed that ciliate diversity and species richness did not show seasonal patterns, though there were significant monthly shifts in species composition. In contrast, the abundance and biomass of micro- and meiofauna showed a seasonal cycle with two distinguishable stages. During the first stage (spring), the feeding type of dominant ciliates and their size spectra suggested that ciliates and meiofauna might be the main diatom consumers and predation pressure from meiofauna on ciliates was low. During the second stage (summer), there was a shift from meiofauna and foraminifer dominance in June to

ciliate dominance in July, coinciding with a shift from high to low the predation pressure from meiofauna on ciliates.

2. The spatial study revealed great differences in ciliate species composition betweens sites. The occurrence of dominant ciliates and their allocation to feeding types indicates that herbivory was the most common and important feeding strategy, while the importance of bacterivory varied significantly among sediments investigated.

3. Statistical analyses showed abiotic factors including temperature, salinity, nitrogen and carbon contents were only sometimes important for certain ciliate and/or meiofaunal groups, but not important in determining ciliate diversity and species richness; in contrast, chlorophyll-*a* concentrations were significantly positively correlated to ciliate diversity and species richness, and the biomass of total ciliate and meiofauna. In addition, significant correlations were found between ciliate diversity and meiofauna abundance and biomass, and between biomass of different groups of benthic organisms, indicating that the actual community structure and potential biotic interactions among organism groups were probably most important factors in the sediments investigated.

4. Abundance and biomass of entire micro- and meiofauna communities varied greatly among sites. In general ciliates dominated in terms of abundance, while meiofauna contributed most of the biomass. Based on biomass ratios and estimated weight-specific metabolic rates, ciliates accounted on average for 34% of the estimated metabolic rate of the micro- and meiofauna consumers in the respective sediment investigated.

Acknowlegments

The authors thank the Dr. J. Matthiessen for assistance in measuring environmental parameters; Dr. Nils Volkenborn for precious discussions and providing some physicochemical data and Dr. Kuidong Xu for valuable discussions. The present work has been financed by the DFG (Deutsche Forschungs Gemeinschaft) grant number BE 2279/3-1, by the Alfred-Wegener-Institute for Polar and Marine Research.

References

Alongi, D.M., 1986. Quantitative estimates of benthic protozoa in tropical marine systems using silica gel: a comparison of methods. Estuar. Coast. Shelf. Sci. 23, 443-450.

Arndt, H., Schiewer, U., Jost, G., Wasmund, N., Walter, T., Heerkloss, R., Arlt, G., Arndt, E.A., 1990. The importance of pelagic and benthic microfauna in a shallow-water community of the Darss-Zingst Estuary, Southern Baltic, during mesocosm experiments. Limnologica. 20, 101-106.

Arndt, H., Dietrich, D., Auer, B., Cleven, E.-J., Gräfenhan, T., Weitere, M., Mylnikov, A.P., 2000. Functional diversity of heterotrophic flagellates in aquatic ecosystems. The Systematic Association Special. Vol. Ser. 59, 240-268.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.A., Thingstad, F., 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. Progr. Ser. 10, 257-263.

Baguley, J.G., Hyde, L.J., Montagna, P.A., 2004. A semi-automated digital microphotographic approach to measure meiofaunal biomass. Limnol. Oceanogr. 2, 181–190.

Bak, R.P.M., Nieuwland, G., 1989. Seasonal fluctuations in benthic protozoan populations at different depths in marine sediments. Neth. J. Sea. Res. 24, 37-44.

Berninger, U.-G., Caron, D.A., Sanders, R.W., Finlay, B.J., 1991. Heterotrophic flagellates of planktonic communities, their characteristics and methods of study. In: The Biology of Free-living Heterotrophic Flagellates. Eds: Patterson, D.J., Larsen, J., The Systematic Association Special Volume No.45, 39-56.

Berninger, U.-G., Epstein, S.S., 1995. Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea sediment. Aquat. Microb. Ecol. 19, 229-236.

Borsheim, K.Y., Bratbak, G., 1987. Cell volume to cell carbon conversion factors for a bacterivorous Monas sp. enriched from seawater. Mar. Ecol. Prog. Ser. 36, 171-175.

Böttcher, M. E. Hespenheide, B., Llobet-Brossa, E., Beardsley, C., Larsen, O., Schramm, A., Wieland, A., Böttcher, G., Berninger, U.-G., Amann, R., 2000. The biogeochemistry, stable isotope geochemistry, and microbial community structure of a temperate intertidal mudflat: an integrated study. Cont. Shelf. Res. 20, 1749-1769.

Burgess, R., 2001. An improved protocol for separating meiofauna from sediments using colloidal silica sols. Mar. Ecol. Prog. Ser. 214, 161–165.

Carey, P.G., 1992. Marine interstitial ciliates: an illustrated key. Chapman & Hall, London. 351 pp.

Dietrich, D., Arndt H., 2000. Biomass partitioning of benthic microbes in a baltic inlet: relationships between bacteria, algae, heterotrophic flagellates and ciliates. Mar. Biol. 136, 309-322.

Edler L., 1979. Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. Baltic. Mar. Biologists. Pub. 5, 1-38.

Epstein, S.S., Gallagher, E.D., 1992. Evidence for facilitation and inhibition of ciliate population growth by meiofauna and macrofauna on a temperate zone sandflat. J. Exp. Mar. Biol. Ecol. 155, 27-39.

Epstein S.S., Shiaris, M.P., 1992. Rates of microbenthic and meiobenthic bacterivory in a temperate muddy tidal flat community. Appl. Environ. Microbiol. 58, 2426-2431.

Epstein, S.S., 1995. Simultaneous enumeration of protozoa and micrometazoa from marine sandy sediments. Aquat. Microb. Ecol. 9, 219-227.

Epstein, S.S., 1997a. Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. Microb. Ecol. 34, 188-198.

Epstein, S.S., 1997b. Microbial food webs in marine sediments. II. Seasonal changes in trophic interactions in a

sandy tidal flat community. Microb. Ecol. 34, 199–209.

Feller, R.J., Warwick R.M., 1988. Energetics. In: Higgins R.P. & Thiel H. (eds) Introduction to the study of meiofauna. Smithsonian Institution Press, Washington, pp 181-196.

Fenchel, T., 1967. The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. Ophelia. 4, 37-121.

Fenchel, T., 1968. The ecology of marine microbenthos II. The food of marine benthic ciliates. Ophelia. 5, 73-121.

Fenchel, T., 1969. The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the mcirofauna communities with special reference to the ciliated protozoa. Ophelia. 6, 1-182.

Fenchel, T., 1974. Intrinsic rate of natural increase: the relationship with body size. Oecologia. 14: 317-326.

Fenchel, T., 1987. Ecology of Protozoa – The Biology of Free-living Phagotrophic Protists. Science Tech Publishers, Madison, Wisconsin, pp197.

Finenko, Z.Z., Piontkovski, S.A., Williams, R., Mishonov, A.V., 2003. Variability of phytoplankton and mesozooplankton biomass in the subtropical and tropical Atlantic Ocean. Mar. Ecol. Prog. Ser. 250, 125–144.

Finlay, B.J., 1982. Procedures for the isolation, cultivation and identification of protozoa. In: Burns R.G., Slater J.H. (eds) Experimentaal microbial ecology. Blackwell, Oxford, pp 44-65.

Finlay, B.J., Maberly, S.C., Cooper, J.I., 1997. Microbial diversity and ecosystem function. Oikos. 80, 209-213.

Finlay, B.J., Esteban, G.F., 1998. Freshwater protozoa: biodiversity and ecological function. Biodiv. Conserv. 7, 1163-1186.

Foissner, W., 1995/96. The infraciliature of *Cryptopharynx setigerus* Kahl, 1928 and *Apocryptopharynx hippocampoides* nov. gen., nov. spec. (Ciliophora, Karyorelictea), with an account on evolution in Loxodid ciliates. Arch. Protistenkd. 146, 309-327.

Foisnner, W., 1997. Infraciliature and systematic position of the marine interstitial ciliates (Protozoa, Ciliophora) *Lopezoterenia torpens* (Kahl, 1931) nov. gen., nov. comb. *Discotricha papillifera* Tuffrau, 1954, and *Paraspathidium fuscum* (Kahl, 1928) Fjeld, 1955. Rev. Soc. Mex. Hist. Nat. 47, 41-63.

Foissner, W., Berger, H., Schaumburg, J., 1999. Identification and ecology of limnetic plankton ciliates. - Bayer. Landesamt für Wasserwirtschaft, Heft 3/99. Infomationsberichte des Bayerischen Landesamtes für Wasserwirtschaft, Munich, pp798.

Garstecki, T., Verhoeven, R., Wickham, S.A., Arndt, H., 2000. Benthic-pelagic coupling: a comparison of the community structure of benthic and planktonic heterotrophic protists in shallow inlets of the southern Baltic. Freshw. Biol. 45,147-167.

Giere, O., 1993. Meiobenthology. The Microscopic Fauna in Aquatic Sediments. Springer-Verlag, Berlin, Heidelberg, New York. 328 pp.

Godhe, A., McQuoid, M.R., 2003. Influence of benthic and pelagic environmental factors on the distribution of dinoflagellate cysts in surface sediments along the Swedish west coast. Aquat. Microb. Ecol. 32, 185-201.

Gradinger, R., Friedrich, C., Spindler M., 1999. Abundance, biomass and composition of the sea ice biota of the Greenland Sea pack ice. Deep Sea Res. 46, 1457–1472.

Hamels, I., Sabbe, K., Muylaert, K., Vyverman, W., 2003. Quantitative importance, composition, and seasonal dynamics of protozoan communities in polyhaline versus freshwater intertidal sediments. Microb. Ecol. 47, 18-29.

Hartwig, E., 1973. Die Ciliaten des Gezeiten-Sandstrandes der Nordseeinsel Sylt II. Ökologie. Mikrofauna Meeresboden, 18, 385-453.

Hausmann, K., Hülsmann, N., Polianski, I., Schade, S., Weitere, M., 2002. Composition of benthic protozoan communities along a depth transect in the eastern Mediterranean Sea. Deep-Sea Res. PT I 49, 1959-1970.

Jansson, B.-O., 1967. The significance of grain size and pore water content for the interstitial fauna of sandy beaches. Oikos. 18, 311-322.

Johansson, M., Gorokhova, E., Larsson, U., 2004. Annual variability in ciliate community structure, potential prey and predators in the open northern Baltic Sea proper. J. Plankton. Res. 26, 67-80.

Kahl, A., 1930-1935. Urtiere oder Protozoa: I: Wimpertiere oder Ciliata (Infusoria), eine Bearbeitung der freilebenden und ectocomensalen Infusorien der Erde, under Ausschluß der marinen Tintinnidae. In Dahl F (ed) Die Tierwelt Deutchlands. Fishcher Verlag, Jena, pp 1-886.

Kemp, P.F., 1988. Bacterivory by benthic ciliates: significance as a carbon source and impact on sediment bacteria. Mar. Ecol. Prog. Ser. 49, 163-169.

Kemp, P.F., 1990. The fate of benthic bacterial production. Rev. Aquat. Sci. 2, 9-124.

Kremp, A., 2001. Effects of cyst resuspension on germination and seeding of two bloom-forming dinoflagellates in the Baltic Sea. Mar. Ecol. Prog. Ser. 216, 57-66.

Lee, W.J., Patterson, B.J., 2002. Abundance and biomass of heterotrophic flagellates, and factors controlling their abundance and distribution in sediments of Botany Bay. Microb. Ecol. 43, 467-481.

Lei, Y., Choi, J.K., Xu, K., Petz, W., 2005. Morphology and infraciliature of three species of Metaurostylopsis (Ciliophora, Stichotrichia): *M. songi* n. sp., *M. salina* n. sp., and *M. marina* (Kahl, 1932) from sediments, saline ponds and coastal waters. J. Eukaryot. Microbiol. (in press).

Lynn, D.H., Small, E.B., 2002. Phylum Ciliophora. In: An illustrated guide to the protozoa. Lee, J.J., Bradbury, P.C., Leedale, G.F. (eds.). Society of Protozoologists, Lawrence, Kansas. pp 371-656.

Magurran, A.E., 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, NJ.

Marcus, N.H., Boero, F., 1998. Minireview: the importance of benthic-pelagic coupling and the forgotten role of life cycles in coastal aquatic systems. Limnol. Oceanogr. 43, 763-768.

Meiners, K., Fehling, J., Granskog, M.A., Spindler, M., 2002. Abundance, biomass and composition of biota in Baltic sea ice and underlying water (March 2000). Polar. Biol. 25, 761-770.

Menden-Deuer, S., Lessard, E.J., 2000. Carbon to volume relationship for dinoflagellates , diatoms, and other protist plankton. Limnol. Oceanogr. 45, 569-579.

McCormick, P.V., Cairns, J.J., 1991. Effects of micrometazoa on the protistan assemblage of a littoral food web. Freshw. Biol. 26, 111-119.

Montagnes, D.J.S., Lynn, D.H., 1993. A quantitative protargol stain (QPS) for ciliates and other protists. In: Handbook of Methods in Aquatic Microbial Ecology, Lewis Publishers, Chapter 28, 229-240.

Müller, H., Stadler, P., Weisse, T., 2002. Seasonal dynamics of cyst formation of strombidiid ciliates in alpine Lake Mondsee, Austria. Aquat. Microb. Ecol. 29, 181–188.

Ohman M.D., Snyder R.A., 1991. Growth kinetics of the omnivorous oligotrich ciliate Strombidium sp. Limnol. Oceanogr. 36, 922-935.

Page, F.C., 1988. A new key to freshwater and soil Gymnamoebae with instructions for culture. CCAP and FBA (Freshwater Biological Association), Ambleside, 122 pp.

Patterson, D.J., Larsen, J., Corliss, J.O., 1989. The ecology of heterotrophic flagellates and ciliates living in marine sediments. Progr. Protistol. 3, 185-277.

Petz, W., 1997. Ecology of the active soil microfauna (Protozoa, Metazoa) of Wilkes Land, East Antarctica. Polar Biol. 18, 33-44.

Porter, K.G., Sherr, E.B., Sherr, B.F., Pace, M., Sanders, R.W., 1985. Protozoa in planktonic food webs. J. Protozool. 32, 409-415.

Putt, M., Stoecker, D.K., 1989. An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34, 1097-1103.

Reid, P.C., Boalch, G.T., 1987. A new method for the identification of dinoflagellate cysts. J. Plankton. Res. 9, 249-253.

Rubino, F., Belmonte, G., Miglietta, A.M., Geraci S., Boero F., 2000. Resting stages of plankton in recent north Adriatic sediments. P.S.Z.N. Mar. Ecol. 21, 263-284.

Sanders R.W., Wickham, S.A., 1993. Planktonic protozoa and metazoa: predation, food quality and population control. Mar. Microb. Food Webs. 7, 197-223.

Santangelo, G., Lucchesi, P., 1992. The interstitial ciliated protozoa of a Mediterranean microcommunity. Hydrobiologica. 230, 79-92.

Sherr, E.B., Sherr, B.F., 1994. Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb. Ecol. 28, 223-235.

Skibbe, O., 1994. An improved quantitative protargol stain for ciliates and other planktonic protests. Arch. Hydrobiol. 130, 339-347.

Starink, M., Bar-Gilissen, M.-J., Bak, R.P.M., Cappenberg, T.E., 1994. Quantitative centrifugation to extract benthic protozoa from freshwater sediments. Appl. Environ. Microbiol. 60, 167-173.

Stoeck, T., Epstein, S., 2003. Novel Eukaryotic lineages inferred from small-subunit rRNA analyses of oxygen-depleted marine environments. Appl. Environ. Microbiol. 69, 2657–2663.

Stoeck, T., Taylor, G.T., Epstein, S.S., 2003. Novel Eukaryotes from the permanently anoxic Cariaco Basin (Caribbean Sea). Appl. Environ. Microbiol. 69, 5656–5663.

Tommasa, L.D., Belmonte, G., Palanques, A., Puig, P., Boero, F., 2000. Resting stages in a submarine canyon: a component of shallow–deep-sea coupling? In: M.B. Jones, J.M.N. Azevedo, A.I. Neto, A.C. Costa, A.M. Frias Martins (eds), Island, Ocean and Deep-Sea Biology. Hydrobiologia. 440, 249–260.

Tommasa, L.D, Danovaro, R., Belmonte, G., Boero, F. 2004. Resting stage abundance in biogenic fraction of surface sediments from the deep Mediterranean Sea. Sci. Mar. 68, 103-111.

Tso, S.F., Taghon, G.L., 1997. Enumeration of protozoa and bacteria in muddy sediment. Microb. Ecol. 33, 144-148.

Wickham, S., Gieseke, A., Berninger, U.-G., 2000. Benthic ciliate identification and enumeration: an improved methodology and its application. Aquat. Microbial. Ecol. 22, 79-91.

Wickham, S., Nagel, S., Hillebrand, H., 2004. Control of epibenthic ciliate communities by grazers and nutrients. Aquat. Microbial. Ecol. 35, 153-162.

Wiebe, P.H., 1988. Functional regression equations for zooplankton displacement volume, wet weight, dry weight, and carbon: a correction. Fish. Bull. 86, 833-835.

Wieltschnig, C., Fischer, U.R., Kirschner, A.K.T., Velimirov, B., 2003. Benthic bacterial production and protozoan predation in a silty freshwater environment. Microb. Ecol. 46, 62–72.

Appendix

Abundance of ciliates (ind. ml^{-1}) in the upper 3 mm layer of sediments at 15 sampling sites Shark River Bay (SR), Green Lane Lake (GLL), Nockamixon Lake (N), Fühlinger Lake (K), Dorum June 2002 (D6), Schöhsee (P), Königshafen (S), Basalt Lake (B), Potsdam Lake (PO), Lake near Hochstetter Fjord (HL), beach on Store Koldewey (KB), Beach on Shannon Island (SB), Melles Lake (M), Duck Lake (D), Ice floe (IF): Note station PO, D6 and S (asterisks) recorded the maximum abundance from more than one samples, while data at the other stations were only based on one sample.

[a] See Table 2. -, not found; +, less than 1 ind. ml^{-1}.

Species / Stations [a]		GLL	N	SR	B	D	HL	IF	KB	M	SB	PO	D6	K	P	S
CLASS KARYORELICTEA Corliss, 1974																
Order Prostomatida Small and Lynn, 1985																
Family Kentrophoridae Jankowski, 1980																
109	*Tracheloraphis kahli*	-	-	-	-	-	-	-	-	-	-	-	23	-	-	-
22	*Tracheloraphis* sp.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
Family Trachelocercidae Kent, 1881																
116	*Trachelocerca* cf. *cylindricolis*	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-
73	*Trachelocerca* sp.	-	-	1	-	-	-	-	-	-	-	-	48	-	-	11
Order Loxodida Jankowski, 1980																
Family Cryptopharyngidae Jankowski, 1980																
25	*Cryptopharynx* cf. *setigerus*	-	-	-	-	-	-	-	-	-	-	-	3	-	-	8
Family Loxodidae Bütschli, 1889																
102	*Remanella* cf. *minuta*	-	-	-	-	-	-	-	-	2	-	1	69	-	5	-
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Order Protoheterotrichida Nouzarède, 1977																
Family Geleiidae Kahl, 1933																
39	*Geleia fossata*	-	-	1	-	-	-	-	-	-	-	-	5	-	-	2

Species / Stations [a]		GLL	N	SR	B	D	HL	IF	KB	M	SB	PO*	D6*	K	P	S*
CLASS HETEROTRICHEA Stein, 1859																
Order Heterotrichida Stein, 1859																
Family Blepharismidae Jankowski in Small and Lynn, 1985																
28	*Blepharisma* sp.1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
70	*Blepharisma* sp.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
106	*Blepharisma* sp.3	-	-	-	-	-	-	-	-	-	-	+	-	-	3	-
Family Condylostomatidae Kahl in Doflein and Reichenow, 1929																
17	*Condylostoma arenarium*	-	-	-	-	-	-	-	-	-	-	-	49	-	3	7
45	*Condylostoma remanei*	-	-	-	-	-	-	-	-	-	-	-	9	-	18	-
Family Peritromidae Stein, 1867																
52	*Peritromus californicus*	-	-	-	-	-	-	-	-	-	-	-	15	-	-	35
CLASS SPIROTRICHEA Bütschli, 1889																
SUBCLASS HYPOTRICHIA Stein, 1859																
Order Euplotida Small and Lynn, 1985																
Family Aspidiscidae Ehrenberg, 1838																
34	*Aspidisca fusca*	-	-	15	-	-	-	-	-	-	-	-	207	-	-	7
4	*Aspidisca lyncaster*	-	-	10	-	-	-	-	1	-	-	-	9	-	-	20
56	*Aspidisca lynceus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
78	*Aspidisca major*	-	-	4	-	-	-	-	-	-	-	-	-	-	-	24
49	*Aspidisca polypoda*	-	-	-	-	-	-	-	-	-	-	3	50	38	5	26
11	*Aspidisca steini*	-	-	-	-	-	-	-	-	-	-	-	6	-	-	48
91	*Aspidisca* sp.	-	1	-	-	-	-	-	-	-	-	4	-	23	13	-
Family Euplotidae Ehrenberg, 1838																
75	*Euplotes charon*	-	-	-	-	-	-	-	-	-	-	-	11	-	-	8
21	*Euplotes rariseta*	-	-	1	-	-	-	-	-	-	-	-	1	-	-	10
74	*Euplotes vannus*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27
119	*Euplotes* sp.	-	-	-	1	-	-	16	-	-	-	-	-	-	8	-
Family Uronychiidae Jankowski, 1979																
41	*Uronychia setigera*	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
36	*Diophrys histrix*	-	-	-	-	-	-	-	-	-	-	-	9	-	-	2
69	*Diophrys scutum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
SUBCLASS CHOREOTRICHIA Small and Lynn, 1985																
Order Choreotrichida Small and Lynn, 1985																
Family Strombidinopsidae Small and Lynn, 1985																
38	*Strombidinopsis* sp.	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-

Species / Stations [a]		GLL	N	SR	B	D	HL	IF	KB	M	SB	PO*	D6*	K	P	S*
SUBCLASS STICHOTRICHIA Small and Lynn, 1985																
Order Stichotrichida FauréFremiet, 1961																
Family Keronidae Dujardin, 1840																
48	*Kerona* sp.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	76
Order Sporadotrichida FauréFremiet, 1961																
Family Oxytrichidae Ehrenberg, 1838																
43	*Tachysoma* cf. *dragescoi*	-	-	-	-	-	-	-	-	-	-	-	3	-	-	8
Order Urostylida Jankowski, 1979																
Family Pseudokeronopsidae																
55	*Psudokeronopsis* cf. *rubra*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
Family Urostylidae Bütschli, 1889																
30	*Holosticha* sp.1	-	-	-	-	-	-	-	-	-	-	-	5	-	-	18
44	*Holosticha* sp.2	-	-	-	-	-	-	-	-	-	1	-	5	-	-	2
Undetermined species																
86	Urostylida gen. sp.1	-	-	-	-	-	-	-	-	-	-	74	-	-	10	-
51	Urostylida gen. sp.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
90	Stichotrichia gen. sp.3	-	-	-	-	-	-	-	-	-	-	4	-	-	-	-
103	Stichotrichia gen. sp.4	-	-	-	1	-	-	-	-	-	-	1	-	-	-	-
121	Stichotrichia gen. sp.5	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
124	Stichotrichia gen. sp.6	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
SUBCLASS OLIGOTRICHIA Bütschli, 1887																
Order Strombidiida Petz & Foissner, 1992																
Family Strombidiidae FauréFremiet, 1970																
13	*Strombidium sauebreyae*	-	-	6	-	-	-	-	-	-	-	-	1	-	-	37
37	*Strombidium stylifer*	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
2	*Strombidium* sp.1	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-
6	*Strombidium* sp.2	-	-	-	-	-	-	-	-	-	-	-	3	-	-	1
29	*Strombidium* sp.3	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2
CLASS LITOSTOMATEA Small & Lynn, 1981																
Order Cyclotrichida Jankowski, 1980																
Family Mesodiniidae Jankowski, 1980																
35	*Mesodinium pupula*	-	-	-	-	-	-	-	-	-	-	-	23	-	-	31
46	*Askenasia* sp.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	7

Species / Stations [a]		GLL	N	SR	B	D	HL	IF	KB	M	SB	PO	D6	K	P	S
Order Haptorida Corliss, 1974																
Family Lacrymariidae de Fromentel, 1876																
27	*Lacrymaria coronata*	-	-	-	-	-	-	-	-	-	-	-	3	-	-	*8*
96	*Lacrymaria* cf. *minuta*	1	1	-	-	-	-	+	-	-	-	1	-	2	3	-
110	*Lacrymaria multinucleata*	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-
112	*Lacrymaria* sp.1	-	-	-	-	-	-	+	-	-	-	-	10	2	10	-
Family Spathidiidae Kahl in Doflein and Reichenow, 1929																
98	*Spathidium* sp.	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Family Trachelophyllidae Kent, 1882																
92	*Chaenea* sp.	-	-	-	-	1	1	+	-	-	-	2	-	-	3	-
105	*Enchelyodon* sp.1	-	1	-	-	-	-	-	-	-	-	1	-	13	26	-
115	*Enchelyodon* sp.2	-	-	-	-	-	-	-	-	-	-	-	19	-	-	-
62	Haptorida gen. sp.1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	7
63	Haptorida gen. sp.2	-	-	-	-	-	-	-	-	-	+	-	-	-	-	2
68	Haptorida gen. sp.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
89	Haptorida gen. sp.4	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-
79	Haptorida gen. sp.5	-	-	-	-	-	-	-	-	-	-	-	2	-	-	4
Order Pleurostomatida Schewiakoff, 1896																
Family Amphileptidae Bütschli, 1889																
67	*Amphileptus agilis*	-	-	-	-	-	-	-	-	-	-	1	-	6	-	16
58	*Amphileptus marina*	-	-	-	-	-	-	-	-	-	-	-	2	-	-	6
Family Litonotidae Kent, 1882																
85	*Litonotus anguilla*	-	-	-	-	-	-	-	-	-	-	-	-	8	13	3
60	*Litonotus fasciola*	-	-	-	-	-	-	-	-	-	-	1	2	6	3	34
83	*Loxophyllum cheatontum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
80	*Loxophyllum compressum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
3	*Loxophyllum helus*	-	-	1	-	-	-	-	-	-	-	-	6	-	-	20
50	*Loxophyllum uninucleatum*	1	-	-	-	-	-	-	-	-	-	+	3	-	3	13
23	*Loxophyllum verrucosum*	-	-	1	-	-	-	-	1	-	-	-	9	-	-	7
65	*Loxophyllum* sp.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
101	*Loxophyllum* sp.2	-	-	-	-	1	-	-	-	-	-	+	-	-	-	-
Undetermined species																
57	Litonotidae gen. sp.1	-	-	-	-	-	-	-	-	-	-	-	3	-	-	6
125	Litonotidae gen. sp.2	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-

Species / Stations [a]		GLL	N	SR	B	D	HL	IF	KB	M	SB	PO	D6	K	P	S
CLASS PHYLLOPHARYNGEA de Puytorac et al., 1974																
SUBCLASS PHYLLOPHARYNGIA Puytorac et al., 1974																
Order Chlamydodontida Deroux, 1976																
Family Chilodonellidae Deroux, 1970																
95	*Chilodonella uncinata*	-	-	-	-	-	5	-	-	1	1	4	-	15	16	-
111	*Chilodonella* sp.1	15	-	-	-	1	-	-	-	3	-	-	13	46	-	-
122	*Chilodonella* sp.2	-	-	-	-	-	-	9	-	-	-	-	-	-	-	-
117	*Thigmogaster* cf. *pardus*	-	3	-	-	-	-	-	-	-	-	-	-	-	5	-
Family Chlamydodontidae Stein, 1859																
1	*Chlamydodon cyclops*	-	-	-	-	-	-	-	-	-	-	-	60	-	-	-
77	*Chlamydodon triquetrus*	-	-	-	-	-	-	-	-	-	-	-	118	-	-	12
64	*Cyrtophoron* cf. *isagogicum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
Family Gastronautidae Deroux, 1994																
107	*Gastronauta derouxi*	-	-	-	1	-	-	-	-	-	-	1	-	33	10	-
Family Lynchellidae Jankowski, 1968																
10	*Atopochilodon distichum*	-	-	3	-	-	-	-	-	-	-	-	14	4	-	32
8	*Chlamydonella* sp.	-	-	3	-	-	-	-	-	-	-	-	5	-	-	-
59	*Chlamydonellopsis* sp.	-	-	14	5	-	-	-	7	-	3	-	11	-	-	7
Order Dysteriida Deroux, 1976																
Family Dysteriidae Claparède & Lachmann, 1858																
61	*Dysteria monostyla*	-	-	-	-	-	-	-	-	-	-	-	2	-	-	10
33	*Dysteria procera*	-	-	5	-	-	-	-	-	-	-	-	2	-	-	34
24	*Dysteria pusilla*	-	-	1	-	-	-	-	-	-	-	-	3	-	-	4
42	*Microdysteria decora*	-	-	-	-	-	-	-	-	-	-	-	8	-	-	20
SUBCLASS SUCTORIA Claparède & Lachmann, 1858																
Order Endogenida Collin, 1912																
Family Acinetidae Stein, 1859																
47	*Acineta* sp.	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
CLASS NASSOPHOREA Small & Lynn, 1981																
Order Synhymeniida de Puytorac et al., 1974																
Family Orthodonellidae Jankowski, 1968																
19	*Eucamptocerca longa*	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-
Family Scaphidiodontidae Deroux, 1978																
20	*Chilodontopsis caudata*	-	-	-	-	-	-	-	-	-	-	-	1	-	-	8
72	*Chilodontopsis hisioensis*	1	-	-	-	1	-	-	-	-	-	-	6	-	-	8
84	*Scaphidiodon navicula*	-	-	-	-	-	-	-	-	-	-	-	-	21	-	3
Order Microthoracida Jankowski, 1967																
Family Discotrichidae Jankowski, 1967																
7	*Discotricha papillifera*	-	-	-	-	-	-	-	1	-	-	-	35	-	-	21
Family Microthoracidae Wrzesniowski, 1870																

Species / Stations [a]		GLL	N	SR	B	D	HL	IF	KB	M	SB	PO	D6	K	P	S
100	*Drepanomonas sphagni*	-	-	-	-	-	2	-	-	-	-	1	-	-	26	-
99	*Microthorax simplex*	-	-	-	1	-	-	-	-	5	-	+	-	12	-	-
104	*Pseudomicrothorax dubius*	6	-	-	-	-	2	-	3	-	-	1	-	34	37	-
Undetermined species																
71	Nassophorea gen. sp.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28
CLASS COLPODEA Small and Lynn, 1981																
Order Bursariomorphida FernándezGaliano, 1978																
Family Bursaridiidae Foissner, 1993																
118	*Bursaridium* cf. *pseudobursaria*	-	-	-	-	-	-	-	-	-	-	-	-	-	39	-
CLASS PROSTOMATEA Schewiakoff, 1896																
Order Prostomatida Schewiakoff, 1896																
Family Metacystidae Kahl, 1926																
54	*Metacystis tesselata*	-	-	-	-	-	-	-	-	-	-	-	11	4	5	7
40	*Metacystis* sp.	-	-	-	-	-	-	-	-	-	-	-	3	-	-	2
Order prorodontida Corliss, 1974																
Family Colepidae Ehrenberg, 1838																
53	*Coleps* sp.	-	-	-	-	-	-	-	-	-	-	-	3	-	3	193
Family Holophryidae Perty, 1852																
26	*Holophrya* cf. *collaris*	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
12	*Holophrya* cf. *sulcata*	-	-	3	-	-	-	-	-	-	-	-	3	-	-	3
14	*Holophrya* sp.1	-	-	-	-	-	-	-	-	-	-	-	4	-	-	163
76	*Holophrya* sp.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6
88	*Holophrya* sp.3	-	-	-	-	1	-	+	-	-	-	4	3	-	5	-
123	*Holophrya* sp.4	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Family Placidae Small and Lynn, 1985																
31	*Spathidiopsis striatus*	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-
Family Prorodontidae Kent, 1881																
81	*Prorodon discolor*	-	-	-	-	-	-	-	-	-	-	-	6	-	-	2
32	*Prorodon morgani*	-	-	-	-	-	-	-	-	-	-	-	17	-	-	7
114	*Prorodon vermiforme*	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-
97	*Prorodon* sp.	10	4	-	-	1	3	1	-	3	-	2	3	-	29	-
CLASS PLAGIOPYLEA Small and Lynn, 1985																
Order Plagiopylida Small and Lynn, 1985																
Family Plagiophylidae Schewiakoff, 1896																
108	*Plagiopyla vestita*	-	-	-	-	-	-	-	1	-	-	-	2	33	-	-

Species / Stations [a]		GLL	N	SR	B	D	HL	IF	KB	M	SB	PO	D6	K	P	S
Family Sonderiidae Small and Lynn, 1985																
113	Sonderia mira	1	-	1	-	-	-	-	-	-	-	-	3	8	5	-
CLASS OLIGOHYMENOPHOREA de Puytorac et al., 1974																
SUBCLASS PENICULIA FauréFremiet in Corliss, 1956																
Order Peniculida FauréFremiet in Corliss, 1956																
Family Frontoniidae kahl, 1926																
87	*Frontonia* cf. *caneti*	1	-	-	-	1	1	-	-	-	-	8	-	-	5	-
9	*Frontonia marina*	-	-	-	-	-	-	-	-	-	-	-	4	-	-	7
SUBCLASS SCUTICOCILIATIA Small, 1967																
Order Philasterida Small, 1967																
Family Uronematidae Thompson, 1964																
5	*Uronema marinum*	-	-	6	-	-	-	-	-	-	-	-	77	-	-	6
Order Pleuronematida FauréFremiet in Corliss, 1956																
Family Pleuronematidae Kent, 1881																
18	*Pleuronema coronatum*	2	-	-	-	-	-	-	-	-	-	-	6	2	3	9
15	*Pleuronema marinum*	-	-	-	-	-	-	-	-	-	-	-	9	-	-	28
82	*Pleuronema* sp.	-	-	-	-	-	-	-	-	-	-	-	3	-	-	8
Family Cyclidiidae Ehrenberg, 1838																
66	*Cyclidium* sp.	-	-	4	-	-	-	-	10	-	4	-	15	-	-	35
Undetermined species																
16	Scuticociliatia gen. sp.1	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1
94	Scuticociliatia gen. sp.2	16	22	-	-	2	-	-	-	4	-	10	-	193	110	-
SUBCLASS PERITRICHIA Stein, 1859																
Undetermined species																
120	Peritrichia gen. sp.2	-	-	-	-	2	6	-	-	-	-	-	-	-	-	-
93	Peritrichia gen. sp.1	-	3	-	-	2	-	-	-	-	-	+	-	-	3	-

Study 4

Functional role of small macrofauna in small benthic communities

K.Stumm[1)], N.Volkenborn[1)], U.-G. Berninger[2)]

[1)] Alfred - Wegener - Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

[2)] Universität Salzburg, Hellbrunner Strasse 34, 5020 Salzburg, Austria

Abstract

The effective top-down control of macrofauna on the structure of microbial benthic communities, especially on heterotrophic nanoflagellates are less thoroughly investigated. Short term experiments were carried out in this study to analyse structuring effects of macrofauna in general and of special macrograzers in particular on the components of microbial communities of marine and freshwater habitats in polar and temperate climate. Common macrograzer species from each investigated habitat like *Hydrobia ulvae*, *Corophium* sp. as well as Chironomid larvae and polychaets were added as single special macrograzers to the sediments in laboratory experiments subsequent to the prior removal of all macrograzers. After incubation times of 24 h to 6 d the abundance of diatoms, cyanobacteria, bacteria, phototropic and heterotrophic nanoflagellates were analysed. In addition one field study was carried out in an intertidal mudflat, where *Arenicola marina* was excluded permanently.

The experiments revealed that the top-down forces of macrograzers on the components of microbial communities were astonishingly small compared to their high biomass and their herewith expected grazing rates. The macrograzers stimulated microbial production by their activity (bioturbation, defaecation) rather than they reduced microbes by grazing. The experiments exhibited that in short term the microbial community changed when a small macrograzer´s key species was missing. In long term the key species were replaced by other macro-, meio- or microfaunal species, which adopted the functional role of the missing key-species. Consequently no effect on total microbial community biomass and abundance could be observed in long term and differences between treatments seemed to be a matter of species composition rather than of total biomass.

1. Introduction

Kuipers et al. (1981) suggest that the small benthic food web, including nano-, microorganisms and small macrofauna, should be regarded as a complete functional unit. The components of the small food web are mainly characterised by their small body sizes, relatively short life spans, high metabolic rates per volume of body size and a small total biomass compared to the macrofauna community. 70% to 80% of all organic matter is said to be consumed and respired within the small food web (Kuipers et al. 1981). Less information is available about the factors determining the relative importance of direct effects (grazing) and indirect effects (like stimulation or repression) within the small benthic food webs.

Primary producers like single-celled microalgae, bacteria and cyanobacteria and furthermore detritus constitute the fundament of the small benthic food web in littoral zones (Barnes & Hughes 1999; study 2).

Polychaets like the lugworm *Arenicola marina*, amphipods like species of *Corophium* sp. and molluscs like the snail *Hydrobia* sp. and the mussel *Macoma balthica* are the most common and abundant small macrofauna organisms in a temperate intertidal flat. In the profundal of temperate lakes *Chironomid* larvae often dominate the small macrofauna in numbers and biomass (Johnson et al. 1989). This small macrofauna in turn serves as an important prey to higher-order consumers (as fish, crabs and birds).

Numerous investigations deal with the analysis of the diet of small macrofauna like *Hydobia*, *Corophium* and Chironomid larvae via a gut content analysis and therewith their rate of ingestion of diatoms and bacteria (Morrisey 1988; Jensen & Kristensen 1990; Gerdol & Hughes 1994; Gerdol & Hughes 1994; Menn & Armonies 1999). However, investigations of the effective top-down effects of macrofauna on the components of a microbial community, especially on heterotrophic flagellates, are rare.

Many of these small benthic macrofaunal organisms are known to feed on detritus. It is furthermore recognised that detritus-feeders digest only a small extent of the bulk of ingested organic material and rather use the attached micro- and nanofauna (Fenchel & Jorgensen 1977). Fenchel et al. (1977) investigated detritus particles and suggest that the number and composition of attached microorganisms is mainly a function of the surface size of the detritus particles. It is assumed that the influence of detrivorous macrograzers on the components of microbial communities depends on the ingested detritus particle sizes and therewith on the amount of microorganisms on detritus particles.

Small macrofauna may not only influence the microbenthic populations by grazing but also by their activities such as bioturbation, defecation, excretion and secretion. This reworking of sediment is known to remarkely change the rate of various biochemical processes in the sediment diagenesis and in turn to stimulate primary and bacterial production (Hargrave 1970; Lopez & Levinton 1987; Grossmann & Reichardt 1991).

The combination of preferred omnivorous feeding strategies, feedback mechanisms and indirect effects forms the basis for a very complex and strongly interconnected trophic structure in the small food web of sediments. The trophic structure within the small benthic food web, including microphytobenthos, bacteria, flagellates, ciliates, meiofauna and small macrofauna, is however less thoroughly investigated.

This study therefore aimed at investigating the top-down effects of small macrograzers on the standing stock of components of the microbenthic community (bacteria, cyanobacteria, diatoms and nanoflagellates (phototrophic and heterotrophic). For a cross system comparison of the functional role of small macrograzers laboratory experiments were conducted with sediments from shallow freshwater and marine environments in Germany, USA and the North East of Greenland. The basic idea of the study was to remove all macrograzers from the samples and to analyse changes of abundance and biomass of the communities over different time periods with and without artificially added macrograzer species in the laboratory. By adding a single small macrograzer species in the abundance as identified in the field (e.g. *Hydrobia*, *Corophium*), the potential role of this special macrograzer species within the small macrograzers guild could be examined as well as the influence of the absence of all other macrograzers. In a field experiment, samples were taken from adjacent areas with and without the lugworm *Arenicola marina*, in order to compare and to trace long term changes.

2. Materials and Methods

Study sites and sampling

This study was carried out in marine and freshwater shallow sediments from sites in the North East of Greenland, the East coast of USA and Germany from August 2001 to December 2003 (Table 1). Detailed information about sampling sites, environmental parameters, community biomass and structures are given in study 2. At all sites, sediment samples were taken at places, where the water depth was between 0.3 m and 1 m. At tidal areas, samples were taken during low tide, when the sediment was exposed to the air. The upper 3 mm (representing the oxic layer) of sediments were collected using round plexiglass cores (inner diameter 36 mm) or with a flat shovel. Three to four samples were taken per station. The samples were transported to the laboratory in a dark cooled container and processed within 4 h.

Table 1: Study sites with position, experiment number, sampling date, temperature and salinity.

sampling site	Exp.	position		date	temperature	salinity
	No.	latitude	longitude		°C	
Dorum, Germany	E1	53º 42´N	8º 29´E	22.10.2001	11	25
	E2	53º 42´N	8º 29´E	05.03.2002	6	25
	E3	53º 42´N	8º 29´E	14.05.2002	16	21
	E4	53º 42´N	8º 29´E	12.08.2002	21	15
	E5	53º 42´N	8º 29´E	22.10.2002	10	15
List/Island of Sylt, Germany	E8	55º 2´N	8º 25´E	01.07.2002	21	31
	E6,E7	55º 2´N	8º 25´E	12.09.2002	21	31
Schöhsee/Plön, Germany	E9	54º 09´N	10º 26´E	13.11.2002	7	0
Fühlinger See/Köln, Germany	E10	50º 58´N	74º 01´E	02.06.2003	24	0
Shark River Bay/PA, USA	E11	40º 10´N	74º 01´W	20.11.2003	12	26
Green Lane Lake/PA, USA	E12	40º 20´N	75º 27´W	03.12.2003	1	0
Nockamixon Lake/PA, USA	E13	40º 28´N	75º 13´W	03.12.2003	7	0
Potsdam Lake/Shannon Island, Greenland	E14, E15	75º 03´N	18º 45´W	26.08.2003	7	3
Melles Lake/Island of Store Koldewey, Greenland	E16	76º 07´N	18º 37´W	02.08.2003	9	3
Duck Lake/Island of Store Koldewey, Greenland	E17	76° 25´N	18° 45' W	22.08.2003	10	0
Basalt Lake, Greenland	E18	72º 43´N	22º 27´W	08.09.2003	8	5
Koldewey Beach, Greenland	E19	76º 06´N	18º 30´W	02.08.2003	2	36

Laboratory experiment's design

Sediment samples were taken from the investigated areas and macrograzers were removed by hand. Afterwards three treatments were set up: "initial" natural sediment fixed after removal of macrograzers; "no" sediment samples which were incubated after removal of macrograzers and "macrograzer" sediment samples which were incubated with one added species of macrograzer (Fig. 1). The treatments (no) and (macrograzer) were incubated for 24 h, in one experiment for 6 d under in situ conditions in the lab. Each treatment was replicated three to four times.

Half of a 3 mm slice of a plexiglass core sample or 2 ml of the shovel sampling measured with a cut of syringe were placed into incubation vessels (cellwells with cover, wells with 36 mm inner diameter and 18 mm depth) and flooded with 2 ml filtrated (0.2 µm) environmental water. After removal of all macrograzers the initial samples were immideatelly fixed by the addition of ice cold glutardialdehyd (f.c. 2%) (Sherr & Sherr 1993). Only one species of macrograzer was added each time to the samples (Fig.1; Table 2). Abundance of macrograzers in samples was chosen to be close to the observed abundance at the sampling site. Treatments (no) and (macrograzer) were incubated in the laboratory under roughly in situ conditions (Table 2). At the end of the incubation time ice cold glutardialdehyd (f.c. 2%) was added to the sample in order to stop incubation. Immediately after fixation the sediment of each sample (initial, no, macrograzer) was transferred into a tube with 20 ml of artificial seawater using a cut of 5 ml pipette syringe and fixed again (f.c. of glutardialdehyd 2%). Samples were stored at 4°C in the dark until further analysis for a maximum time of one week.

During all experiments the tracks of the added macrograzers were noticed as an indicator for their activity during incubation.

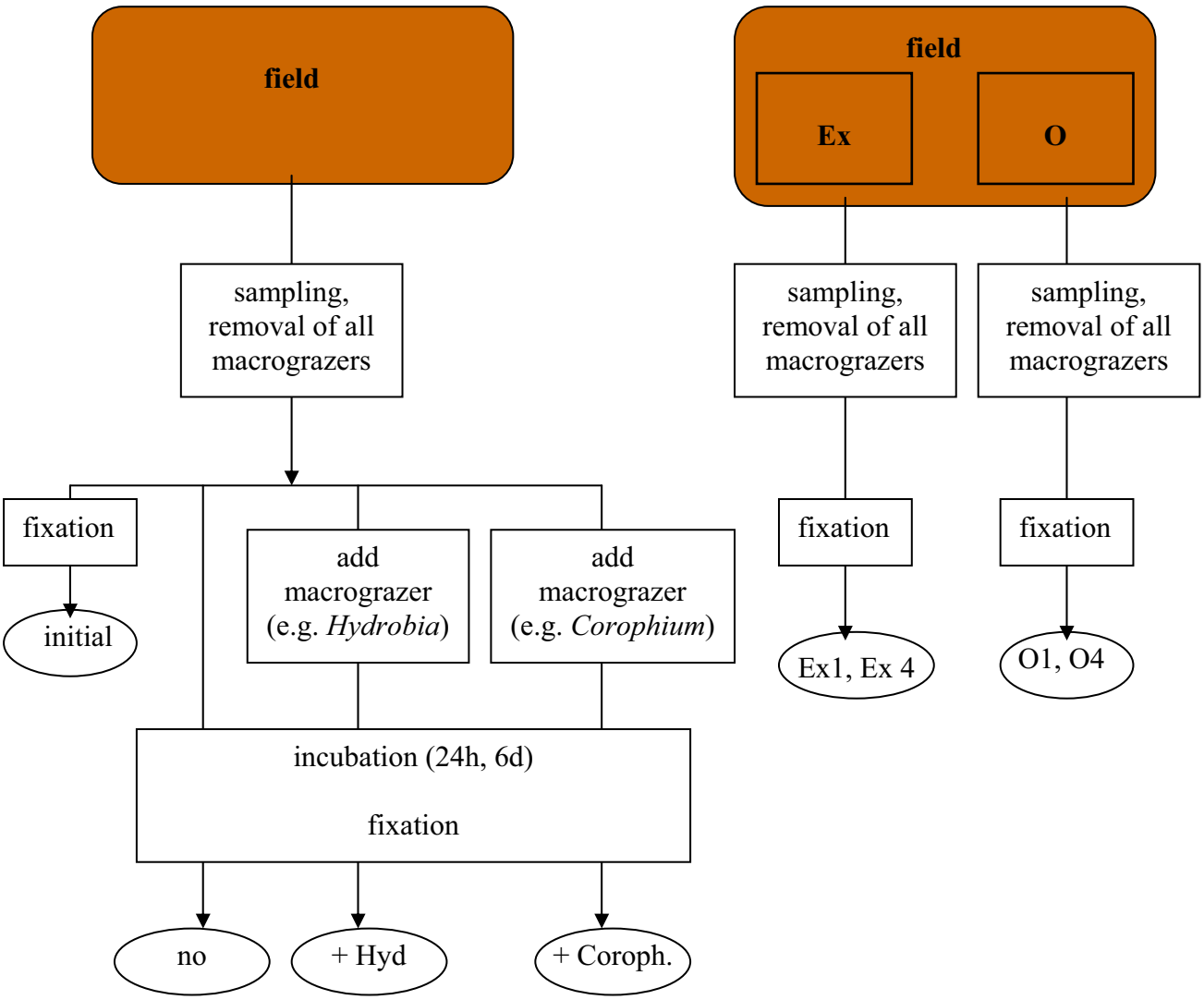


Fig. 1. Design of the experiments

Table 2: Experiment set up with the temperature in the laboratory, the artificial light to dark cycle in h, the added macrograzer species and densities in ind. Cm^{-2} and incubation time in h.

Experiment No.	date	lab temperature [°C]	light : dark	added macrograzer species	macrograzer desities ind.cm^{-2}	incubation time
E1	22.10.2001	15	14h:10h	*Hydrobia ulvae*	0.4	24 h
E2	05.03.2002	6	16h:8h	*Hydrobia ulvae*	0.4	24 h
E3	14.05.2002	15	17h:7h	*Corophium*	0.6	24 h
				Macoma balthica	0.1	24 h
E4	12.08.2002	15	20h:4h	*Corophium* sp.	0.7	24 h
				Bathyporeia sp.	0.5	24 h
E5	22.10.2002	10	14h:10h	*Hydrobia ulvae*	0.4	24 h
				Corophium sp.	0.5	24 h
E7	12.09.2002	20	14h:10h	*Arenicola marina*		24 h
E6				*mixed Polychaets*	0.5	24 h
				Hydrobia ulvae	0.4	24 h
E8	01.07.2003	20	14h:10h	*Arenicola marina*		16 month
E9	13.11.2001	13	12h:12h	*Potamopyrgus antipodarum*	0.2	24 h
E10	02.06.2003	23	0h:24h	Chironomid larvae	0.4	24 h
			0h:24h	*Gammarus roselli*	0.5	24 h
E11	20.11.2003	4	24h:0h			24 h
E12	03.12.2003	4	24h:0h			24 h
E13	03.12.2003	4	24h:0h			24 h
E14	26.08.2003	4	24h:0h	Chironomid larvae	0.3	24 h
E15	26.08.2003	4	24h:0h	Chironomid larvae	0.3	6 d
E16	02.08.2003	4	24h:0h			24 h
E17	22.08.2003	4	24h:0h			24 h
E18	08.09.2003	4	24h:0h			24 h
E19	02.08.2003	4	24h:0h			24 h

In situ *Arenicola marina* exclusion experiment

The *A. marina* field experiment was carried out to test the effects of the exclusion of *Arenicola marina* on the benthic microbial community. The influences of the sampling area position within a mudflat and of experimental manipulations were studied simultaneously.

A. marina exclusion

Within the tidal flat of a bay in the north of the island of Sylt experimental fields (each with an area of 20 x 20 m) were manipulated in spring 2002 to exclude *A. marina* from intertidal sand (Volkenbron, 2005). Exclusion was achieved by inserting a 1 mm meshed net in 10 cm depth within the sediment, blocking the burrow shafts of *A. marina*. To bury the net into the

sediment (exclusion: Ex areas) surface sediment was excavated with a small backhoe. The reference areas (reference: O areas) were not manipulated and *A. marina* was present with an abundance of 30 ind. m^{-2} to 40 ind. m^{-2}. The effect of disturbance by backhoe dredging on the sediment community was analysed by the establishment of control plots, which were disturbed in the same way as the exclusion areas but without placing a net. Since it turned out that dredging had no long-term effects on various sediment parameters sampling for this study was focussed on the exclusion and reference plots (Volkenborn et al. 2005). The exclusion plots were established within a mid intertidal medium sand (emersion period 6 to 7 hours per tide; 330-340 µm grain size median) and within a low intertidal fine sand (emersion period 3 to 4 hours per tide; 200-220 µm grain size median). Further detailed information on preparation and environmental position is shown in Volkenborn et al. (2005).

Sampling

Samples were taken during low tide from the exclusion and reference areas of the mid intertidal site during September 2002 and of the low and the mid intertidal site during July 2003 (Fig. 1, Table 1). The upper 3 mm (within the oxic layer) of sediments were collected with a flat shovel. 2 ml of the shovel sampling measured with a cut of syringe were placed into a tube, flooded with 2 ml filtrated (0.2 µm) environmental water and fixed by adding ice cold glutardialdehyd (f.c. 2%). Samples were stored at 4°C in the dark until further analysis for a maximum time of one week.

Determination of microbial organisms' abundance and biomass

The organisms were detached chemically with PPI (Tetrasodiumpyrophosphate, f.c. 0.5-10 mM) and Tween 80 (f.c.1-10 µg ml^{-1}) and detached physically by gentle sonification on ice (Branson, Sonifer 250, pulses for 30 s at 60 W). The supernatant was filtered using a black polycarbonate filter (Osmonics 0.2 µm) and stained with DAPI (4`.6-Diamino-2-phenylindol, working solution 50 µg ml^{-1}, f.c. of supernatant 5 µg ml^{-1}). Finally, the filter was embedded in fluorescence-free immersion oil (AppliChem) and stored at -20°C until microscopical analysis (Sherr et al. 1993; Velji & Albright 1993; Epstein & Rossel 1995). The slides were examined using epifluorescence microscopy (Zeiss, Axioscop2 plus, *1000 magnification). For the determination of bacteria abundance a minimum of 500 cells per slide on 24 fields were counted. For the count of cyanobacteria, diatoms and PNF abundance a minimum of 30 random fields were evaluated using the autofluorescence of the photosynthetic pigments (Waterbury et al. 1986; Maclassac & Stoeckner 1993). For the count of HNF abundance only

cells with a definite nucleus were counted on a minimum of 30 random fields, while cells with irregular shapes were excluded (Sherr et al. 1993). PNF and HNF were split in size groups according to their lengths, 2 - 5 µm = "small HNF and PNF", 5 -10 µm = "medium sized PNF and HNF", >10 µm = "large HNF and PNF". This size split was introduced in order to allow the study of cell size related interactions of the organisms with the other components of the food web. The cell sizes of organisms of all different groups were measured and the biovolume was estimated using simple geometrical shapes from the literature (Edler 1979). The biomass was calculated by converting cell biovolume using different conversion factors from the literature (detailed description in study 1).

Data analysis

Assumptions

Some assumptions had to be made in order to consider all the mostly unevitably imperfections, associated with the transfer of natural systems into a laboratory environment.

(1) We regarded the initial sample as baseline or control sample. We assumed that, if we could have incubated the sediment under completely natural conditions (including all macrograzers as worms, crabs, fish, birds) the microbial community would not have changed.

(2) We presumed for our laboratory experiments, that changes in production were exclusively caused by the absence or presence of macrograzers respectively, not by laboratory effects.

(3) It was presumed that macrograzer kept natural feeding preferences and feeding manner like in the field.

Statistical analysis

A one-way analysis of variance (ANOVA) was used to test the significance of differences in abundance between treatments in all investigated organism groups of all experiments. Abundance data were transformed to the fourth root prior to the analysis in order to normalise data. Furthermore the equality of the variances and the normality of the residuals were tested. The Scheffé test was used as "post hoc test" for the pair wise comparisons of mean values. Differences between abundance were regarded as significant if both the significance levels (p) of ANOVA and Scheffé test were $p < 0.05$ and highly significant with p-values < 0.001.

3. Results

Laboratory experiments

Dorum (E1 to E5)

In Dorum five experiments with macrograzeres were carried out from October 2001 to October 2002 (Table 2). *Hydrobia* cf *ulvae* was found in an abundance of 4300 ind. m^{-2} in April 2002 (personal observation). Also *Corophium* sp. and *Bathypoeria* sp. (both Amphipoda) were highly abundant. Two detrivorous species of *Corophium* were found, *C. volutator* (Pallas 1766) and *C. arenarium* Crawford 1937, but species were not separated because of taxonomic difficulties during the experimental setup. *Corophium* sp. was found in densities of 1351 ind. m^{-2} in April 2002. The density of *Bathyporeia* sp. was not enumerated, only during the sampling in August 2002 conspicuously high abundance was observed. During May 2002 the density of the mussel *Macoma balthica* was around 405 ind. m^{-2}. Other small and large macrograzers could not be observed. But naturally many other organisms were present within intertidal sediments, a fact to be kept in mind while analyzing changes in microbial organisma' abundance in our laboratory experiments (see assumptions). In the experiments *Hydrobia* sp. was added in a density of 3930 ind. m^{-2}, *Corophium* sp. with 4931 ind. m^{-2} to 6878 ind. m^{-2}, *Bahyiporeia* sp. with 4913 ind. m^{-2} and *Macoma balthica* with 1039 ind. m^{-2} (Table 2).

Exclusion of macrograzers (no)

During E1, E2, E3 and E5 the absence of macrograzers did not affect the abundance of bacteria, cyanobacteria, diatoms, PNF, HNF and microbial total biomass (Appendix Table 10,11). During E4 large HNF increased whereas all other microorganisms were not affected by the absence of macrograzers (Table 3, Fig. 3). The total microbial biomass significantly increased only during E4 (p=0.0472)

Table 3: Results of ANOVA and Scheffé post hoc tests of laboratory experiment E4, comparison of "initial" samples to "no" samples (absence of macrograzers)

E4	treatments	Scheffé p	
Large HNF	initial : no	0.0487	increase
Total Biomass	initial : no	0.0472	increase

Hydrobia sp. (E1, E2 and E5)

During E1 (October 2001) small PNF (p= 0.332) significantly decreased in the presence of *Hydrobia* (Fig. 2, Appendix Table 11). During E2 (March 2002) large PNF (p=0.025) increased significantly whereas in both E1 and E2 no other microgranisms or total microbial biomass were affected (Table 3). The presence of *Hydrobia* did not affect the abundance of microorganisms during E5 (October 2002).

Abundance of small PNF in laboratory experiment E1

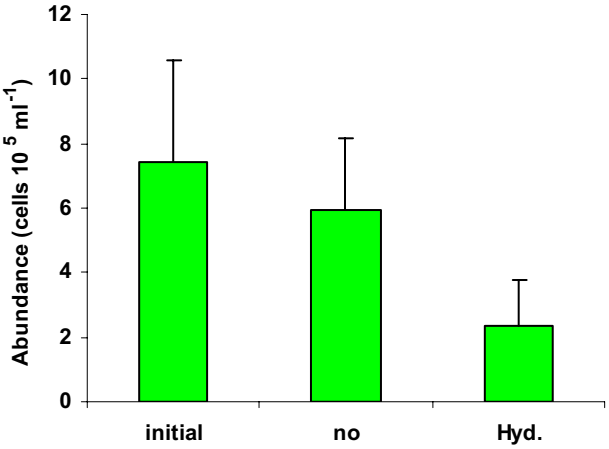


Fig. 2: Abundance (cells ml^{-1}) of small phototrophic nanoflagellates (2-5 µm) of laboratory experiment E1, with standard deviation (n=3).

Macoma cf *baltica* (E3)

The presence of *Macoma* sp. during E3 (May 2002) did not cause noticeable changes in the abundance of observed microorganisms and total microbial biomass (Appendix Table 10,11).

Corophium sp. (E3, E4 and E5)

During E3 (May 2002) and E5 (October 2002) the presence of *Corophium* sp. had no obvious influence on the abundance of all observed microorganisms and biomass (Appendix Table 10,11). During E4 (August 2002) the presence of *Corophium* sp. resulted in an significant increase of bacteria, small HNF and diatoms (Table 4, Fig. 3). Simultaneously the abundance of medium PNF decreased in the presence of *Corophium* sp. (Table 4, Fig. 3). Large HNF increased with *Corophium* sp.. It has to be mentioned that also in the absence of all macrograzers a significant increase of large HNF was found (see exclusion of macrograzer). Total microbial biomass significantly increased in the presence of *Corophium* sp. during E4.

Table 4: Results of ANOVA and Scheffé post hoc tests of laboratory experiment E4, comparison of "initial" samples to "+Coroph" samples (presence of *Corophium* sp. as single macrograzer) and "no" (absence of macrograzes) samples to "+Coroph" samples.

E4	treatments	Scheffé p	
Diatoms	initial : +Coroph	0.001	increase
	no : +Coroph	0.0155	increase
Medium PNF	initial : +Coroph	0.0139	decrease
Bacteria	initial : +Coroph	0.0075	increase
Large HNF	initial : +Coroph	0.044	increase
Small HNF	initial : +Coroph	0.0023	increase
	no : +Corophium	0.0325	increase
Total Biomass	initial : +Coroph	0.0019	increased

Bathiporeia sp. (E4)

The presence of *Bathiporeia* sp. during E4 (August 2002) caused an increase of bacteria, small HNF and diatoms (Table 5; Fig. 3). Simultaneously medium PNF decreased. Further the total microbial biomass increased significantly in the presence of *Bathiporeia* sp.. It should be mentioned that several microorganism groups showed the same responses (increasing or decreasing abundance) to the presence of *Corophium* sp. and *Bathyporeia* sp. during E4. The Amphipods *Corophium* sp. and *Bathiporeia* sp. seemed to affect microbial organisms in the same way or at least their activity led to the same results.

Table 5: Results of ANOVA and Scheffé post hoc tests of laboratory experiment E4, comparison of "initial" samples to "+Bathy" samples (presence of *Bathyporeia* sp. as single macrograzer) and "no" (absence of macrograzes) samples to "+Bathy" samples.

E4	treatments	Scheffé p	
Diatoms	initial : +Bathy	0.0033	increase
Medium PNF	initial : +Bathy	0.0139	decrease
Bacteria	initial : +Bathy	0.0018	increase
	no : +Bathy	0.0208	increase
Small HNF	initial : +Bathy	0.0191	increase
Total Biomass	initial : +Bathy	0.0004	increase

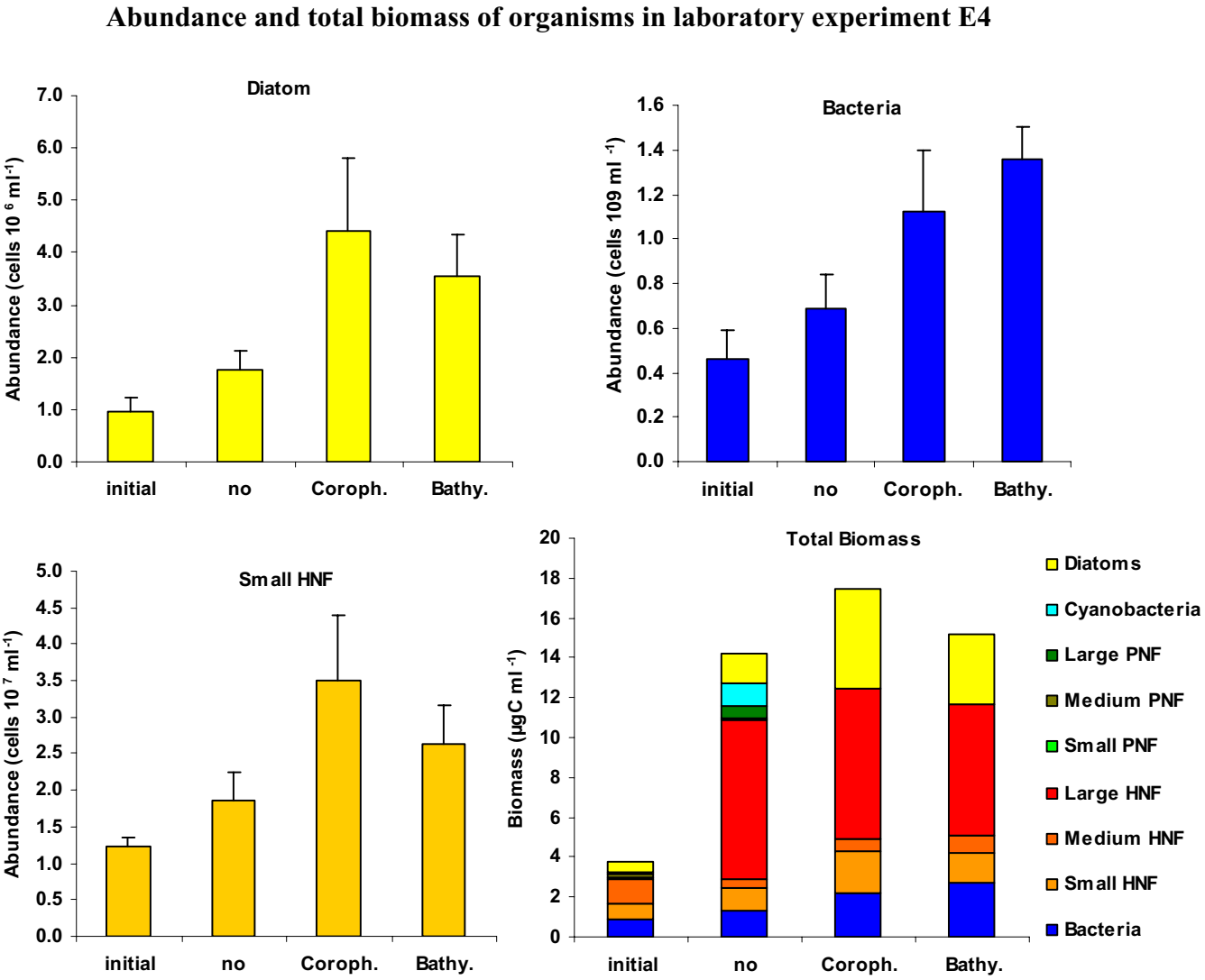


Fig. 3: Abundance (cells ml^{-1}) of diatoms, bacteria, small heterotrophic nanoflagellates (HNF 2-5 µm) and total biomass of laboratory experiment E4, with standard deviation (n=3), and total biomass (µgC ml^{-1}).

Schöhsee, Plön 2001 (E9)

Potamopyrgus antipodarum

In November 2001 an experiment was set up with sediment from the Schöhsee in Plön (E9). The common small freshwater snail *P. antipodarum* at a density of 1612 ind. m^{-2} was added as macrograzer. Snails had been active during incubation indicated by their tracks on the sediment (personal observation). However within 24 h no significant changes of bacteria, diatoms, cyanobacteria, PNF and HNF abundance were observed as a result of the absence of macrograzers or the presence of *P. antipodarum* (Appendix Table 10,11). The total microbial biomass did also not change significantly. During an experiment in August 2001 (data not presented here) HNF increased in the absence of macrograzers, whereas no change was found in the presence of *P. antipodarum*.

Fühlinger See, Cologne (E10)

Chironomid larvae and *Gammarus* cf *roselli*

In June 2003 an experiment was carried out with Chironomid larvae and *Gammarus* sp. in natural sediment of the Fühlinger See (E10). The densities used in the treatments were 4157 ind. m^{-2} for Chironomid larvae and 5196 ind. m^{-2} for *Gammarus* sp.. After an incubation of 24 h no significant changes of the observed organisms' abundance and total microbial biomass were found as a result of the absence of all macrograzers or the presence of one species of macrograzers (Appendix Table 10,11).

Potsdam Lake (E14 and E15)

Chironomid larvae

Two experiments were set up in August 2003 with the sediment from the artic Potsdam Lake. E14 was a short term experiment without macrograzers, lasting 24 h, whereas in E15 Chironomid larvae were added to one part of the treatments for 6 d. During E14 (no macrograzers) small HNF significantly increased, whereas bacteria, cyanobacteria, diatoms, PNF and medium and large HNF were not affected (Table 6, Fig. 4).

In E15 Chironomid larvae were added in a density of 3118 ind. m^{-2}. The presence of macrograzers led to a significant increase of bacteria and to a decrease of cyanobacteria (Table 6, Fig. 4). Medium PNF decreased significantly in both the absence of all macrograzers and the presence of Chironomid larvae. However the total microbial biomass did not show significant changes during the experiment.

Table 6: Results of ANOVA and Scheffé post hoc tests of laboratory experiment E14 and E15, comparison of "initial" samples to "no" (absence of macrograzes) samples and "initial" samples to "+Chiro" samples (presence of Chironomid larvae as single macrograzer).

Exp. No.	organism	treatment	Scheffé p	
E14	Small HNF	initial : no	0.0124	increase
E15	Bacteria	initial : +Chiro	0.0307	increase
	Cyanobacteria	initial : +Chiro	0.0061	decrease
	Medium PNF	initial : no	<0.0001	decrease
		initial : +Chiro	<0.0001	decrease

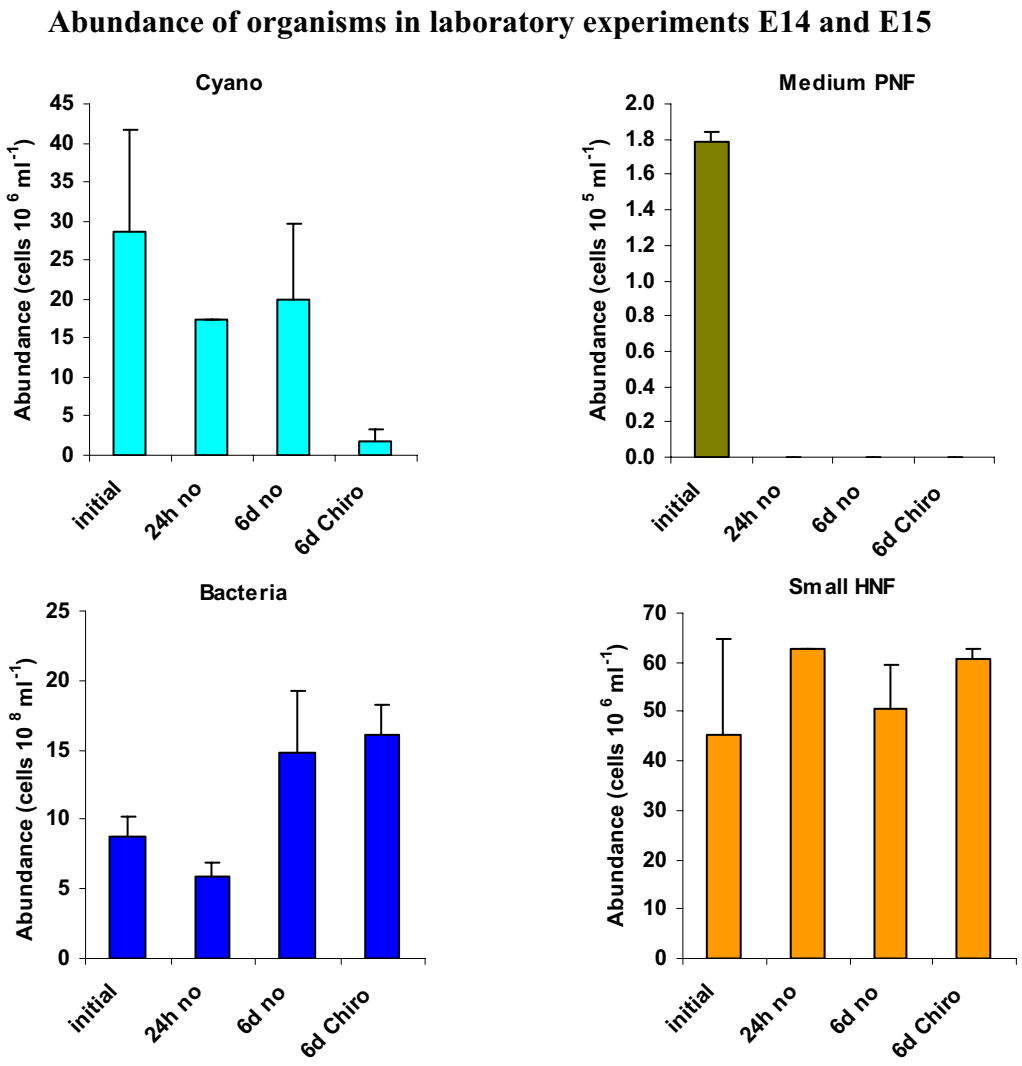


Fig. 4: Abundance (cells ml^{-1}) of cyanobacteria, medium phototropic nanoflagellates (PNF), bacteria and small heterotrophic nanoflagellates (HNF) of laboratory experiment E14 (initial : 24h no) and E15 (initial : 6d no: 6d Chiro), with standard deviation (n=3).

Sylt (E6)

Hydrobia c.f. *ulvae*, mixed Polychaets

The short term experiment (E6) was set up in September 2002 with natural sediment. *Hydrobia* sp. (density 3930 ind. m^{-2}) and mixed polychaets (density 4913 ind. m^{-2}) were added to the treatments, respectively. The additional polychaets were composed of *Scoloplos armiger* (O.F. Müller, 1776), *Pygospio elegans* Claparède, 1863, *Polydora ciliata* (Johnston, 1838) and *Spio filicornis* (O.F. Müller, 1766) collected from the sediment. All worms were around 2 cm in length. Visible tracks proved that worms and *Hydrobia* sp. had been active during incubation. No significant changes of the abundance of bacteria, medium and large HNF, small and medium PNF, cyanobacteria and diatoms were observed in the presence or absence of macroorganisms (Appendix Table 10,11). However, small HNF significantly increased in the presence of *Hydrobia* sp. and polychaets, respectively, whereas no change

was observed in the absence of macrograzers (Table 7, Fig. 5). Medium PNF increased in all treatments but the rise was significant only in the absence of macrograzer. Total microbial biomass however did not change during E6.

Table 7: Results of ANOVA and Scheffé post hoc tests of laboratory experiment E6, comparison of "initial" samples to "no" samples (absence of macrograzers) and "initial" samples to "+Hyd" and "+Poly" samples (presence of *Hydrobia* and mixed polychaets single macrograzer, respectively).

E6	treatments	Scheffé p	
Small HNF	initial : +Hyd initial : +Poly	0.0337 0.0248	increase
Medium PNF	initial :no	0.0352	increase

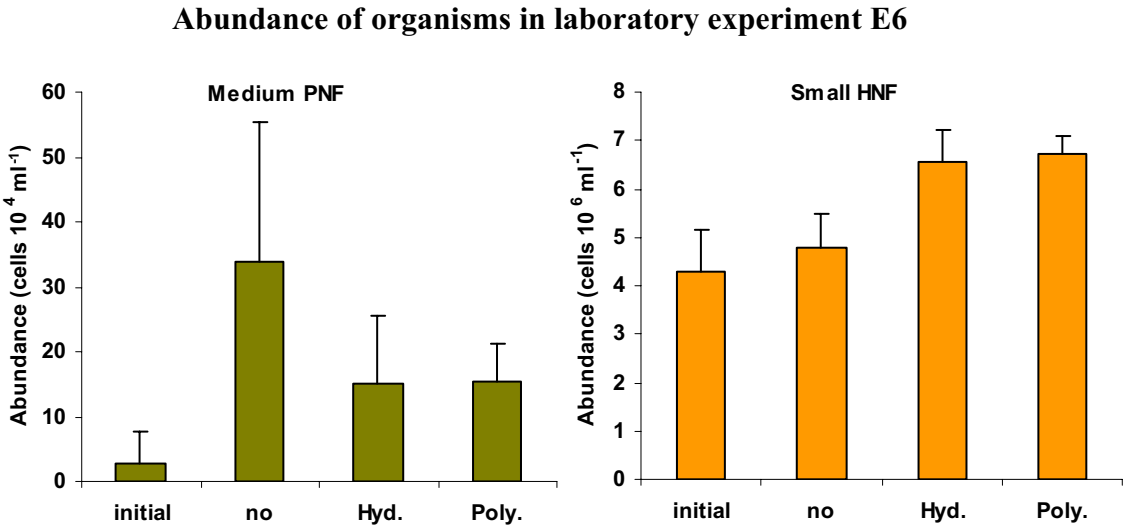


Fig. 5: Abundance (cells ml^{-1}) of medium phototrophic nanoflagellates (PNF) and small heterotrophic nanoflagellates (HNF) of laboratory experiment E6, with standard deviation (n=3).

Different other sites (E11 to E19)

Exclusion of macrograzers (no)

Several experiments were set up without adding special macrograzers since the sediment in the field exhibited the absence of abundant macrograzers. This was not a proof for the total absence of macrograzers, but during the sampling they were not found in mentionable numbers. However these experiments were useful for the understanding of the influence and function of macrograzers within the small food web. In Green Lane Lake (E12) the abundance and total microbial biomass of investigated microorganisms were not affected by the absence of macrograzers. In Shark River Bay sediment (E11) the absence of macrograzers for 24h led to a significant increase of bacteria abundance while diatoms decreased (Table 8, Fig. 6). During the experiment with sediment from Nockamixon Lake (E13) only small HNF increased significantly after 24h in the absence of macrograzers (Table 8, Fig. 6). Within the

arctic areas in Melles Lake (E16), Duck Lake (E17), Basalt Lake (E18) and Store Koldewey Beach (E19) the absence of macrograzers did within 24 h not affect the abundance and the total microbial biomass.

Table 8: Results of ANOVA and Scheffé post hoc tests of laboratory experiment E11 and 13, comparison of "initial" samples to "no" samples (absence of macrograzers).

Exp. No.	organism	treatments	Scheffé p	
E11	Bacteria	initial :no	0.0124	increase
	Diatoms	initial :no	0.0013	decrease
E13	Small HNF	initial : no	0.0177	increase

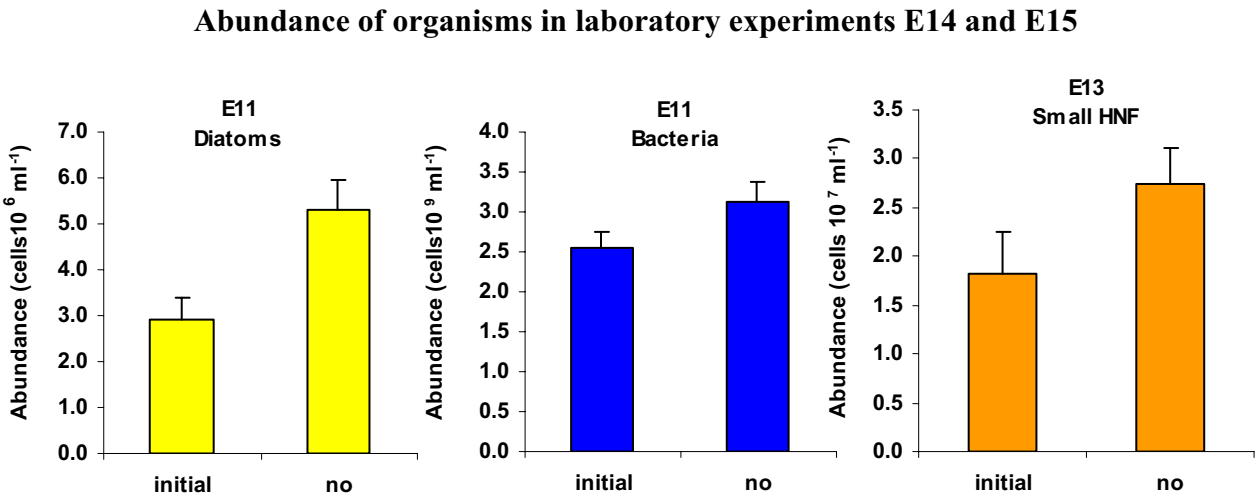


Fig. 6: Abundance (cells ml^{-1}) of diatoms and bacteria of laboratory experiment E11 as well as of small heterotrophic nanoflagellates (HNF) of laboratory experiment E13, with standard deviation (n=3).

Field experiment

Sylt (E7, E8) *Arenicola marina* exclusion

The long term exclusion fields were sampled twice, once in September 2002 (six month after exclusion; E7) and once in July 2003 (16 month after exclusion, E8). The exclusion of *A. marina* resulted in an obvious change of the sediment chemistry. According to Volkenborn & Reise (2005) distinct increases of organic carbon and nitrogen content were found within the *A. marina* free areas (Ex1 and Ex4; data not presented). Chlorophyll-*a* was also consistently higher in *A. marina* exclusion areas (Volkenborn & Reise 2005). In E7 exclusion (Ex4) and reference (O4) areas in the mid intertidal medium sand were sampled. We found significantly higher abundance of diatoms, medium PNF, bacteria and small HNF within the

A. marina exclusion (Ex4) field (Table 9, Fig. 7). The total microbial biomass was also significantly higher in the absence of *A. marina* (Table 9, Appendix Table 10,11).

During E8 (16 month after exclusion) we sampled the experimental plots in the mid (Ex4, O4) and low (Ex1, O1) intertidal area. After 16 month of exclusion the previously significant differences found in E7 (six month after exclusion) were less clear. Bacteria were still significantly higher in *A. marina* exclusions (Table 9, Fig. 8). Diatoms, large PNF, small HNF and large HNF were significantly higher only in Ex1, whereas in Ex4 only cyanobacteria were significantly higher (Table 9, Fig. 8).

A comparison between the mid and low intertidal clusters (O1:O4 and Ex1:Ex4) exhibited that bacteria and small PNF were always more abundant in low intertidal areas than in the mid intertidal ones (Table 9). Additional differences between the *A. marina* free areas in low and mid intertidal areas were found. Abundance of diatoms, large PNF and small HNF were significantly higher in the low intertidal fine sediment than in the mid intertidal medium sand.

Table 9: Results of ANOVA and Scheffé post hoc tests of the field experiments E7 and E8, comparison of "O" samples (untreated natural sediment) to "Ex" samples (exclusion of *Arenicola marina*).

Exp. No.	organism	treatments	Scheffé p	
E7	Diatoms	O4 : Ex 4	0.0177	increase
	Medium PNF	O4 : Ex 4	0.0342	increase
	Bacteria	O4 : Ex 4	0.0041	increase
	Small HNF	O4 : Ex 4	0.0043	increase
	Total Biomass	O4 : Ex 4	0,0172	increase
E8	Bacteria	O1 : Ex1	0.0002	increase
	Bacteria	O4 : Ex4	0.0009	increase
	Diatoms	O1 : Ex1	0.0076	increase
	Large PNF	O1 : Ex1	0.0338	increase
	Small HNF	O1 : Ex1	0.0482	increase
	Large HNF	O1 : Ex1	0.0303	increase
	Cyanobacteria	O4 : Ex4	0.009	increase
	Small PNF	O1 :O4	0.0457	increase
	Bacteria	O1 :O4	<0.0001	increase
	Diatoms	Ex1 : Ex4	0.0101	increase
	Small PNF	Ex1 : Ex 4	0.0052	increase
	Large PNF	Ex1 : Ex 4	0.0321	increase
	Bacteria	Ex1 : Ex 4	0.0002	increase
	Small HNF	Ex1 : Ex 4	0.0052	increase

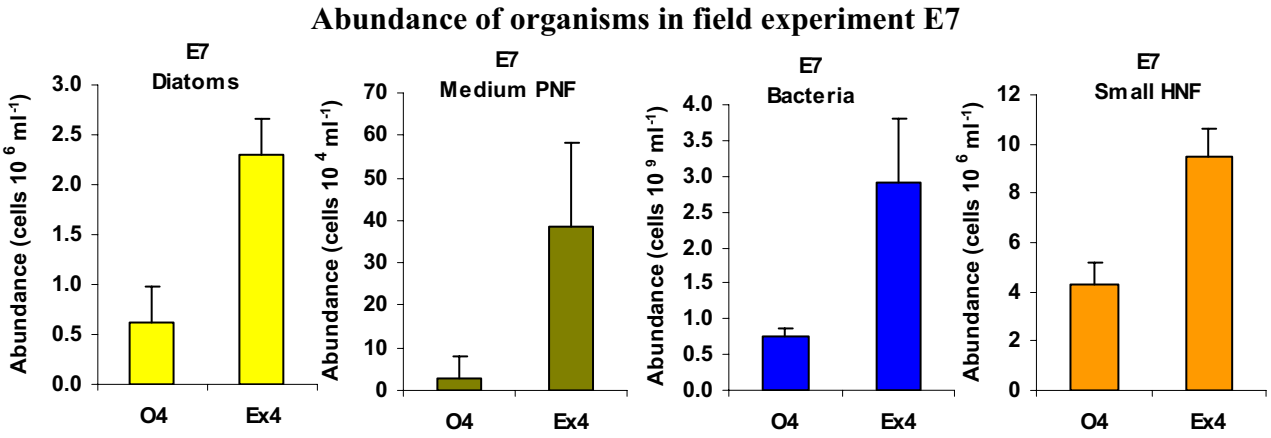


Fig. 7: Abundance (cells ml^{-1}) of diatoms, medium phototrophic nanoflagellates (PNF) and small heterotrophic nanoflagellates (HNF) of the field experiment E7, with standard deviation (n=3).

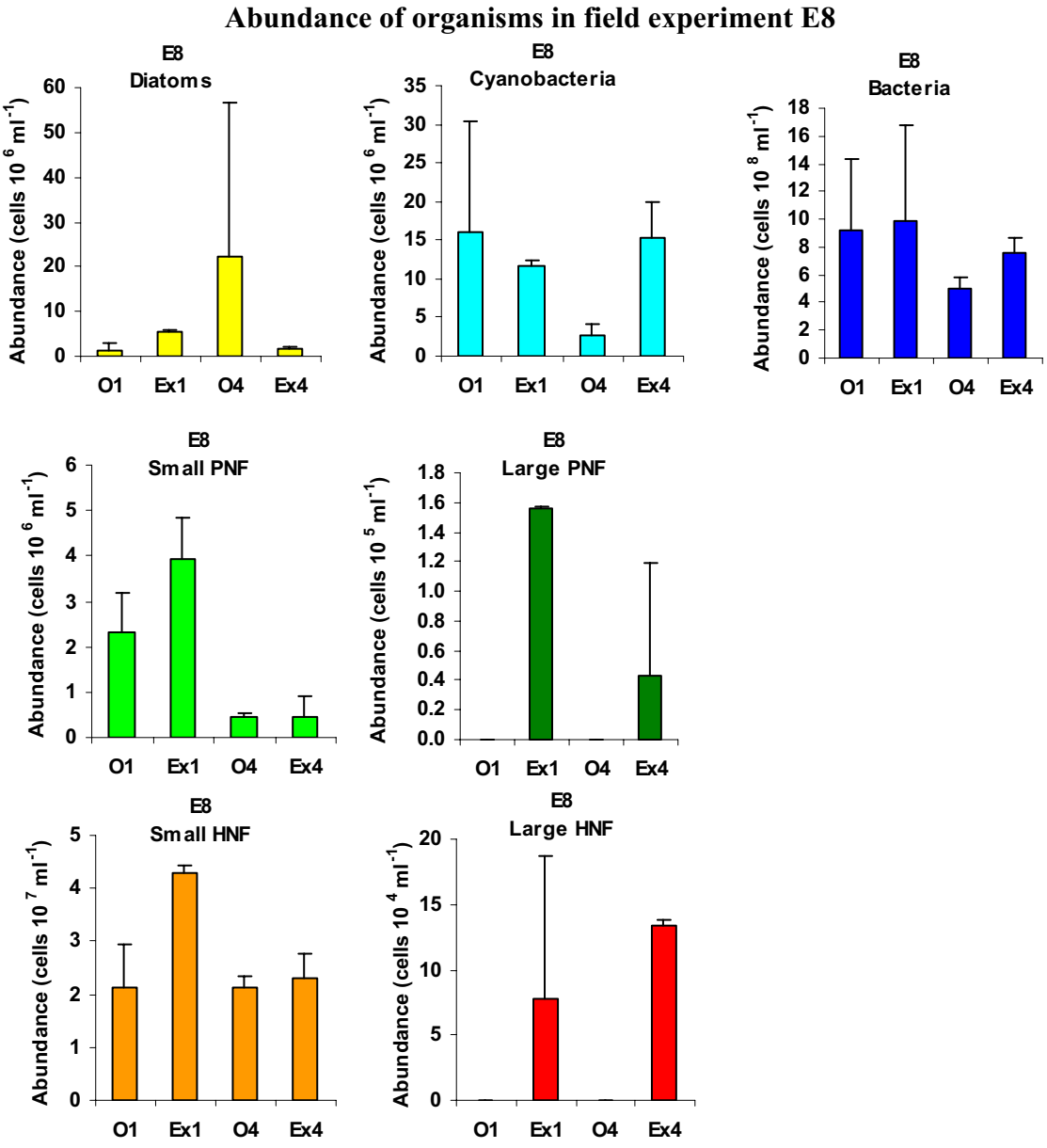


Fig. 8: Abundance (cells ml^{-1}) of diatoms, cyanobacteria, bacteria, large phototrophic nanoflagellates (PNF), small phototrophic nanoflagellates, small heterotrophic nanoflagellates (HNF) and large heterotrophic nanoflagellates of the field experiment E8, with standard deviation (n=3).

4. Discussion

The factors controlling the abundance and distribution of components of the microbial food webs in sediments are still sparsely understood and the debate amongst ecologists continues as to the question whether the fundamental control is executed by bottom-up factors (such as resources) or top-down factors (such as predation) (Power 1992). This author suggests that a clear top-down effect can be expected, when short-lived primary producers quickly balance predation by rapid growth and single species, furthermore when a guild directly affects the trophic level beneath via cascading effects, in other words, if the trophic links are chain like and not web like (Begon et al. 1996 and references therein). It is known that a high primary production can mask every top-down effect (Begon et al. 1996). However, many of these findings are gathered from theoretical models and reflections with only three or four trophic levels and species. A closer look at realistic communities revealed that trophic levels were composed of numerous species with diverse preferences forming a web like structure. Species composition and environmental parameters as forcing factors could again change with season (study 1). The effects of higher trophic levels influencing those beneath are either caused by grazing (trophic cascading) or by indirect effects or by both simultaneously. Indirect effects, like stimulation and inhibition, are effects effective because of one organism´s presence and activity onto another organism, grazing exepted. Stimulation for instance via bioturbation, fecal pellets recycling and sloppy feeding might enhance e.g. nutrients concentration and O_2 saturation, which in turn might increase microbial production. Inhibition might be caused by competition, enhanced turbulence from bioturbation or inhibitory exudations, which decrease anothers organisms production or even kill it.

For the discussion of the results some assumptions had to be made (material and methods). As a consequence of these assumptions, a decrease of microorganisms' abundance in the presence of a single added macrograzer species in natural densities could not be regarded as direct grazing. The grazer should keep grazing rates and preferences like afield (see assumption 3) while the prey organisms should grow and therefore balance losses like in the field (assumption 2). Communities in the field would otherwise collapse every day by overgrazing and regenerate completely within 24 h, which seems to be unlikely. Consequently decreasing abundance was interpreted to be caused by a lack of stimulation or by changes in the cascading effects in the absence of macrograzers other than the single added macrograzer species. Increasing abundance might indicate the lack of grazing pressure for instance in the absence of other macrograzers than the single added macrograzer species. No change in abundance during an experiment was interpreted in a way that the added macrograzer could

not have been the main direct predator and its structuring force by cascading seemed also to be, at most, marginal. In turn other small predators like meiofauna, ciliates and large HNF might be the major predators. Comprehensive comparisons of "initial" and "macrograzer" samples and in particular of the "no" samples were accomplished on this base of interpretation of the study results.

Effects of Molluscs

Hydrobia cf *ulvae* Pennant, 1777

The presence of *Hydrobia*. sp resulted in an increased abundance of medium and large PNF and small HNF during spring and late summer whereas during late autumn small PNF decreased. In most experiments the presence of *Hydrobia*. sp had no effect on the microbial organisms. *Hydrobia*. sp ingests sediment particles of 20 µm to 200 µm via deposit feeding and browsing. The particle size selection by *Hydrobia*. sp depends on the animals body size and on the quality of the available food (Levinton & Bianchi 1981; Levinton 1987; Morrisey 1988; Blanchard et al. 2000). Within their buccal cavity they scrape off particle attached microbes with the help of their radula and spite out the rest (Lopez & Kofoed 1980). Diatoms seem to belong to the main components of the gut content but cells may survive the gut residence time of 30-40 min without being digested, thus not contributing to the feeding of the grazer (Lopez & Kofoed 1980). Cyanobacteria, bacteria and other particle associated microorganisms are used as additional nutrition, even cropopharie is observed (Levinton & Bianchi 1981; López-Figueroa & Niell 1987; Morrisey 1988; Bianchi & Levinton 1984). The findings of this study led to he assumption that even if *Hydrobia*. sp was abundant and feeding rates on detritus and attached organisms could be assumed to be high, their top-down forces were rather marginal. This fits to the findings of Blanchard et al. (2000) who report that only 0.4% to 1.2% of the microalgal biomass is ingested by snails. *Hydrobia*. sp is known to decrease ingestion rate per individual in high abundance situation, caused by density-dependent effects rather than by food limitation. Blanchard et al. (2000) exhibit the threshold density to be between 1.4 and 2.5 snails cm^{-2}. In this study *Hydrobia*. sp was added in a density of 0.39 snails cm^{-2}, representing the density found in the field, much lower than the density-dependent threshold and lower than the maximum abundance observed in other sediments (7.5 ind. cm^{-2}; Hagerthey et al. 2002). A density dependent reduction in feeding seemed therefore to be unlikely during our experiments.

The activity of *Hydrobia*. sp mixes the upper millimeter of the surface sediment layer horizontally and affects the distribution of nutrients and might stimulate primary production

(Lopez & Levinton 1987). It seemed that the snail´s activity improved the conditions for their potential prey rather than it affected it negatively by grazing.

Potamopyrgus antipodarum (Gray, 1843)

In freshwater sediments *P. antipodarum* reaches an abundance of 150 000 ind. m^{-2} (Aberle-Malzahn 2004; Richadrs et al. 2005). The density of *P. antipodarum* within Schöhsee sediment was low (150 ind. m^{-2}). For our experiment we used a much higher density (1136 m^{-2}) to increase possible grazing forces in order to improve their detection during the short-term experiments. But the used density did not at all exceed densities found in other field studies (Richadrs et al. 2005). However, no significant effects on the abundance of diatoms, cyanobacteria, bacteria and PNF during November within 24 h could be identified assignable to the presence of *P. antipodarum* or to the absence of macrofauna. The high density of *P. antipodarum* in the laboratory experiments might have caused a density dependent decrease of their feeding activity, like it is known for *Hydrobia*, although such an effect has not yet been described for *P. antipodarum*. An increasing abundance of HNF was observed during August in the absence of macrofauna whereas abundance did not change in the presence of *P. antipodarum*. *P. antipodarum* ingests particles comparable to *H. ulvae* (Nielsen & Kofoed 1982). James et al. (2000) reported that the stomach contents of these organisms are composed of diatoms (62%), organic matter (32%) and animals (2%). We assumed that the production of HNF was higher during summer than during winter, due to higher temperatures. Consequently the abundance increased faster when the main predator was missing. These findings led to the assumption that at least during summer *P. antipodarum* was a key species structuring HNF abundance by top-down forces. All other potential prey microorganisms seemed to balance grazing by high growth rates, which again was possibly stimulated by snails bioturbation.

Macoma cf *balthica* (Linnaeus, 1758)

During this study we did not identify significant changes in the abundance and biomass of observed microorganisms in the presence of *Macoma* sp.. The clam *Macoma* sp. can feed by filtrating particles of suspensions, in addition by pipetting detritus, diatoms and other microogranisms with its siphon from the sediment surface and it can facultatively switch between suspension- and deposit-feeding in response to the availability of suspended food particles (Olaffson 1986; Levinton 1976; Lin & Hines 1994; Westheide & Rieger 1996).

Grazing of *Macoma* sp. seemed not to be high enough to affect the standing stock of benthic microorganisms.

Effects of species of Amphipoda

Amphipods are widespread throughout diverse ranges of tropical, temperate and arctic intertidal sandy shore habitats. They often dominate benthic macrofaunal communities in terms of both numbers and biomass (Fenchel et al. 1975, Wijnsma et al. 1999; Dittmann 2000). Amphipods fall into 7 major feeding categories: suspension-feeders, surface detrivores, buried detrivores, scavengers, carnivores, commensals and grazers on living food (Biernbaum 1979; Gerdol & Hughes 1994; Fenchel et al. 1975; Nielsen & Kofoed 1982). Feeding methods differ between species, habitats and sometimes between seasons and developmental stages.

Corophium sp. Latr. and *Bathyporeia* sp. Lindstöm 1855

In Dorum two species of Corophium were found. *C. volutator* (Pallas 1766) and *C. arenarium* Crawford 1937. Due to taxonomic difficulties during the experimental set up we used a mixture of both species as added macrograzer. Both intertidal species are feeding on deposit by raking the surface with their antennae, capturing particles out of suspensions or scrabbing organic matter from particles (Gerdol & Hughes 1994). They avoid competition by preferring different prey sizes and/or habitat separation (Jensen & Kristensen 1990). *C. volutator* is known to ingest particles of 4 µm to 20 µm in fine sediments, whereas *C. arenarium* uses particle sizes between 4 µm and 60 µm in coarser sandy sediments (Morrisey 1988). The bulk of gut content in both species are diatoms, which were crushed with mandibles and digested effectively (Morrisey 1988; Gerdol & Hughes 1994). Large organic particles can also be ingested and associated microorganisms can be used as additional nutrition (Gerdol & Hughes 1994). With the help of fluorescently labelled algae Gerdol & Hughes (1994) measured ingestion rates of 4000 diatoms per h in an estuary sediment.

Bathyporeia sp. lives in the interstices of fine sand within the upper 5 cm. The animal is lying upside down in a small cavity with the interstices as only connection to the water above. They are feeding on particles adhering on sand grains but not on moving ciliates (d´Udekem d`Acoz 2004 2004). Stomach contents consists of unidentifiable matter and crushed diatom frustules, therefore they should be considered as selective deposit feeders. (d´Udekem d`Acoz 2004).

During May (E3) and October (E5) no significant changes of diatoms or other microorganisms were found in the presence of *Corophium* sp. or absence of macrograzers. During August (E4) however several significant changes of bacteria, diatoms, medium and large PNF and small HNF were observed. Bacteria, diatoms and small HNF increased only in the presence of *Corophium* sp. or *Bathyporeia* sp.. These changes could be explained as a stimulation of the production of bacteria, diatoms and small HNF because of the activities of both added macrograzers. In the absence of macrograzers the prey abundance did not change significantly, which could be explained by either the absence of stimulation by amphipods activity or the absence of grazing by other macrograzers. *Corophium* sp. bioturbates the sediment vertically. It increases porosity and affects sediment chemistry which in turn may stimulate primary production (Gerdol & Hughes 1994). Medium PNF decreased in this study in the absence of macrograzers but in the presence of amphipods also. The absence of macrograzers and the consequent lack of stimulating effects seemed to be more important than amphipods grazing. In contrast large HNF increased in the absence of macrograzers as well as in the presence of amphipods (significantly only with *Corophium* sp.). *Bathyporeia* sp. is assumed to feed at least to a small extent on large HNF, other macroorgnisms revealed to be probably the main predators of large HNF.

The predation of *Corophium* sp. and *Bathyporeia* sp. seemed not to cause strong top-down effects on the bulk of microorganisms. Only during summer some specific microbenthic organisms groups exhibited some grazing impacts. It could be assumed that the activity of Amphipodes affected the microbial community rather positively by stimulation than negatively by grazing.

Gammarus c.f. *roseli* Gervais 1835

In the experiment during June 2003 (E10) *Gammarus* sp. did not affect the components of the microbial community in a temperate shallow lake sediment. *Gammarus* sp. are traditionally regarded as herbivorous shredders. Recent studies however suggest that species of the genus Gammarus should be viewed also as predators, where food availability and quality are supposed to decide beween the two feeding types (Kelly et al. 2002). The study of three species of the genus Gammarus in the surf zone of a Korean sandy shore shows that two species (*Synchelidium* sp.) are carnivours, consuming mainly benthic harpacticoid copepods, whereas the diet of *G. japonica* consists of copepods and detritus (Yu et al. 2003). The missing impact of these species in this study might be allocated to the fact that *Gammarus* sp. ingested either the complete microbial production or they ingested microorganisms in such

low numbers, that differences in microorganisms abundance were hidden in the displayed standard deviation.

Effects of Chironomid larvae

During the experiment in a freshwater lake (Fühlinger See, Cologne) in June 2003 (E10) the abundance of bacteria, cyanobacteria, diatoms, PNF and HNF were not affected by the presence of Chironomid larvae or the absence of macrofauna. Changes in the microbial community in the presence/absence of macrograzers were however observed within the sediment of Potsdam lake (E14 and E15) during August 2003. After a 6 d incubation the presence of Chironomid larvae led to an increasing bacteria abundance. The lack of grazing pressure caused by the lack of other macrograzers than Chironomid larvae could explain this trend. In study 5 we found that ciliate biomass decreased only in the presence of Chironomids, whereas the absence of macrograzers did not cause any change, to be attributed to a decreased grazing pressure on bacteria as result of the lower ciliate abundance. On the other hand Chironomids activity could have stimulated bacteria production. In contrast, the abundance of cyanobacteria decreased only in the presence of Chironomids, whereas no change was found when all macrograzers were absent. This could be interpreted in a way that Chironomids fed selectively on cyanobacteria, while the stimulation effects from macrograzers other than Chironomids were missing. Medium PNF were affected by the absence of other macrograzers but also in the presence of Chironomids. It is assumed that macrograzers, other than Chironomids, had caused a cascading effect or a stimulation, which was missing, when only Chironomids were present. Small HNF increased after 24 h incubation, but after 6 d of incubation the numbers decreased again to the initial abundance in all treatments. It could be assumed that small HNF increased due to a higher production, which in turn might have been caused by the lack of macrograzers, but after 6 d other grazers might have taken over the feeding pressure. These other grazers, feeding on small HNF, were either not analysed or an increasing abundance was not detected due to a shift in an undetected species composition. Another explanation was a change in feeding preferences of grazers, due to a higher availability of small HNF. Other findings, described in detail in study 5, support the assumption that a species shift in ciliates can cause a change in feeding pressure on small HNF during the 6 d of incubation.

Chironomid larvae often dominate (in numbers and biomass) the profundal of temperate freshwater lakes with densities of more than 2000 ind. m^{-2} (Johnson et al. 1989). These animals can be classified as collectors-gatherers or collectors-suspension feeders (Johnson et

al. 1989). The stomach content analysis reveals that 71% of the content consists of organic matter, 10% of diatoms and 7% of animal remains (James et al. 2000). Investigations on *Chironomous plumosus* (Linnaeus) show that that they forage selectively for sedimented cyanobacteria (Johnson et al. 1989). Hence it could be assumed that Chironomids in Potsdam lake fed selectively on cyanobacteria, whereas their activity in turn stimulated at least bacterial production. The absence of other small macrograzers caused a significant change in the species composition of ciliates and herewith in feeding pressure on some components, observable in the development of medium PNF and small HNF.

Polychaets

Polychaets, studied during the laboratory experiment, were composed of *Scoloplos armiger*, Capitellidae, *Pygospio elegans* and *Spio* sp.. All worms had more or less equal sizes of 2 to 3 cm in length and a few millimetres in diameter. Polychaetes are a diverse group with likewise diverse feeding types and methods (Fauchald & Jumars, 1979). Carnivores are equipped with various mandible apparatus whereas microphages ingest either the whole sediment, slurping the microorganisms from the grains with the help of their muscular pharynx (sand licker) or they are picking up the microorganisms with their tentacles and palpes from the sediment surfaces and from the interstitial (picker) (Westheide et al. 1996). *Arenicola marina* (Linnaeus 1758) and *Scoloplos armiger* (O.F. Müller, 1776) are typical substrate feeders, ingesting the sediment and digest organisms attached to grains. *Pygospio elegans* Claparède, 1863, *Polydora ciliata* (Johnston, 1838) and *Spio filicornis* (O.F. Müller, 1766) are equipped with tentacles or palpes which they use to pick up microorganisms and detritus particles from the sediment. Thus the mixture of polychaetes added during the laboratory experiment was composed of different substrate feeders (*Scolops armiger*, Capitellidae), sand lickers and pickers (*Pygospio elegans*, *Spio sp.)* all mainly feeding on detritus and microorganisms. The presence of these polychaetes resulted in an increase of small HNF whereas the absence of macrograzers had no measurable effect. Increases could be explained by the lack of grazing pressure while production remained on the same level, but polychaetes must have had either an additional cascading effect on HNF feeders (such as ciliates, like in Potsdam Lake) or a stimulation impact on HNF production by their activity. In contrast to that the abundance of medium PNF increased only in the absence of macrograzers. Here polychaetes might be regarded as key species controlling the abundance of medium PNF. For all other microorganisms the macrofauna seemed to play a minor role.

Arenicola marina

The large funnel-feeding polychaete *A. marina* (Linnaeus, 1758) is the most apparent origin of bioturbation in intertidal flats of the Wadden Sea. The abundance reaches up to 40 ind. m^{-2} (Beukema, 1976; Reise, 1985). The sediment's biochemistry and biology are significantly affected by the lugworm's activities. The burrow ventilation brings oxygen into the anoxic environment. Their feeding activity by underground uptake of sediment is enormous (up to 1 cm^3 of sediment h^{-1}) (Grossmann & Reichardt 1991; Volkenborn & Reise 2005). It could be shown that the long term exclusion of lugworms led to an increase of organic carbon and nitrogen content (Volkenborn et al. 2005) and higher abundance of tube building polychaetes and copepods, whereas cilitate's abundance was not affected (Volkenborn & Reise 2005; study 5). After six month of lugworm's exclusion the abundance of bacteria, small HNF, medium PNF and diatoms significantly increased in the mid intertiald cluster (Ex4:O4). But after further 10 months, only bacteria abundance was still higher and cyanobacteria abundance was significantly higher in Ex4. These findings led to the assumption that the functional role of *A. marina* within the small food web was adopted by other organisms (such as *Nereis divesicolor*, nematods, copepods, other ciliate species) especially after a complete season including a larvae settlement event during spring.

The comparison of the investigated microoorganisms' abundance between the two locations within the tidal flat showed that bacteria, and small PNF were significantly more abundant within the sediments near the low intertide than in the mid interdial area, regardless the presence or absence of *A. marina*. The sediment near the low intertideal area was characterised by finer sand with a higher organic content compared to the mid intertidal area (Volkenborn et al. 2005), both parameters known to affect the abundance of bacteria and flagellates (Fenchel 1987). The different environmental conditions in the mid and low intertidal areas seemed rather to be responsible for the differences of the microbial communities in both areas than the presence or absence of *A. marina*. The presence of *A. marina* had considerable effects on the environment in terms of both abiotic and biotic factors, like microorganism abundance. But the environmental conditions induced by the presence of lugworms, seemed to have stronger influences on the microbial communities than the presence of lugworms as grazers.

Effects of macrograzers exclusion

The responses of microorganisms to sediment incubations without macrograzers were overall inconsistent. In some experiments the number of HNF increased (E4; E9; E13; E14). We

assumed that the decreasing grazing pressure as shown in Potsdam Lake (E14) was the main triggering factor. It is questionable whether the incubation times of 24 h were too short to cause noticeable effects on organisms' abundance. Especially protists however are known to react quickly to changed conditions. The generation times of nanoflagellelates range from 2,8 h to 231 h and those of ciliates could last only 3,6 h for bacterivorous and 11,5 h for algivores (Wetzel 2001).

Conclusion

The results of this study revealed that the absence of all macrograzers affected the microbial communities at least and only in short term. The influence of single macrograzers had a much smaller effect than previously expected. According to Epstein & Gallagher (1992) and Walters & Moriatry (1993) the impacts of macrograzers on benthic microorganisms seem to be species-specific. During the experiments of this study selective feeding by the added macrograzer species was found only rarely. As an example Chironomid larvae are known to feed selectively on cyanobacteria and during the experiment the changes of abundance of cyanobacteria was supposed to be caused by direct grazing by Chironomid larvae. Levinton & Bianchi (1981) suppose that the components of the microbenthic community balances grazing immediately by high production. High production rates might this way mask clear top-down effects (Begon et al. 1996).

Microbial components of benthic communities are characterised by short generation times. A possible rapid species shift of both prey organisms and predator probably may lead in a change of the trophic structure.

Small macrofaunal organisms seemed to prefer feeding omnivorously on detritus-attached microorganisms rather than specialised on single prey species. This feeding strategy could be the basis for the creation of a very complex and strongly interconnected food web in sediments.

Direct top-down effects of macrograzers on the composition of microbial community by grazing seemed to be astonishingly small compared to the macrograzers high biomass and grazing rates known from literature data. The activity of macrograzers therefore was suggested to play a more important role by stimulating prey production than by decreasing prey organisms abundance by grazing. Single macrofauna key species were identified, whose absence influenced the microbial community at least in short term. In the field experiment macrograzer species revealed to be replaceable by other species with a comparable feeding

strategy in long term. These findings turned out to hold true for marine and freshwater systems in temperate and arctic regions.

According to the theory of Fretwell (1977) the benthic microbial community seemed to be highly variable in terms of their species composition, whereas total biomass seemed to be basically determined by the availability of carbon and nutrients.

Acknowledgments

The authors thank for their help: T.Burgmer and the crew of FS Polarstern on sampling in Arctic regions, Dr. J. Matthiessen for assistance at measuring environmental parameters; Dr. W. Wosniock for statistical help; Dr. Gritta Veit-Köhler and Dr. Henning Reiss for the taxonomic determination of the Amphipods; Dr. W. Stumm for reading the proofs. The present work has been financed by the DFG (German Research Community) grant number BE 2279/3-1, by the Alfred-Wegener-Institute for Polar and Marine Research and by the German Social Security System.

References

Aberle-Malzahn, N., 2004. The microphytobenthos and its role in aquatic food webs. Ph.D. Thesis, Christian-Albrechts-Universität Kiel, Germany, unpublished.

Barnes, R.S.K., Hughes, R.N., 1999. An introduction to marine ecology. Blackwell Science Ltd, London, pp286.

Begon, M. E., Harper, J. L., Townsend, C. R. , 1996. Ecology. Oxford, Blackwell Science, pp738.

Beukema, J. J., Flach, E.C., Dekker, R., Starink, M., 1999. A long-term study of the recovery of the macrozoobenthos on large defaunated plots on a tidal flat in the Wadden Sea. J. Sea Res. 42, 235-254.

Bianchi, T.S., Levinton, J.S., 1984. The importance of microalgae, bacteria and particulate organic matter in the somatic growth of *Hydrobia totteni*. J. Mar. Res. 42 431-443.

Biernbaum, C.K., 1979. Influence of sedimentary factors on the distribution of benthic amphipods of Fishers Island Sound. J. Exp. Mar. Biol. Ecol. 38; 201–223.

Blanchard, G.F., Guarini, J.-M., Provot,L., Richard, P., Sauriau P.-G., 2000. Measurement of ingestion rate of *Hydrobia ulvae* (Pennant) on intertidal epipelic microalgae: The effect of mud snail density. J. Exp. Mar. Biol. Ecol. 255, 247-260.

Dittmann, S., 2000. Zonation of benthic communities in a tropical tidal flat of north-east Australia. J. Sea Res. 43, 33-51.

d´Udekem d`Acoz, C., 2004. The genus Bathyporeia Lindström, 1855, in western Europe (Crustacea: Amphipoda: Pontoporeiidae). Zoologische Verhandeling Leiden 348.

Edler, L., 1979. Recommendations for marine biological studies in the Baltic Sea: Phytoplankton and Chlorophyll. The Baltic Marine Biologists 5, 1-37.

Epstein, S.S., 1997. Microbial food webs in marine sediments. I. Trophic interactions and grazing rates in two tidal flat communities. Microb. Ecol. 34, 188-198.

Epstein, S.S., Burkovsky, I.V., Shiaris, M.P., 1992. Ciliate grazing on bacteria, flagellates, and microalgae in a temperate zone sandy tidal flat: ingestion rates and food niche partitioning. J. Exp. Mar. Biol. Ecol. 165, 103-123.

Epstein, S.S., Gallagher, E.D., 1992. Evidence for faciliation and inhibition of ciliate population growth by meiofauna and macrofauna on a temperate zone sandflat. J. Exp. Mar. Biol. Ecol. 155, 27-39.

Fauchald, K., Jumars, P. A., 1979. The diet of worms: a study of polychaete feeding guilds. Oceanography and Marine Biology: an Annual Review 17, 193-284.

Fenchel, T., 1975. The quantitative importance of the benthic microfauna of an Arctic tundra pond. Hydrobiologia 46, 445-464.

Fenchel, T. M., Jorgensen, B. B., 1977. Detritus food chains of aquatic ecosystems: the role of bacteria. Adv. Microb. Ecol. 1, 1-58.

Fenchel, T., 1987. Ecology of Protozoa. The biology of free-living phagotrophic Protists. Science Tech, Wisconsin, pp197.

Fenchel, T.M., Jorgensen, B.B., 1977. Detritus food chains of aquatic ecosystems: the role of bacteria. Adv. Microb. Ecol. 1, 1-58.

Fretwell, S. D., 1977. The regulation of plant communities by food chains exploiting them. Perspec. Biol. Med. 20, 169-185.

Gerdol, V., Hughes, R.G., 1994. Effect of *Corophium volutator* on the abundance of benthic diatoms, bacteria

and sediment stability in two estuaries in southeastern England. Mar. Ecol. Prog. Ser. 114, 109-115.

Gerdol, V., Hughes, R.G., 1994. Feeding behaviour and diet of *Corophium volutator* in an estuary in southeastern England. Mar. Ecol. Prog. Ser. 114, 103-108.

Grossmann, S., Reichardt, W., 1991. Impact of *Arenicola marina* on bacteria in intertidal sediments. Mar. Ecol. Prog. Ser. 77, 85-93.

Hagerthey, S.E., Defew, E.C., Paterson, D.M., 2002. Influence of *Corophium volutator* and *Hydrobia ulvae* on intertidal benthic diatom assemblages under different nutrient and temperature regimes. Mar. Ecol. Prog. Ser. 245, 47-59.

Hargrave, B.T., 1970. The effect of a deposit-feeding Amphipod on the metabolism of benthic microflora. Limnol. Oceanogr. 15, 21-30.

James, M., Hawes, I., Weatherhead, M., Stanger, C., Gibbs, M.. 2000. Carbon flow in the littoral food web of an oligotrophic lake. Hydrobiologia 441, 93-106.

Jensen, K.T., Kristensen L.D., 1990. A field experiment on competition between *Corophium volutator* (Pallas) and *Corophium arenarium* Crawford (Crustacea:Amphipoda): effects on survival, reproduction and recruitment. J. Exp. Mar. Biol. Ecol. 137, 1-24.

Johnson, R.K., Boström, B., van de Bund, W., 1989. Interactions between *Chironomus plumosus* (L.) and the microbial community in surficial sediments of a shallow, eutrphic lake. Limnol. Oceanogr. 34, 992-1003.

Kelly, D.W.; Dick, J.T.A., Montgomery, W.I., 2002. The functional role of Gammarus (Crustacea, Amphipoda): shredders, predators, or both? Hydrobiologia 485 (1-3), 199-203.

Kuipers, B.R., De Wilde, P.A.W.J., Creutzberg, F., 1981. Energy flow in a tidal flat ecosystem. Mar. Ecol. Prog. Ser. 5, 215-221.

Levinton, J.S., 1976. Deposit feeders, their resources, and the study of resource limitaion. Biology of benthic organisms. 11th European Symossium on Marine Biology 117-141.

Levinton, J.S., 1987. The body size-prey size hypothesis and *Hydrobia*. Ecology 68, 229-231.

Levinton, J.S., Bianchi, T.S., 1981. Nutrition and food limitation of deposit-feeders. I. The role of microbes in the growth of mud snails (Hydrobiidae). J. Mar. Res. 39, 531-545.

Lin, J., Hines A.H., 1994. Effects of suspended food availability on the feeding mode and burial depth of the Baltic clam, *Macoma balthica*. Oikos 69, 28-36.

Lopez, G.R., Kofoed, L.H., 1980. Epipsammic browsing and deposit-feeding in mud snails (Hydrobiidae). J. Mar. Res. 38, 585-599.

Lopez, G.R., Levinton, J.S., 1987. Ecology of deposit-feeding animals in marine sediments. The Quarterly Review of Biology 62, 235-260.

López-Figueroa, F., Niell, F.X. 1987. Feeding behavior of *Hydrobia ulvae* (Pennant) in microcosms. J. Exp. Mar. Biol. Ecol. 114, 153-167.

Maclassac, E. A., Stoeckner, J. G., 1993. Enumeration of phototrophic picoplancton by autofluorescence microscopy. In: Handbook of Methods in Aquatic Microbial Ecology. Eds. Kemp, P.F., Sherr, B. F., Sherr, E. B., Cole, J. J., pp. 187-197. Boca Raton, FL, USA.

Menn, I., Armonies, W., 1999. Predatory Promesostoma species (Plathelminthes, Rhabdocoela) in the Wadden Sea. J. Sea Res. 41, 309-320.

Montagna, P.A., 1995. Rates of metazoan meiofaunal microbivory: A review. Vie et milieu Paris 45, 1-9.

Morrisey, D.J., 1988. Differences in the effects of grazing by deposit-feeders *Hydrobia ulvae* Pennant (Gastropoda Prosobranchia) an *Corophium arenarium* Crawford (Amphipoda) on sediment microalgal populations I. Qualitative differences. J. Exp. Mar. Biol. Ecol. 118, 33-42.

Morrisey, D.J., 1988. Differences in the effects of grazing by deposit-feeders *Hydrobia ulvae* Pennant (Gastropoda Prosobranchia) an *Corophium arenarium* Crawford (Amphipoda) on sediment microalgal populations II. Quantitative effects. J. Exp. Mar. Biol. Ecol. 118, 43-54.

Nielsen, M.V., Kofoed, L.H., 1982. Selective feeding and epipsammic browsing by the deposit-feeding amphipod *Corophium volutator*. Mar. Ecol. Prog. Ser. 10, 81-88.

Olafsson, B.E., 1986. Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica* in field experiments. J. Anim. Ecol. 55, 517-526.

Power, M.E., 1992. Top-down and bottom-up forces in food webs: Do plants have primacy. Ecology 73, 733-746.

Reise, K., 1985. Tidal flat ecology. Springer-Verlag, Heidelberg, pp191.

Richards, D.C., Falter, C.M., Myers, R., Lester, G.T., 2005. Native versus invasive Mollusks in Hells Canyon, Snake River, USA. Abstracts for the 4th NZMS Conference.

Sherr, E.B., Sherr, B.F., 1993. Protistan grazing rates via uptake of flourescencly labelled prey. In: Handbook of Methods in Aquatic Microbial Ecology. Eds: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole J.J., Lewis Boca Raton, Florida, USA, pp 695-701.

Yu, O.H., Suh, H.-L., Shirayama, Y., 2003. Feeding ecology of three amphipod species *Synchelidium lenorostralum*, *S. trioostegitum* and *Gitanopsis japonica* in the surf zone of a sandy shore. Mar. Ecol. Prog. Ser. 258, 189–199.

Volkenborn, N., 2005.Ecosystem engineering in intertidal sand bi tue lugworm Arenicola marina. PhD Thesis, University of Bremen.

Volkenborn, N., Hedtkamp, S.I.C., van Beusekom, J.E.E., Reise, K., 2005. Intertidal habitat succession inhibited by lugworm bioturbation. Estuar. Coast. Shelf Sci., submitted.

Volkenborn, N. Reise, K. 2005. Cumulative sediment mediated effects of Arenicola marina on polychaete functional diversity revealed by large-scale experimental lugworm exclusion. submitted

Walters, K., Moriarty, D.J.W., 1993. The effect of complex trophic interactions on a marine microbenthic community. Ecology 74, 1475-1489.

Waterbury, J. B., Watson, S.W., Valois, F.W., Franks,D.G., 1986. Biological and ecological characterization of the marine unicellular cyanobacterium Synechococcus. Canad. Bull. Fish. Aquat. Sci. 214, 71-120.

Wetzel, R.G., 2001. Limnology. Academic Press, London, pp1006.

Wijnsma, G., Wolff, W.J., Meijboom, A., Duiven, P., De Vlas, J., 1999. Species richness and distribution of benthic tidal flat fauna of the Banc d'Arguin, Mauritania. Oceanologica Acta 22, 233-243.

Westheide, R., Rieger, R.M., 2003. Spezielle Zoologie, Bd.1: Einzeller und Wirbellose Tiere. Spektrum Akademischer Verlag, Heidelberg, pp919.

Appendix

Table 10: Abundance of organisms (cells ml^{-1} sediment) with standard deviation (sd) n=3. Phototrophic and heterotrophic nanoflagelltates (PNF and HNF) of different size classes: small = 2 - 5 µm; medium = 5 - 10 µm and larger = >10µm). Abundance at the beginning of the experiment (initial); abundance after 24 h incubation without macrograzer (24h no); abundance after 24 h incubation with *Hydrobia ulvae* (24 h Hyd.); *Macoma balthica* (Mac.); *Corophium* sp. (Coroph.); *Bathyporia* sp. (Bathy.); mixed Polychaets (Poly.); *Arenicola marina* free area near high tide line (G4); near the low tide line (G1); untreated area near the high tide line (O4) near the low tide line (O1); *Potamopyrgus antipodarum* (Pot.); Chironomid larvae (Chiro.); *Gammarus* sp. (Gam.).

Exp.		Bacteria		Small HNF		Medium HNF		Large HNF	
No.		$x10^9$	sd	$x10^6$	sd	$x10^6$	sd	$x10^3$	sd
E1	initial	1.70	0.52	16.10	10.46	0.68	0.27	38.45	45.18
	no	1.75	0.69	22.62	7.19	0.74	0.51	133.96	203.40
	Hyd	1.23	0.61	13.68	14.29	0.50	0.56	26.51	53.02
E2	initial	0.32	0.17	1.12	0.87	0.15	0.14	146.34	49.32
	no	0.21	0.10	1.21	0.26	0.10	0.08	81.20	13.69
	Hyd	0.26	0.05	1.18	0.20	0.11	0.04	146.46	40.14
E3	initial	0.95	0.15	5.32	1.10	0.30	0.27	103.54	179.34
	no	0.70	0.28	5.30	0.86	0.40	0.14	121.39	107.32
	Mac	0.65	0.18	4.87	1.95	0.42	0.25	112.36	50.72
	Coroph	0.67	0.19	4.60	2.52	0.48	0.31	111.86	59.66
E4	initial	0.46	0.13	12.21	1.43	0.53	0.30	0.00	0.00
	no	0.69	0.16	18.71	3.72	0.19	0.09	252.54	236.44
	Coroph	1.12	0.28	35.12	8.82	0.26	0.25	237.10	119.85
	Bathy	1.36	0.15	26.27	5.31	0.37	0.07	206.68	215.70
E5	initial	1.48	0.42	12.53	4.00	0.31	0.17	125.05	142.48
	no	1.16	0.15	14.95	2.10	0.18	0.09	32.86	28.58
	Hyd	1.19	0.38	19.75	5.61	0.42	0.40	194.17	8.65
	Coroph	1.20	0.31	19.21	4.85	0.24	0.05	101.50	45.04
E6	initial	0.75	0.11	4.29	0.87	0.29	0.15	28.33	25.40
	no	0.86	0.05	4.77	0.73	0.24	0.08	19.48	16.93
	Hyd	0.86	0.13	6.56	0.65	0.48	0.06	0.00	0.00
	Poly	0.74	0.03	6.72	0.37	0.36	0.25	0.00	0.00
E7	initial	0.75	0.11	4.29	0.87	0.29	0.15	28.33	25.40
	Ex4	2.91	0.89	9.46	1.16	0.59	0.16	101.49	62.66
E8	O1	0.92	0.51	21.33	7.96	0.14	0.14	0.00	0.00
	Ex1	0.99	0.69	43.07	1.40	0.00	0.00	77.64	109.80
	O4	0.50	0.07	21.30	2.03	0.18	0.08	0.00	0.00
	Ex4	0.76	0.10	22.93	4.82	0.40	0.34	134.40	3.72
E9	initial	1.82	0.09	33.53	8.99	0.68	0.37	89.07	154.27
	no	1.63	0.23	43.50	9.88	0.23	0.21	137.46	238.08
	Pot	1.56	0.14	37.53	1.91	0.18	0.08	45.48	78.77
E10	initial	0.34	0.03	7.00	0.29	0.27	0.03	0.00	0.00
	no	0.45	0.05	6.29	0.81	0.10	0.10	14.05	24.34
	Chiro	0.67	0.43	10.47	7.43	0.13	0.04	14.30	24.76
	Gam	0.45	0.05	5.12	0.31	0.06	0.05	42.93	0.52
E11	initial	2.56	0.20	127.08	14.37	0.66	1.04	114.70	229.40
	no	3.12	0.25	126.67	27.69	0.12	0.24	0.00	0.00
E12	initial	1.21	0.63	42.08	10.64	0.08	0.16	111.32	141.61
E13	initial	0.68	0.25	18.18	4.26	0.11	0.13	89.26	84.15
	no	0.73	0.10	27.52	3.58	0.06	0.11	55.89	71.16
E14	initial	0.87	0.14	45.31	19.43	1.08	0.21	61.60	106.69
	no	0.95	0.19	125.18	21.12	0.81	0.87	0.00	0.00

Exp.		Bacteria		Small HNF		Medium HNF		Large HNF	
No.		x10^9	sd	x10^6	sd	No.		x10^9	sd
E15	initial	0.87	0.14	45.31	19.43	1.08	0.21	61.60	106.69
	no	1.48	0.44	50.76	8.64	0.55	0.47	828.73	812.42
	Chiro	1.61	0.21	60.56	2.20	1.39	0.95	0.00	0.00
E16	initial	1.14	0.26	71.31	19.62	0.41	0.71	0.00	0.00
	no	1.13	0.23	83.65	21.25	1.25	1.01	0.00	0.00
E17	initial	0.30	0.06	17.75	0.94	0.17	0.07	41.28	47.67
	no	0.34	0.06	22.88	7.08	0.14	0.18	82.28	81.73
E18	initial	0.09	0.03	5.10	1.16	0.03	0.02	20.23	23.36
	no	0.11	0.02	7.32	1.71	0.03	0.04	20.65	23.86
E19	initial	0.02	0.00	2.42	0.96	0.13	0.24	0.00	0.00
	no	0.02	0.00	1.27	0.88	0.02	0.05	0.00	0.00

Continuation of Table 10.

Exp.		Small PNF		Medium PNF		Large PNF		Cyano		Diatom	
No.		x10^6	sd	x10^3	sd	x10^3	sd	x10^6	sd	x10^6	sd
E1	initial	0.74	0.31	415.83	232.31	0.00	0.00	1.54	1.73	1.04	0.47
	no	0.60	0.22	206.23	195.77	0.00	0.00	0.18	0.13	0.50	0.32
	Hyd	0.23	0.14	148.08	113.52	26.54	53.08	0.30	0.43	0.43	0.39
E2	initial	0.11	0.12	33.80	30.85	0.00	0.00	0.22	0.25	1.57	1.38
	no	0.16	0.10	86.40	78.73	41.76	38.93	0.00	0.00	0.76	0.17
	Hyd	0.11	0.01	82.67	78.40	73.03	8.72	0.00	0.00	1.18	0.21
E3	initial	0.25	0.23	327.27	125.48	23.44	40.60	0.24	0.22	1.31	0.50
	no	0.30	0.08	189.40	188.76	0.00	0.00	0.20	0.17	0.92	0.22
	Mac	0.39	0.17	169.33	149.39	0.00	0.00	0.54	0.73	0.86	0.32
	Coroph	0.56	0.30	171.97	242.17	59.86	103.67	0.15	0.26	0.58	0.31
E4	initial	0.29	0.16	157.28	113.87	0.00	0.00	5.27	1.37	0.96	0.28
	no	0.26	0.19	29.71	51.46	29.71	51.46	12.81	3.93	1.75	0.38
	Coroph	0.26	0.07	0.00	0.00	0.00	0.00	13.99	13.28	4.41	1.38
	Bathy	0.14	0.14	0.00	0.00	0.00	0.00	20.05	9.29	3.54	0.80
E5	initial	0.24	0.11	303.54	121.91	114.97	50.97	5.04	2.43	1.49	0.24
	no	0.17	0.05	183.93	64.49	15.54	26.91	1.88	1.63	2.41	0.52
	Hyd	0.23	0.18	113.92	103.66	38.72	34.85	2.58	2.92	3.43	2.01
	Coroph	0.40	0.19	175.35	125.58	104.27	121.80	2.78	1.28	1.93	0.33
E6	initial	0.40	0.46	28.42	49.23	40.31	36.73	17.40	17.58	0.62	0.36
	no	0.28	0.03	339.54	215.76	29.67	30.60	29.76	17.95	0.99	0.26
	Hyd	0.18	0.02	151.47	103.52	0.00	0.00	8.88	8.49	1.49	0.37
	Poly	0.16	0.05	154.62	56.50	11.81	20.45	18.89	20.21	1.44	0.36
E7	initial	0.40	0.46	28.42	49.23	40.31	36.73	17.40	17.58	0.62	0.36
	Ex4	0.85	0.22	383.86	198.76	104.21	91.91	23.96	5.70	2.29	0.37
E8	O1	2.33	0.86	619.70	955.93	0.00	0.00	16.16	14.27	1.36	1.42
	Ex1	3.91	0.92	0.00	0.00	156.32	1.48	11.73	0.77	5.47	0.27
	O4	0.46	0.07	0.00	0.00	0.00	0.00	2.68	1.54	22.03	34.48
	Ex4	0.45	0.45	0.00	0.00	43.53	75.40	15.23	4.73	1.57	0.66
E9	initial	1.24	0.79	194.31	114.39	42.21	73.11	23.24	6.28	5.54	3.46
	no	2.26	1.06	134.71	3.38	45.24	78.36	42.55	13.85	6.16	2.24
	Pot	1.99	0.50	90.96	157.55	89.87	77.87	35.82	13.78	5.98	1.56
E10	initial	0.64	0.07	45.79	5.07	63.30	89.51	2.72	1.88	0.95	0.27
	no	0.24	0.09	28.55	24.73	29.17	25.26	3.71	0.44	0.68	0.38
	Chiro	0.56	0.30	42.60	73.79	0.00	0.00	4.97	3.45	1.16	0.69
	Gam	0.19	0.11	71.74	25.45	14.46	25.04	2.58	0.45	0.46	0.26
E11	initial	4.09	1.36	953.60	832.29	365.60	448.75	4.32	1.09	29.18	4.95
	no	2.48	0.83	252.69	505.38	0.00	0.00	8.38	9.42	53.11	6.62

Exp. No.		Small PNF x10^6	sd	Medium PNF x10^3	sd	Large PNF x10^3	No.	Cyano	x10^6	Diatom sd	x10^3
E12	initial	1.47	1.18	37.47	74.93	39.41	78.83	24.54	10.63	19.62	5.56
	no	0.61	0.41	200.83	174.04	104.88	90.89	27.14	10.06	29.22	5.92
E13	initial	0.16	0.19	343.92	399.92	18.06	36.12	5.01	2.17	1.16	0.60
	no	0.50	0.19	130.34	72.36	74.45	61.29	4.70	4.19	1.73	1.56
E14	initial	1.23	1.19	178.77	5.51	0.00	0.00	28.60	13.15	1.71	0.68
	no	1.48	1.11	270.63	468.75	0.00	0.00	27.50	16.59	0.92	1.59
E15	initial	1.23	1.19	178.77	5.51	0.00	0.00	28.60	13.15	1.71	0.68
	no	1.11	0.47	0.00	0.00	0.00	0.00	19.88	9.76	2.46	1.43
	Chiro	1.10	1.27	0.00	0.00	0.00	0.00	1.88	1.44	2.82	2.04
E16	initial	0.82	1.42	0.00	0.00	0.00	0.00	11.50	14.71	3.69	3.27
	no	0.95	1.22	0.00	0.00	0.00	0.00	3.11	4.66	3.43	2.71
E17	initial	0.06	0.08	124.27	80.62	0.00	0.00	6.64	4.90	0.29	0.20
	no	0.31	0.20	55.60	96.30	55.60	96.30	5.87	2.99	0.42	0.29
E18	initial	0.07	0.04	41.29	57.81	20.44	40.87	3.21	1.60	0.46	0.40
	no	0.06	0.04	81.34	46.26	0.00	0.00	2.83	1.01	0.32	0.12
E19	initial	0.07	0.11	16.53	19.10	0.00	0.00	0.01	0.02	0.02	0.02
	no	0.56	1.12	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.06

Table 11: Results of statistical analysis (ANOVA) of all experiments and components. 0: not significant, +: significant increase; -: significant decrease.

Exp. No.	treatment	Bacteria	Small HNF	Medium HNF	Large HNF	Small PNF	Medium PNF	Large PNF	Cyano	Diatoms	Total Biomass
E1	initial:no	0	0	0	0	0	0	0	0	0	0
E1	initial:+Hyd	0	0	0	0	0	0	0	0	0	0
E1	no:Hyd	0	0	0	0	0	0	0	0	0	0
E2	initial:no	0	0	0	0	0	0	0	0	0	0
E2	initial:+Hyd	0	0	0	0	0	0	+	0	0	0
E2	no:Hyd	0	0	0	0	0	0	0	0	0	0
E4	initial:no	0	0	0	+	0	0	0	0	0	+
E4	initial:Coroph	+	+	0	+	0	0	0	0	+	+
E4	initial:Bathy	+	+	0	0	0	-	0	0	+	+
E4	no:Coroph	0	+	0	0	0	-	0	0	+	0
E4	no:Bathy	+	0	0	0	0	0	0	0	0	0
E4	Coroph:Bathy	0	0	0	0	0	0	0	0	0	0
E5	initial:no	0	0	0	0	0	0	0	0	0	0
E5	initial:Hyd	0	0	0	0	0	0	0	0	0	0
E5	initial:Coroph	0	0	0	0	0	0	0	0	0	0
E5	no:Hyd	0	0	0	0	0	0	0	0	0	0
E5	no:Coroph	0	0	0	0	0	0	0	0	0	0
E5	Hyd:Coroph	0	0	0	0	0	0	0	0	0	0
E6	initial:no	0	0	0	0	0	0	0	0	0	0
E6	initial:Poly	0	0	0	0	0	0	0	0	0	0
E6	initial:Hyd	0	0	0	0	0	0	0	0	0	0
E6	no:Poly	0	0	0	0	0	0	0	0	0	0
E6	no:Hyd	0	0	0	0	0	0	0	0	0	0
E6	Hyd:Poly	0	0	0	0	0	0	0	0	0	0
E7	O4:Ex4	+	+	0	0	0	+	0	0	+	+
E8	O1:Ex1	+	0	0	0	0	0	0	0	0	0
E8	O1:O4	-	0	0	0	0	0	0	0	0	0
E8	Ex1:Ex4	-	-	0	0	-	0	0	0	0	0
E8	O(1,4):Ex(1,4)	0	0	0	0	0	0	0	0	0	0
E9	initial:no	0	0	0	0	0	0	0	0	0	0
E9	initial:Pot	0	0	0	0	0	0	0	0	0	0
E9	no:Pot	0	0	0	0	0	0	0	0	0	0
E10	initial:no	0	0	0	0	0	0	0	0	0	0
E10	initial:Chiro	0	0	0	0	0	0	0	0	0	0
E10	initial:Gam	0	0	0	0	0	0	0	0	0	0
E10	no:Chiro	0	0	0	0	0	0	0	0	0	0
E10	no:Gam	0	0	0	0	0	0	0	0	0	0
E10	Chiro:Gam	0	0	0	0	0	0	0	0	0	0
E11	initial:no	+	0	0	0	0	0	0	0	+	0
E12	initial:no	0	+	0	0	0	0	0	0	0	0

Exp. No.	treatment	Bacteria	Small HNF	Medium HNF	Large HNF	Small PNF	Medium PNF	Large PNF	Cyano	Diatoms	Total Biomass
E13	initial:no	0	+	0	0	0	0	0	0	0	0
E14	initial:no	0	0	0	0	0	0	0	0	0	0
E15	initial:no	0	0	0	0	0	0	0	0	0	0
E15	initial:Chiro	0	0	0	0	0	0	0	-	0	0
E15	no:Chiro	0	0	0	0	0	0	0	-	0	0
E16	initial:no	0	0	0	0	0	0	0	0	0	0
E17	initial:no	0	0	0	0	0	0	0	0	0	0
E18	initial:no	0	0	0	0	0	0	0	0	0	0
E19	initial:no	0	0	0	0	0	0	0	0	0	0

Study 5

Control of benthic ciliate community structure and meiofauna by grazers in marine; brackish and freshwater sediments: a cross system comparison

Yanli Lei[1; 3)]; Karen Stumm[2)]; Stephen A. Wickham[3)] and Ulrike-G. Berninger[3)]

[1)] Institute of Oceanology; Chinese Academy of Science; 266071 Qingdao; PR China

[2)] Alfred-Wegener-Institute for Polar and Marine Research. Am Handelshafen 12; 27570 Bremerhaven; Germany

[3)] Universität Salzburg; FB Organismische Biologie; Hellbrunnerstrasse 34; A-5020 Salzburg; Austria

Abstract

Three laboratory and one *in situ* experiments were conducted to investigate potential top-down control of ciliate communities and meiofauna in marine; brackish and freshwater sediments. The laboratory experiments were established by adding some macrometazoans: small snails; mud shrimps; polychaetes; and chironomid larvae. The field experiment manipulated the presence/absence of the polychaete; *Arenicola marina*; from mid and low intertidal mudflats; respectively. The laboratory experiments showed that there were no significant grazing effects of individual macrograzers on neither ciliates nor meiofauna. Ciliate species richness and diversity were significantly increased by grazer addition in the freshwater sediment; and several dominant ciliate species significantly increased or decreased depending on addition of different grazers in the marine and brackish sediments. However; incubation resulted in significant decreases of ciliate abundance and biomass; and meiofauna abundance in the Potsdam Lake experiment. The field experiment revealed that ciliate abundance; biomass; species richness; and diversity were not significantly affected by the exclusion of *Arenicola*. However; the species composition was distinctly changed; and there were functional shifts between carnivorous dominance and omnivorous/herbivorous dominance due to the shifts of dominant species. Meiofauna abundance was significantly enhanced or decreased; depending on sampling dates and sites. Remarkably; with the increase of the dominant carnivorous ciliate biomass; there were tendencies of the decrease of meiofauna abundance; and *vice versa*. As the predacious ciliates were frequently dominant in sediments; they might play an important role in the microbial food web and thus obscure the effects of macrograzer predation on meiofauna and ciliates. Both ciliates and meiofauna were conspicuously influenced by the sampling location (different tidal levels) or by interactions between sampling location and grazer's exclusion; than by *Arenicola* exclusion; they were also influenced by physical disturbance; suggesting the potential nontrophic interactions between biotic and abiotic factors may sometimes play a more important role than a single factor (e.g. predation) in the benthic microbial food web within our study.

1. Introduction

Many protozoa are substrate-associated; also many of those that are commonly found in the plankton. Comparing to planktonic microbial food web studies; limited information is available about the significance of interactions between protozoa and metazoa within benthic microbial assemblages. The primary quantitative studies conducted by Krogh and Spärck (1936) and Mare (1942) revealed a great amount of protozoa present in benthic environments (Carey 1992). Later studies (Fenchel 1967; 1968; 1969; 1975; 1978; Hartwig 1973; 1977) showed that protozoa perform various functional roles in benthic microbial communities. As one of the most important components of the protozoa; benthic ciliates are increasingly attractive for both taxonomic and ecological research because of their rich morphological adaptations; diverse trophic strategies; and overall ecological importance in sediments (Montagana 1984; Patterson et al 1989; Berninger & Epstein 1995; Epstein 1997; Böttcher et al. 2000; Shimeta et al. 2001; Hayward et al. 2003; Wieltschnig et al. 2003; Lei et al. 2005).

A variety of predation–related impacts on benthic ciliate have been documented in laboratory experiments and field *in situ* studies in different habitats. Taylor (1980) observed that oligochaetes significantly reduced the abundance of benthic freshwater ciliates in a laboratory experiment. McCormick (1991) demonstrated that benthic ciliate abundances decreased with elevated densities of snails and mayfly nymphs in a stream; McCormick & Cairns (1991) found further that the addition of micrometazoa (assemblages of rotifers and of rotifers/cladocerans/copepods) resulted in a significant decrease in abundance of benthic freshwater ciliates in a laboratory microcosms. More recently; Wickham et al. (2000) documented that meiobenthos (e.g. ostracods) reduced ciliate abundance and significantly affected ciliate diversity in the 5-10 mm layer of a brackish water sediment. Interestingly; Hillebrand et al. (2002) showed the opposite result: epibenthic ciliate abundance decreased when macrozoobenthos were removed in littoral of a lake and brackish water; thus excluding a direct predation effect of macrozoobenthos on ciliates. These studies suggest that the effects of predators in controlling benthic ciliate and meiofauna are complex; and may often be indirect and unexpected; with both enhancement and suppression possible. On the other hand; Garstecki et al. (2000) found that in contrast to their contribution to zooplankton biomass; heterotrophic protist biomass was much lower than that of meiofauna in a brackish non-tidal inlet sediment; and the quantitative importance of heterotrophic protists differs between the pelagia and the benthos. It is likely that the interaction between protozoa and metazoa; and the actual importance that predation plays in any given community; is dependent on many aspects of the particular community and habitat (Nielsen 2001; Blumenshine & Hambright 2003).

Many factors have been postulated to affect potential ciliate and meiofauna responses to larger predators; such as life history of resident species; food limitation; biotic and physical disturbance; and the community composition (McCormick & Cairns 1991; Coull 1999; Posey et al. 1999; Kneitel & Chase 2004). Further; the feeding typs; and therefore the ecological functions of ciliates are extremely diverse; including bacterivores; algivores; carnivores; omnivores and histophages etc. Furthermore; some ciliates are able to prey on metazoans (Sanders & Wickham 1993; author's personal observations). Such complexities are often overlooked in ecological studies when only quantitative aspects are examined. Accordingly; good taxonomic resolution is required in order to obtain insights into the complexity of the benthic microbial food web (Hausmann et al. 2002). However; few studies have been conducted on the role of macrograzers in regulating benthic ciliate community structures in sediments (Wickham et al. 2004).

To address the above questions; we conducted a series of laboratory and field *in situ* experiments by adding or excluding certain groups of macrograzers to investigate their potential top-down control of ciliates and meiofauna in sediments. Experiments were carried out through two complementary approaches: quantitative studies of meiofauna and protozoa; among which ciliates were taxonomically studied as well. These studies had three primary objectives: 1) to detect whether benthic ciliates and meiofauna exhibit significant responses to addition and exclusion of small macrofauna in laboratory and field environments; 2) to test; besides predation effects; whether other factors exert influence on ciliates and meiofauna; such as different intertidal areas and physical disturbance; and 3) to determine whether ciliate diversity and species composition; together with changes in the dominant species; will be altered by experimental manipulations.

2. Material and Methods

Study sites and sampling

The samples for the three laboratory experiments and the field experiment originated from a marine sediment of Sylt; a brackish sediment of Dorum; and a freshwater sediment of Potsdam Lake; respectively. The field (*Arenicola*) experiment was conducted in Sylt; an island in the North of Germany (55° 02' N; 8° 25' E); where *Arenicola marina* (Polychaeta) was very abundant. The tides change around every 6 h with water difference of 3 m. Dorum is located near Bremerhaven; German costal of the North Sea (53° 42' N; 8° 29' E). The salinity of the sediment was usually influenced by a wind-induced water tongue from the nearby Weser River; and varies from 10 PSU to 31 PSU. Potsdam Lake is a shallow lake in the North East of Greenland (75° 03.48' N; 18° 45.86' W). The maximal depth of the lake is 0.7 m. Detailed information on the sampling sites are shown in Table 1; Study 2 and 3.

The marine and brackish sediments were sampled around low tide. For Sylt and Dorum experiments; sampling was conducted around 500 m from the shoreline; for the *Arenicola* field experiment sampling was done at the mid and low intertidal flat. The sediments were sampled by using round plexiglass cores (36 mm inner diameter) inserted about 50 mm into the sediment. The cores were stoppered at the top; extracted; and then stoppered at the bottom. For the Potsdam Lake experiment samples were collected by carefully removing an undisturbed section of the sediment with a shovel. The samples were stored in cooling boxes and transported to the laboratory immediately. In the laboratory the upper 3 mm layer (within the oxic zone) of each sediment was sliced off and transferred into tubes; where samples were slightly diluted with 2 ml filtrated (0.2 µm) seawater originating from sampling sites and immediately fixed in 2% ice-cold glutaraldehyde (f.c. 2%). For laboratory experiments; further 2-3 ml of the sediments were transferred into cellwells (36 mm inner diameter and 1.7 cm depth) and incubated. To stop the experiment the samples were fixed with ice cold glutaraldehyde and transferred with 20 ml of artificial seawater into tubes and fixed again.

Table 1: Basic sediment information of the three sampling sites. Note the samples were taken from 3 mm upper layers of sediments.

Sites	Date	Salinity (psu)	Habitats	Temperature (°C)	Silt and clay contents (<63µm)	Location
Sylt	12.09.2002	31	Marine tidal flat	19	1%	55º 02' N; 08º 25' E
Dorum	22.10.2002	15	Brackish tidal flat	10	10%	53º 42' N; 08º 29' E
Potsdam Lake	26.08.2003	4	Freshwater sediment	7	40%	75º 03' N; 18º 46' W

Table 2: Laboratory experimental design.

Experiments	Macrograzers	Densities of macrograzers	Incubation times	Treatments with Macrograzers	Treatments without Macrograzers
Sylt	*Hydrobia ulvae* Mixed polychaetes	2 ind. ml^{-1} 2 ind. ml^{-1}	t_1 = 30 min t_2 = 1 d	t_1H; t_2H t1P; t_2P	t_0N; t_1N; t_2N
Dorum	*Hydrobia ulvae* *Corophium volutator*	2 ind. ml^{-1} 2 ind. ml^{-1}	t_1 = 30 min t_2 = 1 d	t_1H; t_2H t_1C; t_2C	t_0N; t_1N; t_2N
Potsdam Lake	Chironomid larvae	1 ind. ml^{-1}	t_3 = 5 d	t_3CHL	t_0N; t_3N

Laboratory (Sylt; Dorum and Potsdam Lake) experimental design

The laboratory experiments were established to test the grazing impacts on benthic ciliate and meiofauna by certain small macrofauna originating from each sampling site (Table 2): a small snail *Hydrobia ulvae*; a little mud shrimp *Corophium volutator*; mixed polychaetes and chironomid larvae. The Sylt and Dorum laboratory experimental design was the cross-classified manipulation of 3 levels of grazers and 2 lengths of incubation; with 3 replicates per treatment combination. In the Sylt laboratory experiment (Table 2) four individuals of *Hydrobia ulvae* and 5 individuals of the mixed polychaete assemblage were used in each treatment; giving a density of macrograzers about 2 ind. ml^{-1}. Incubations lasted 30 min (t_1) or 1 d (t_2). The cellwells were placed in an environmental chamber at 20 °C and illuminated from above on a 14:10 h light:dark cycle. In the Dorum laboratory experiment (Table 2) four individuals of *Hydrobia ulvae* and *Corophium* cf *volutator*; respectively; were added to each treatment; giving a macrograzers density of about 2 ind. ml^{-1}; and incubated 30 min or 1 d. The cellwells were placed in an environmental chamber at 10 °C and illuminated from above on a 14:10 h light:dark cycle. In the Potsdam Lake laboratory experiment (Table 2) three individuals of chironomid larvae were added to each treatment; giving a density of macrograzers of about 1 ind. ml^{-1}; and incubated for 5 days (t_3). Thus; only 2 levels of grazers and 1 level of incubation were involved in this experiment; with 3 replicates per treatment. The cellwells were placed in an environmental chamber at 4 °C and illuminated from above on a 24: 0 h light:dark cycle; corresponding to the field *in situ* condition.

The following abbreviations are used in the three laboratory experiments (Table 2): t_0N; samples at initial time (without macrograzers); t_1N; t_2N and t_3N; the samples were incubated for 30 min; 1 d and 5 d; respectively; without macrograzers addition; t_1C; t_1H and t_1P; samples were incubated for 30 min with the addition of *Corophium volutator* (C); *Hydrobia ulvae* (H) and mixed polychaetes (P); respectively; t_2C; t_2H and t_2P; samples were incubated for 1 d with the addition of *Corophium volutator* (C); *Hydrobia ulvae* (H) and mixed polychaetes (P); respectively; t_3CHL is sample was incubated for 5 d with the addition of chironomid larvae.

Arenicola field experiment

The *Arenicola* field experiment was conducted to test the effects of exclusion of macrograzers (*Arenicola*) and physical disturbance (dredging event) on benthic ciliates and meiofauna; simultaneously; to test whether there were interactions between experimental manipulations and study sites. The experiment was carried out from March 2002 to July 2003 at an intertidal mudflat; and two studies sites; the low and mid intertidal areas were selected (Fig. 1). The distance between two areas is about 130 m. A lugworm; *Arenicola marina* (Polychaeta) was abundant at both areas (Volkenborn's personal observations). Further detailed information on preparation and environmental position is shown in Volkenborn et al. (2005).

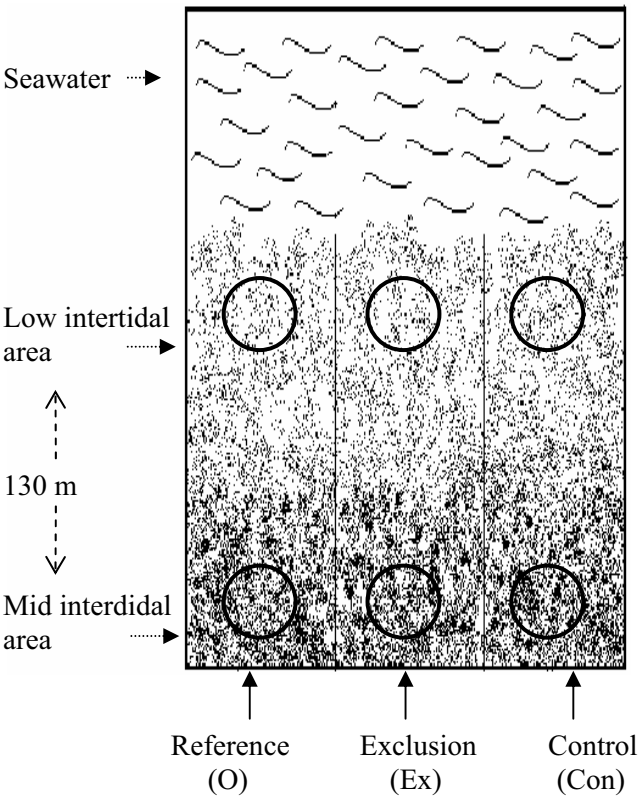


Fig. 1: Schematic drawing of *Arenicola* field experimental design. The experiment was conducted an intertidal mudflat of Sylt from March 2002 to July 2003; with a factorial combination of 2 levels of sampling areas (low and mid intertidal areas) and 3 levels of experimental manipulations (Reference; Exclusion and Control). The low and mid intertidal areas are flooded 9-10 h; respectively; 6-7 h per day; and the distance between the two areas is about 130 m. The three experimental treatments were conducted at each area: Reference treatment; where the sediment was not manipulated and *Arenicola* was present at natural densities; Exclusion treatment; where the sediment was dredged and *Arenicola* was removed by sieving; Control treatment; where the sediment was dredged but *Arenicola* was still remained.

Arenicola is a deposit-feeder dwelling in J- or U-shaped burrows down to a sediment depth up to 0.4 m.; and has a 1- to 2-year old generation time. The lugworm is an intense bioturbator ingesting sediment at a rate several times its own body weight per day (Flach & Beukema 1994; Timmermann & Andersen 2003).

Three experimental treatments were conducted at each study area (Fig. 1). The first is the Reference treatment ("O"); the sediment was not manipulated and *Arenicola* was present at natural densities. The second is the Exclusion treatment ("Ex"); where the sediment was dug by dredge and *Arenicola* was sieved out by a mesh; simultaneously; a 1 mm mesh was inserted horizontally into 10 cm depth of the sediment. This procedure was aimed to vertically block the borrow channels of the lug worms and thus prevent immigrations. This treatment site was not found to be resettled by adult *Arenicola* during the experimental period (Volkenborn's personal observations). The third is the Control treatment ("Con"); which was created to test for effects of the dredging. In this treatment the sediment was only dredged but without using the mesh. The manipulations of the Exclusion and the Control treatments were done within two months; that is; from early March to late April; 2002.

The experiment was conducted with a factorial combination of 2 levels of sampling areas (mid and low intertidal areas) and 3 levels of experimental manipulations (Reference: Ref; Exclusion: Ex and Control: O); with 3 replicates per treatment (Fig. 1; Table 3). Two samplings were carried out on Sep 11; 2002 (the first sampling) and on July 1; 2003 (the second sampling); respectively; that is; about 6 months; respectively; and 16 months after the experiment was manipulated. In the first sampling we sampled only at the mid intertidal area for the Reference and the Exclusion treatments; thus data were unavailable on the Control treatment at this area; and on all treatments at the low intertidal area. In the second sampling all three treatments at both areas were included (Table 3).

Table 3: *Arenicola* field experimental design; and ciliate dominant species; feeding types; and biomass and abundance contributions in each treatment.

Sampling date	Area	Treatments	Dominant species	Feeding types[b]	Biomass (%)	Abundance (%)
Sep 11; 2002 (1st sampling[a])	**Mid** intertidal	Exclusion	*Condylostoma arenarium*	Carnivorous	51	10
		Reference	*Condylostoma arenarium*	Carnivorous	24	2
			Coleps sp.	Omnivorous	21	49
		Control	nd	nd	nd	nd
Jul 1; 2003 (2nd sampling)	**Mid** intertidal	Exclusion	*Coleps* sp.	Omnivorous	22	16
			Kerona sp.	Omnivorous	18	10
		Reference	*Trachelocerca* sp.	Carnivorous	46	3
		Control	*Trachelocerca* sp.	Carnivorous	42	2
Jul 1; 2003 (2nd sampling)	**Low** intertidal	Exclusion	*Trachelocerca* sp.	Carnivorous	28	2
			Condylostoma arenarium	Carnivorous	18	1
		Reference	*Aspidisca fusca*	Omnivorous	24	15
			Frontonia marina	Herbivorous	17	1
		Control	*Prorodon morgani*	Herbivorous	23	1
			Aspidisca fusca	Omnivorous	18	15

[a] in the first sampling data were unavailable on Control treatment at mid intertidal area and treatments at low intertidal area. nd; no data.
[b] Feeding types of dominant species of ciliates were mainly determined by our observations; that is; food vacuoles contents in protargol impregnated specimens and/or live cells.

Extraction; staining and analysis

The protozoa and meiofauna were separated from the sediments by using centrifugation in silicagel (Percoll®) gradients (Epstein 1995; Starink et al. 1994). The Percoll gradients were prepared after Alongi (1986); Epstein (1995) and Burgess (2001) with minor modifications. Briefly; 6 ml of 50% Percoll solution in twice concentrated artificial seawater were placed into centrifuge tubes; 2 ml of 100% Percoll solution were gently injected into the 50% Percoll solution from the bottom of the tubes; and then the stacked Percoll gradient was obtained. 1 ml to 3 ml of the sediment sample was placed on the top of the gradient and centrifuged at 4°C for 15 min at 3026 g in an swing out rotor. The samples were gently (< 5 mm Hg) filtered onto 1.2 µm pore-size cellulose nitrate filters and impregnated by the Quantitative Protargol Stain (QPS) method (Montagnes & Lynn 1993; Skibbe 1994). Using this method; ciliate's infraciliature and nuclear apparatus were revealed. Combining live observation and QPS; ciliates could be identified generally to genus level; and almost half to species level. In general; the filters were counted in their entirety and at least 20 ciliates were counted; rarely half of the filters were counted when the ciliates were too abundant; for instance; more than 500 cells per filter. Ciliates were identified using relevant literature, for example, Kahl (1930-1935), Carey (1992), Foissner et al. (1999), Lynn and Small (2002), etc. Measurements were performed during counting. Ciliate biovolume was estimated using the measured lengths and

widths and common geometric equations. Biovolume was converted to carbon content; allowing for shrinking due to fixation; using a conversion factor of 0.14 pg C μm^{-3} (Putt and Stoecker 1989). Species diversity was measured using the Shannon-Wiener index (*H'*). This is calculated as $H' = - \Sigma p_i \times \ln (p_i)$ where p_i is the proportion of the total ciliate abundance made up by species *i*. The maximum diversity occurs when each species has the same proportion of the total abundance; and is equal to the natural logarithm of the species richness (the total number of species). Similarity in community composition between two study areas or between treatments (*Arenicola* field experiment) was measured using the Jaccard measure of similarity. This is measured as: $C_j = j / (a + b - j)$ where *a* and *b* are the number of morphotypes in samples A and B; and *j* is the number of morphotypes found in both A and B (Magurran 1988). C_j ranges from 0 to 1; and is the proportion of shared morphotypes. Those ciliate species which occupied beyond 15% of biomass or abundance of total ciliates; were regarded as dominant species. Feeding types of dominant species of ciliates were mainly determined by our observations; for example; contents of food vacuoles in protargol impregnated specimens and/or live cells. Meiofauna were identified to group level using Giere (1993).

Statistical Analysis

For laboratory experimental analysis; incubation was included as a factor in a 2-way factorial ANOVA (Grazer × Incubation) in Dorum and Sylt experiments. Two-sample t-Test was employed to test grazing effects of grazers and incubation; respectively; in Potsdam Lake experiment. For field experimental analysis study site was included as a factor in the 2-way factorial ANOVA (Grazer × Area). Abundance; biomass and richness data were log-transformed prior to analysis; in order to equalize variance between treatments; but untransformed data are present in the figures. Because the data for ciliates were employed in 5 separate analyses (abundance; biomass; species richness; diversity and dominant species of each community); the α-level used for these tests was adjusted from 0.05 to 0.01; effects with a p-value between 0.01 and 0.05 were considered marginally significant. However; the α-level for meiofauna testing remained 0.05 because the data were used only once. Pearson correlation analyses were carried out to test relationship between log-transformed abundances of ciliates and meiofauna. All data analysis was carried out using SAS; 6.12.

3. Results

Laboratory (Sylt; Dorum and Potsdam Lake) experiments

Sylt experiment

A total 51 ciliate species were observed in this experiment. Ciliate species richness ranged from 8 to 12; and diversity from 1.3 to 2.2. They were both highest in t_2N treatment; and lowest in t_0N treatment (Fig. 2a). Ciliate abundance varied between 390 and 677 ind. ml^{-1}; and biomass between 1.57 and 7.38 μg C ml^{-1}. They were both lowest in t_0N treatment; and highest in t_1P treatment (Fig. 2b). The ciliate community was dominated by a large (ca. 500 μm long); carnivorous heterotrich *Condylostoma arenarium* contributing on average 45% of the total biomass. In contrast; two small (50-60 μm long) ciliates; a herbivorous *Peritromus californicus* and a omnivorous *Coleps* sp. occupied most of the abundance (Fig. 2c).

The entire meiofauna abundance varied between 186 ind. ml^{-1} (t_1P treatment) and 386 ind. ml^{-1} (t_2H treatment). Numerically; copepods were the predominant meiofaunal group; and their abundance ranged from 172 ind. ml^{-1} to 366 ind. ml^{-1}. In addition; few nematodes were observed; and their abundance varied between 0 ind. ml^{-1} and 20 ind. ml^{-1} (Fig. 2d).

There were no significant effects for either grazer addition or grazer × incubation interaction on ciliates (species richness and diversity; and abundance and biomass; and dominant species) and meiofauna (abundance) in this experiment (Table 4). Only a dominant ciliate; *Peritromus californicus* was marginally significantly reduced by *Hydrobia ulvae* while enhanced by addition of polychaetes (Fig. 2c; Table 4).

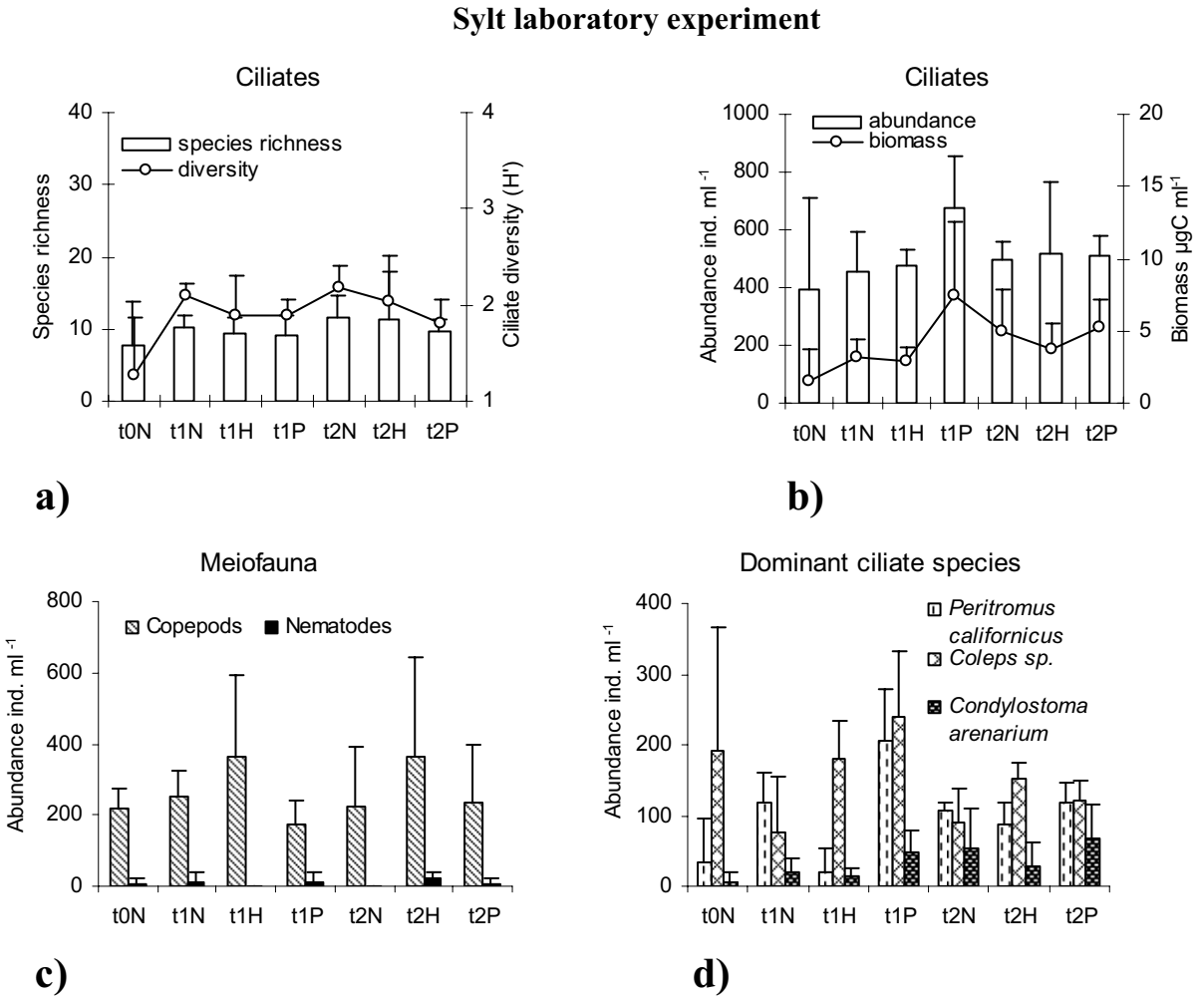


Fig. 2: Ciliate species richness and Shannon-Wiener diversity (a); abundance and biomass (b) and abundance of dominant species (**c**); and abundances of main groups of meiofauna (**d**) in the Sylt laboratory experiment. t_0N; sample at initial time (without macrograzers); t_1N; t_1H and t_1P are; respectively; the samples were incubated 30 min without macrograzers (N); with addition of *Hydrobia ulvae* (H) and mixed polychaetes (P); t_2N; t_2H and t_2P are; respectively; the samples were incubated 1 d without macrograzers (N); with addition of *Hydrobia ulvae* (H) and mixed polychaetes (P). Error bars are 1 SD of the means of untransformed data; but for the statistical analyses; log-transformed data were used.

Table 4: p-values for the three laboratory (Sylt; Dorum and Potsdam Lake) experiments.

Sylt experiment	Grazer	Incubation	Grazer × Incubation
Ciliates			
Abundance	0.3368	0.8086	0.4571
Biomass	0.2336	0.8942	0.3984
Speciess richness	0.5707	0.5183	0.7499
Diversity	0.3574	0.3702	0.9532
Peritromus californicus	*0.0231*	*0.0256*	*0.0418*
Colep sp.	0.1604	0.4254	0.7197
Condylostoma arenarium	0.3386	0.8934	0.7852
Meiofauna (abundance)			
Copepods	0.7778	0.9126	0.6643
Nematodes	0.6656	0.6287	0.4444
Dorum experiment	Grazer	Incubation	Grazer × Incubation
Ciliates			
Abundance	0.0759	0.3239	0.1273
Biomass	0.0519	0.9947	0.6271
Speciess richness	0.2066	0.2044	0.2362
Diversity	0.6309	0.1338	0.0847
Chlamydodon cyclops	0.1633	0.6803	0.1597
Aspidisca lyncaster	*0.0469*	*0.0115*	0.2869
Discotricha pappillifera	0.8197	0.1621	0.2773
Meiofauna (abundance)			
Copepods	0.3262	0.8960	0.1416
Nematodes	0.1408	0.2784	0.1706
Tardigrades	0.8254	0.3936	0.9080
Potsdam Lake experiment	Grazer	Incubation	Grazer × Incubation
Ciliates			nd
Abundance	0.5392	**0.0001**	nd
Biomass	0.2473	**0.0002**	nd
Speciess richness	*0.0391*	0.0589	nd
Diversity	*0.0363*	0.4198	nd
Frontonia cf. *caneti*	0.7215	**0.0005**	nd
Aspidisca sp.	0.3919	0.2899	nd
Urosylida g.sp.1	0.4473	**0.0004**	nd
Meiofauna (abundance)			nd
Nematodes	0.4864	*0.0507*	nd
Rotifers	0.9216	**0.0386**	nd
Tardigrades	0.8304	**0.0099**	nd

Notes: Ciliate abundance; biomass and species richness were log-transformed prior to analysis. Because the same data for ciliates were test in 5 separate analyses (abundance; biomass; species richness; diversity and dominant species); the α-level used for these testes was adjusted from 0.05 to 0.01; and is marked in bold; effects with a p-value between 0.01 and 0.05 were considered as marginally significant trends; and are marked in italics. The data used for meiofauna analyses were only employed once; thus α-level remained 0.05 and is marked in bold; effects with a p-level between 0.05 and 0.1 was considered as marginally significant trends; and is marked in italics. nd; not determined.

Dorum experiment

A total of 50 ciliate species were observed in this experiment. Species richness ranged between 21 and 29; and diversity between 2.4 and 2.7. They were both highest in t_2C treatment; while species richness was lowest in t_1N treatment; and diversity was lowest in t_0N treatment (Fig. 3a). Ciliate abundance varied between 122 and 269 ind. ml^{-1}; and biomass between 0.50 and 1.13 µg C ml^{-1}. They were both lowest in t_1N treatment; and highest in t_1C treatment (Fig. 3b). The ciliate community was dominated by a middle-sized (ca. 100 µm long) herbivorous species (mainly feeding on large diatoms); *Clamydodon cyclops*; which contributed to on average 45% of biomass across all treatments. In addition; two small (30-40 µm long) ciliates; an omnivorous *Aspidisca lyncaster* and a herbivorous *Discotricha papillifera* were also abundant (Fig. 3c).

The entire meiofauna abundance ranged from 23 ind. ml^{-1} (t_1N treatment) to 69 ind. ml^{-1} (t_1H treatment). Copepods were the most abundant meiofaunal group; followed by nematodes and tardigrades (Fig. 3d). The abundance of copepods varied between 15 and 57 ind. ml^{-1}. Nematode abundance varied between 6 and 23 ind. ml^{-1}; while the abundance of tardigrades was very low; ranging from 1 to 4 ind. ml^{-1} (Fig. 3d).

There were also no significant effects for either grazer addition or grazer × incubation interaction on ciliates (species richness and diversity; and abundance and biomass; and dominant species) and meiofauna (abundance) in this experiment (Table 4). However; there was a nonsignificant trend toward higher ciliate biomass with adding of both grazers; especially for the dominant species; *Aspidisca lyncaster*; which was marginally significantly increased (Fig. 3c; Table 4). But there was no constant trend on meiofauna (Fig. 3d). In addition; significantly positive correlations between nematode abundance and ciliate abundance were observed (Table 5).

Table 5: Correlations (Pearon's *r* values) between ciliate abundance and meiofauna abundance in the three laboratory (Sylt; Dorum and Potsdam Lake) experiments.

Laboratory experiments	Copepods	Nematodes	Tardigrades	Ostracods	Rotifers
Sylt experiment	-0.1837 (p 0.4253)	nd	nd	nd	nd
Dorum experiment	0.0907 (p 0.6958)	0.4577 (p **0.0369**)	nd	nd	nd
Potsdam Lake experiment	nd	0.8746 (p **0.0020**)	0.8892 (p **0.0013**)	nd	0.8452 (p **0.0041**)

Notes: The data used for the correlation tests were only employed once; thus the α-level was 0.05; and is marked in bold. nd; not determined.

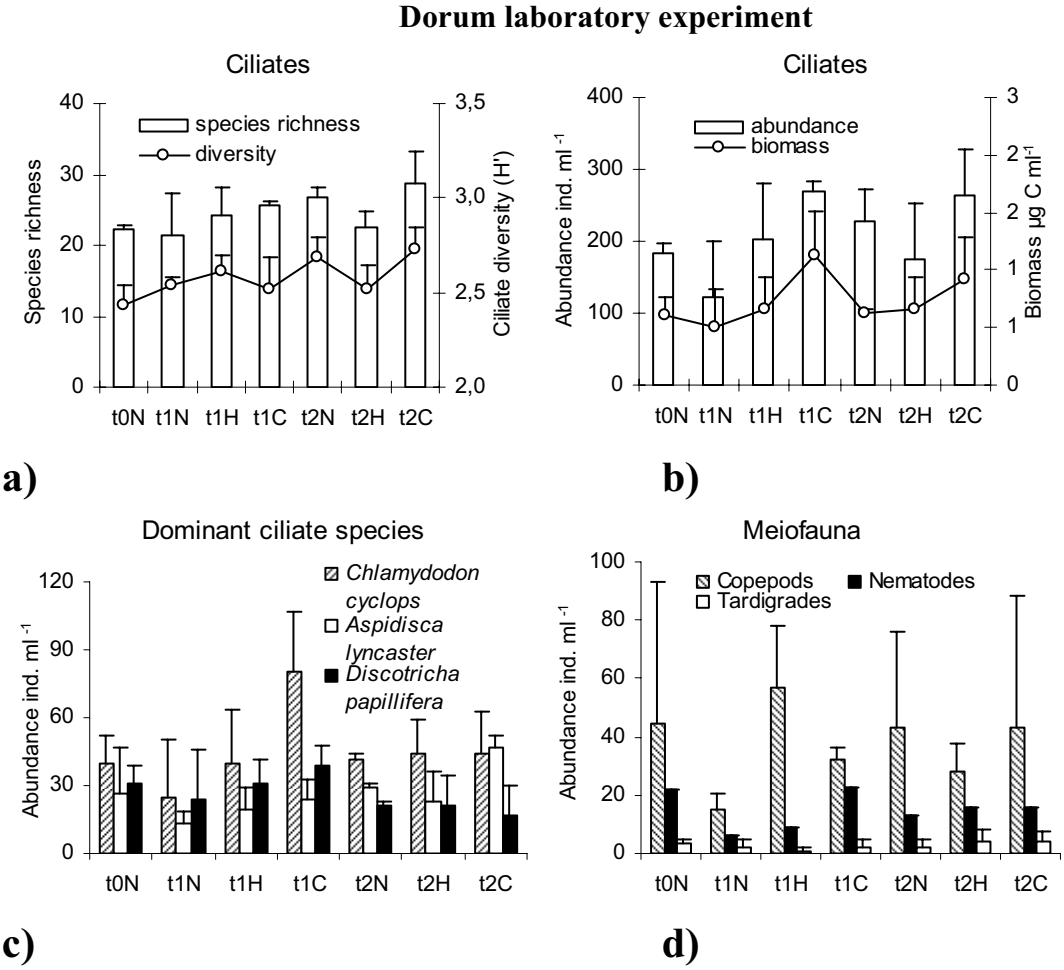


Fig. 3: Species richness and Shannon-Wiener diversity (**a**); total abundance and biomass (**b**) and abundances of dominant species of ciliate (**c**); and abundances of main groups of meiofauna (**d**) in the Dorum laboratory experiment. t_0N; sample at initial time (without macrograzers); t_1N; t_1H and t_1C are; respectively; the samples were incubated 30 min without macrograzers (N); with addition of *Hydrobia ulvae* (H) and *Corophium volutator* (C); t_2N; t_2H and t_2C are; respectively; the samples were incubated 1 d without macrograzers (N); with addition of *Hydrobia ulvae* (H) and *Corophium volutator* (C). Error bars are 1 SD of the means of untransformed data; but for the statistical analyses; log-transformed data were used.

Potsdam Lake experiment

A total of 26 ciliate species were observed in this experiment. Ciliate species richness ranged between 10 and 14; and diversity between 1.5 and 1.9; and they were both highest in t_3CHL treatment (Fig. 4a). Ciliate abundance varied between 64 and 121 ind. ml^{-1}; and biomass between 0.11 and 0.15 µg C ml^{-1}; and they were both highest in t_0N treatment (Fig. 4b). The ciliate community was dominated by a herbivorous species; *Frontonia* cf. *caneti* (on average 49% biomass contribution); while a bacterivorous stichotrichous ciliate (Urostylida gen. sp.) was most abundant (57% abundance contribution) (Fig. 4c).

The entire meiofauna abundance varied from 27 ind. ml^{-1} (t_3N treatment) to 174 ind. ml^{-1} (t_0N treatment). Rotifers were the most abundant meiofaunal group in this experiment; followed by nematodes and tardigrades (Fig. 4d). Mean abundance of the three groups was

41 ind. ml^{-1}; 35 ind. ml^{-1} and 4 ind. ml^{-1}; respectively. The abundances of all meiofunal groups were highest in the initial treatment (Fig. 4d).

There were also no significant effects of grazer addition on either ciliates (species richness and diversity; and abundance and biomass; and dominant species) or meiofauna (abundance) in this experiment (Table 4). However; ciliate species richness and diversity were marginally significantly increased by grazer addition (Fig. 4a). In contrast; incubation resulted in significant decreases of ciliate abundance; biomass and dominant species; and abundance of meiofauna (rotifers and tardigrades) (Fig. 4b; d; Table 4). In addition; significantly positive correlations between meiofauna (rotifers; nematodes and tardigrades) abundance and ciliate abundance were observed (Table 5).

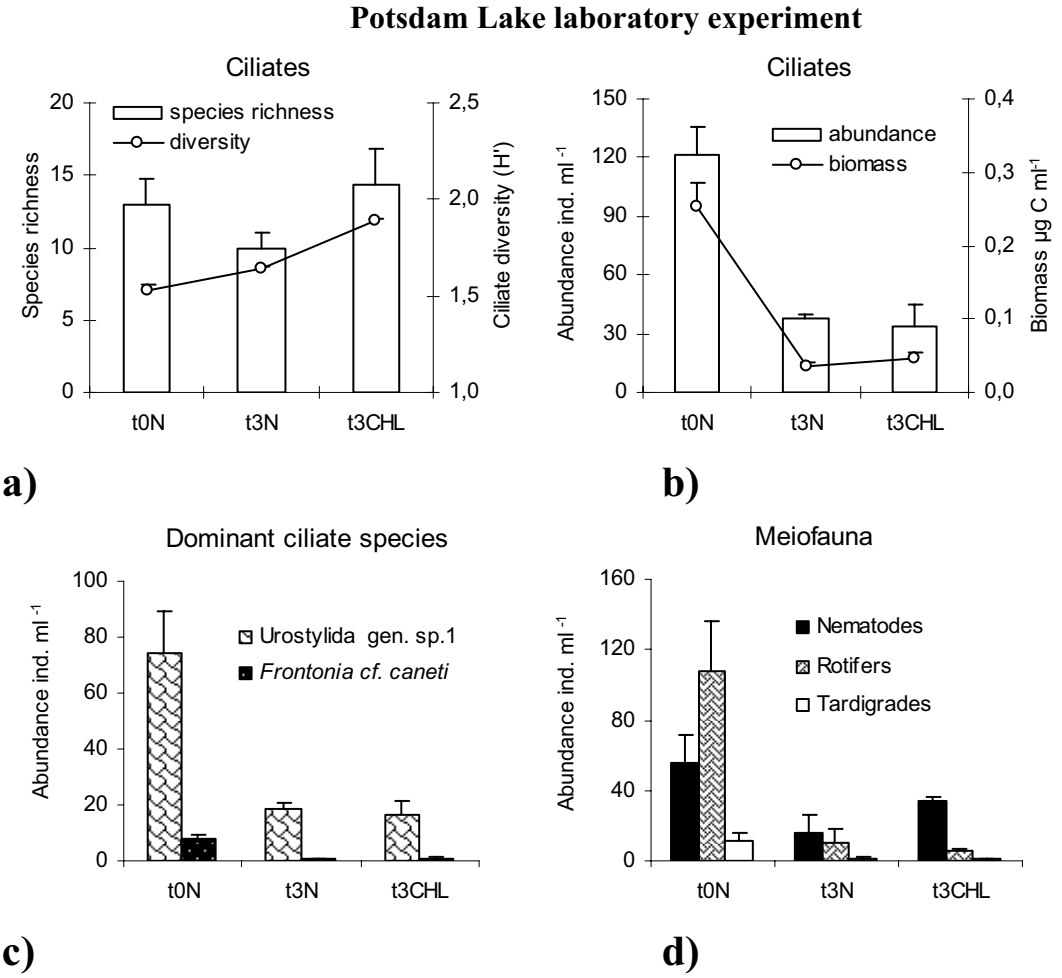


Fig. 4: Species richness and Shannon-Wiener diversity (a); total abundance and biomass (b) and abundances of dominant species of ciliate (c) and abundances of main groups of meiofauna (d) in the Potsdam Lake laboratory experiment. t_0N; sample at initial time (without macrograzers); t_3N; the sample was incubated 5 d without macrograzers; t_3CHL; the sample was incubated 5 d with addition of chironomid larvae. Error bars are 1 SD of the means of untransformed data; but for the statistical analyses; log-transformed data were used.

***Arenicola* field experiment**

The first sampling at mid intertidal area

A total of 50 ciliate species were observed in this sampling. Ciliate species richness varied between 8 and 31; and diversity between 1.2 and 2.8. Ciliate abundance ranged from 390 ind. ml^{-1} to 881 ind. ml^{-1}; and biomass from 1.57 μg C ml^{-1} to 9.99 μg C ml^{-1} (Fig. 5a; b). The ciliate community in the Reference treatment was dominated by a small omnivorous *Coleps* sp (ca. 50 μm in length) and a large (ca. 500 μm long) carnivorous herterotrich; *Condylostoma arenarium* (Fig. 5c; Table 3). In the Exclusion treatment; only *C. arenarium* dominated the ciliate community both in terms of abundance and biomass (Fig. 5c; d; Table 3); in addition; *Frontonia marina*; *Clamydodon triquetrus*; *Peritromus californicus*; and *Coleps* sp. were also abundant in this treatment (Fig. 5c). There was relatively little overlap in the species composition of ciliate communites between the Reference and the Exclusion treatments; that is; only 32% of the taxa were shared (Table 7). The entire meiofauna abundance in the Exclusion treatment was 108 ind. ml^{-1}; which was only half of that in the Reference treatment (229 ind. ml^{-1}). Copepods were the predominant meiofaunal group; and their abundance in the Reference treatment was much higher than that in the Exclusion treatment (Fig. 5e). In addition; nematodes and ostracods were also observed but in low numbers (Fig. 5e).

Statistical analyses showed that there were no significant effects of *Arenicola* exclusion on either ciliates (species richness and diversity; abundance and biomass) or meiofauna (abundance); though ciliate abundance and biomass were much higher in the *Arenicola* exclusion treatment than those in the *Arenicola* presence treatment; and there was a reversed situation for meiofauna abundance (Fig. 5a-e; Table 6). However; ciliate species richness and diversity; and the dominant species; *Condylostoma arenarium*; were marginally significantly increased by *Arenicola* exclusion (Fig. 5a; d; Table 3; 6).

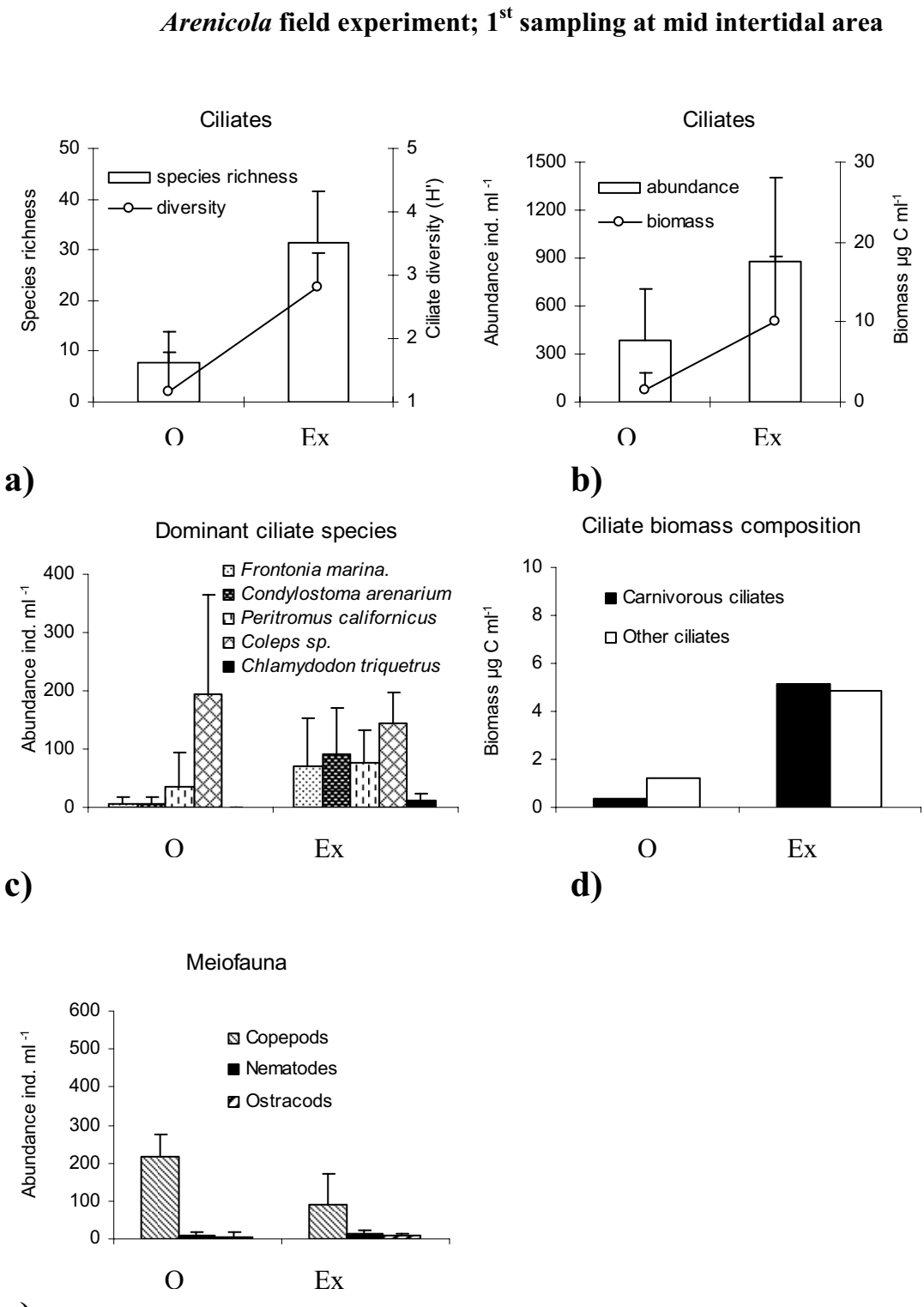


Fig. 5: Ciliate species richness and Shannon-Wiener diversity (a); abundance and biomass (b); abundance of dominant species (c); biomass composition grouped by carnivorous (*Condylostoma arenarium*) and other ciliates (d); and meiofauna abundance (e) in the *Arenicola* field experiment in the first sampling (Sep 11; 2002) at the mid intertidal area. O; Reference treatment; where the sediment was not manipulated and *Arenicola* was present at natural densities; EX; Exclusion treatment; where sediment was dredged and *Arenicola* was removed by sieving. Error bars are 1 SD of the means of untransformed data; but for the statistical analyses; log-transformed data were used.

Table 6: *p*-values for the *Arenicola* field experiment.

	Grazer [a] (1st sampling)	**Grazer** [a] (2nd sampling)	**Area** [b] (2nd sampling)	**Grazer** [a] **× Area** [b] (2nd sampling)
Ciliates				
Abundance	0.2514	0.0985	**0.0002**	*0.0109*
Biomass	0.0982	0.0785	**0.0022**	0.7191
Speciess richness	*0.0444*	*0.0460*	0.3197	**0.0063**
Diversity	*0.0325*	**0.0068**	0.8122	**0.0017**
Frontonia marina	0.0845	0.9790	0.4995	0.4692
Condylostoma arenarium	*0.0456*	0.3966	0.3370	0.3966
Mesodinium pupula	0.1227	0.5894	**0.0041**	**0.0001**
Peritromus californicus	0.1926	*0.0103*	**0.0038**	*0.0103*
Coleps sp.	0.5818	*0.0408*	**0.0001**	0.2751
Chlamydonellopsis sp.	0.6472	**0.0001**	**0.0001**	**0.0001**
Microdysteria decora	0.2969	0.1700	**0.0005**	0.0748
Trachelocerca sp.	nd	0.9286	0.0786	0.0678
Aspidisca fusca	0.4346	**0.0005**	**0.0104**	0.2986
Meiofauna (abundance)				
Copepods	0.1135	**0.0053**	**0.0001**	**0.0003**
Nematodes	0.5664	**0.0339**	**0.0001**	**0.0148**
Ostracods	0.6247	**0.0044**	**0.0001**	**0.0001**

Notes: Ciliate abundance; biomass and species richness were log-transformed prior to analysis. Because the same data for ciliate community were test in 5 separate analyses (abundance; biomass; species richness; diversity and dominant species); the α-level used for these testes was adjusted from 0.05 to 0.01; and is marked in bold; effects with a p-value between 0.01 and 0.05 were considered as marginally significant trends; and are marked in italics. The data used for meiofauna analyses were only employed once; thus α-level remained 0.05 and is marked in bold. nd; not determined.

[a] the factor 'Grazer' includes the effects of *Arenicola* exclusion and dredging event.

[b] the factor 'Area' includes the mid and the low intertidal areas.

nd; no data due to the species was not observed.

Table 7: Jaccard similarity index; a measure of proprotion of shared species of ciliate communities between the three experimental treatments (O; EX and CON) at the low and the mid intertidal areas; respectively; or between the low and the mid intertidal areas in each treatment in the *Arenicola* field experiment. O; *Arenicola* presence treatment; EX; *Arenicola* exclusion treatment; CON; control treatment. The bolds indicates high similarity in ciliate species composition between the Reference and the Control treatments at both areas in the second sampling.

Between treatments	Mid intertidal area		Low intertidal area	Samplings
O × EX	0.320		-	1st sampling
O × CON	**0.741**		**0.579**	2nd sampling
O × EX	0.390		0.366	2nd sampling
EX × CON	0.474		0.429	2nd sampling
Between areas	**O**	**EX**	**CON**	
Mid intertidal × Low intertidal	0.390	0.400	0.471	2nd sampling

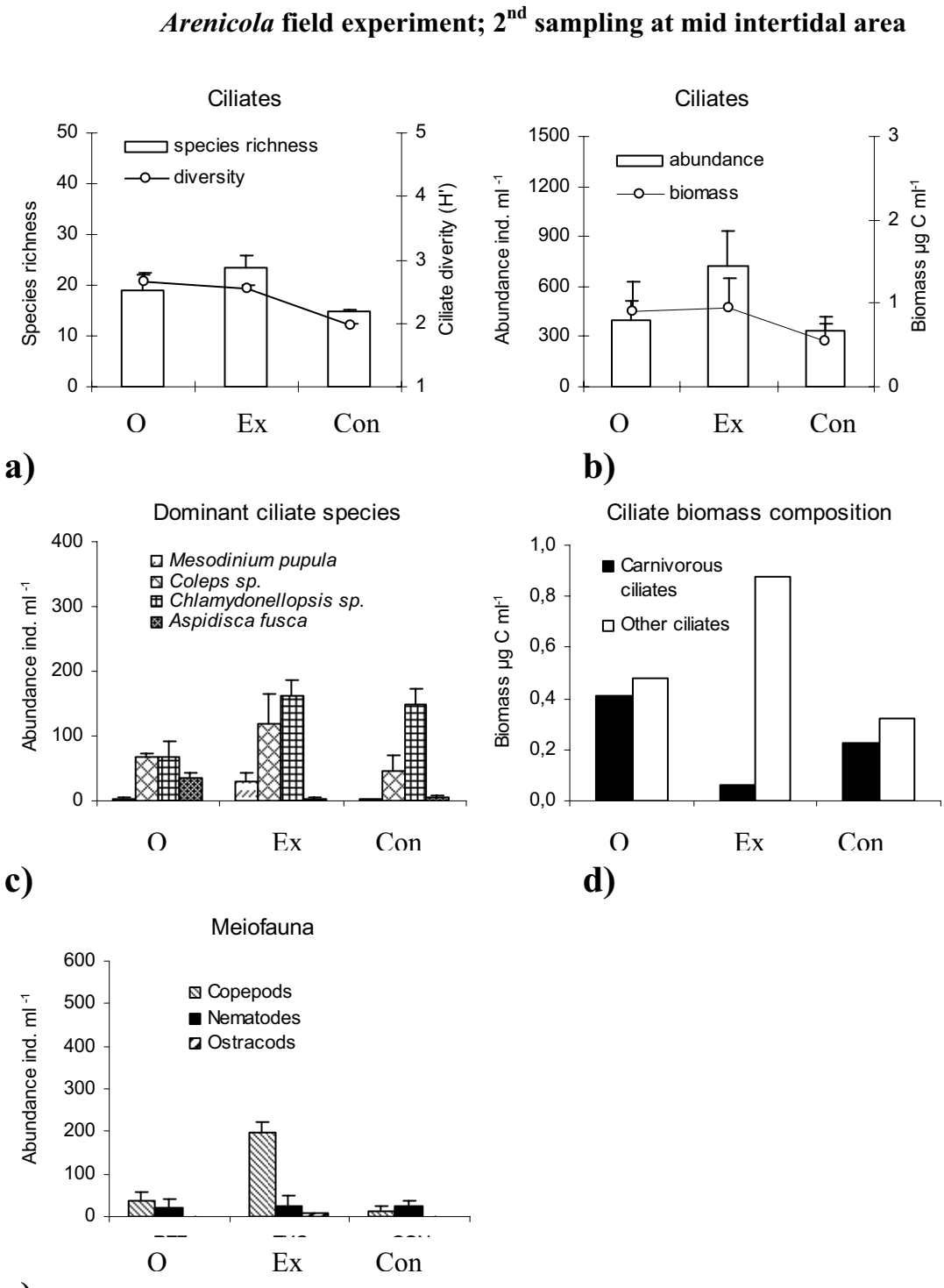


Fig. 6: Ciliate species richness and Shannon-Wiener diversity (a); abundance and biomass (b); abundance of dominant species (c); and biomass composition grouped by carnivorous (*Trachelocerca* sp.) and other ciliates (d); and meiofauna abundance (e) in the *Arenicola* field experiment in the second sampling (Jul 1; 2003) at the mid intertidal area. REF; Reference treatment; where the sediment was not manipulated and *Arenicola* was present at natural densities; EXC; Exclusion treatment; where sediment was dredged and *Arenicola* was removed by sieving; CON; Control treatment; where the sediment was dredged only and *Arenicola* was still remained. Error bars are 1 SD of the means of untransformed data; but for the statistical analyses; log-transformed data were used.

At the mid intertidal area (Fig. 6); the ciliate communities in the Reference and the Control treatments area were dominant by the same species; a large (about 700 μm long) carnivorous karyorelictid; *Trachelocerca* sp. (Fig 6d; Table 3). However; the ciliate community in the Exclusion treatment was dominated by two small to middle-sized (50-60 μm long) omnivorous ciliates; *Coleps* sp. and *Kerona* sp. In addition; a very small (ca. 20 μm long) herbivorous *Clamydonellopsis* sp. was the most abundant species in all treatments at this area (Fig. 6c). At the low intertidal area (Fig. 7); the ciliate community in the Reference treatment was dominated by a small (about 35 μm long) omnivorous *Aspidisca fusca*; and a middle-sized (about 120 μm long) herbivorous *Frontonia marina* (Table 3). In the Control treatment; ciliate community was also dominated by *Aspidisca fusca*; and a large (ca. 250 μm long) herbivorous *Prorodon morgani*. In addition; the very small (ca. 20 μm long) *Mesodinium pupula* was very abundant in the above two treatments at this area (Fig. 7c). In the Exclusion treatment; ciliate community was dominated by two large carnivorous species; *Trachelocerca* sp. and *Condylostoma arenarium* (Fig. 7d; Table 3).

Most dominant ciliate species were significantly affected by either Grazer or Area; or by their interactions; but different species showed unequal responses to *Arenicola* exclusion and dredging at the two areas (Fig. 6c; 7c; Table 6): *Trachelocerca* sp. was obviously reduced at the mid intertidal area while enhanced at the low intertidal area by *Arenicola* exclusion; but not influenced by dredging (Fig. 6d; Table 3). However; the difference was nonsignificant due to a large difference being masked by a still larger within-replicate variance. *Clamydonellopsis* sp. was significantly increased at the mid intertidal area but completely vanished at the low intertidal area by *Arenicola* exclusion; while dredging resulted in an enhancement of this species at both areas; consequently; Grazer × Area interaction was significant on this species. *Aspidisca fusca* was significantly reduced by *Arenicola* exclusion at both areas; and was significantly reduced by dredging only at the mid intertidal area (Fig. 6c; 7c; Table 6). *Mesodinium pupula* was significantly affected by Area and Grazer × Area interactions. *Peritromus californicus* and *Coleps* sp. was marginally significantly affected by Grazer and significantly impacted by Area. *Frontonia marina* and *Condylostoma arenarium* showed no responses to any experimental manipulations (Table 6).

A total of 53 ciliate species were found in the second sampling; and among them 39 species were also observed in the first sampling. Comparing community similarity index between areas showed that ciliate species composition of any treatment (Reference; Exclusion and Control treatment) at the mid intertidal area was different from that at the low intertidal area; that is; less than 50% of species were shared in any corresponding treatment between the two

areas (Table 7). Comparing the community similarity index beween treatments revealed that relatively more species were shared between the Reference and the Control treatments both at the mid (J = 0.741) and the low intertidal area (J = 0.579). In contrast; similarity indexes between the Exclusion and any other treatments were rather low; and no more than 50% species were shared each other in either area (Table 7).

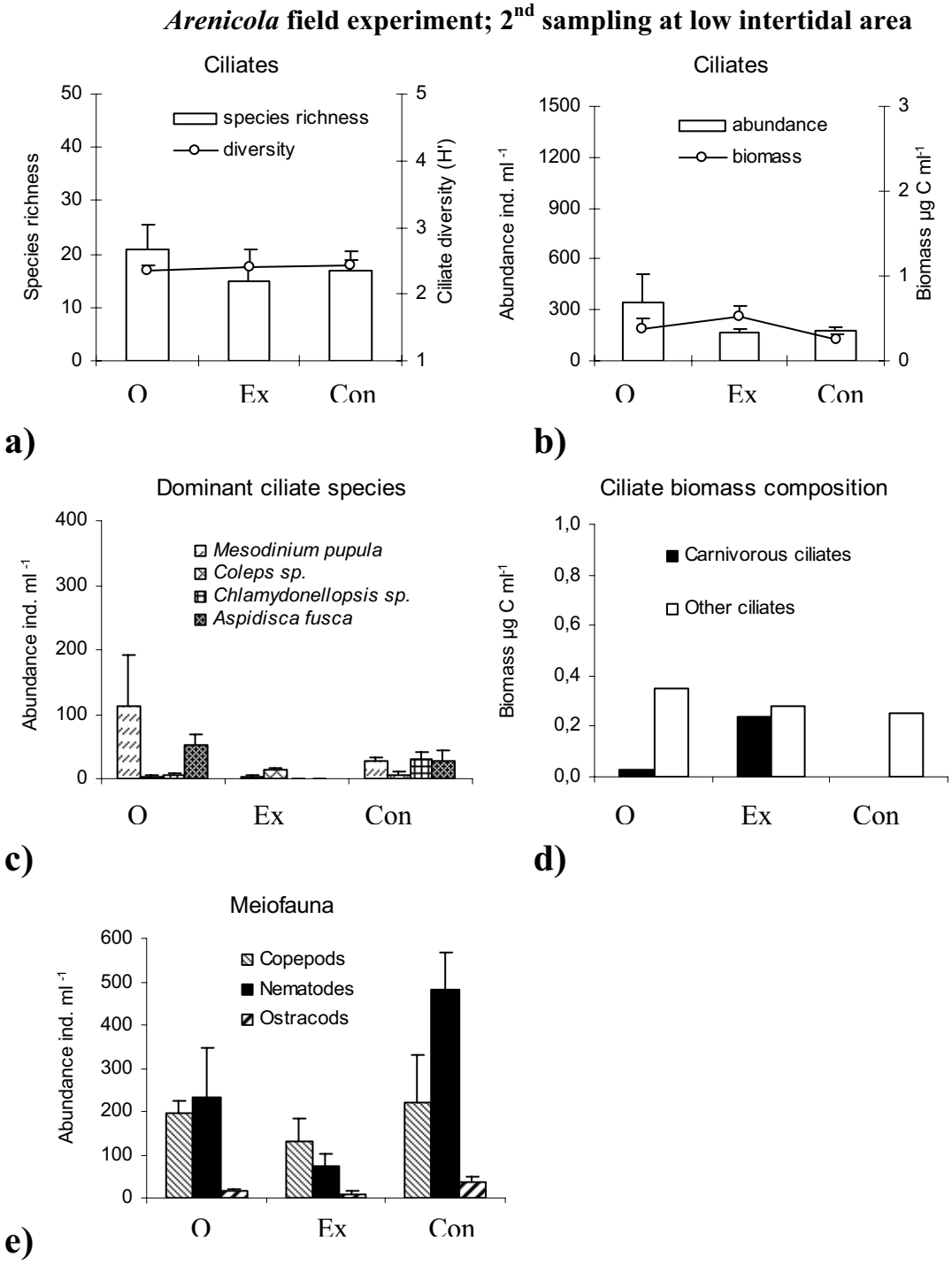


Fig. 7: Ciliate species richness and Shannon-Wiener diversity (a); abundance and biomass (b); abundance of dominant species (c); and biomass composition grouped by carnivorous (*Trachelocerca* sp. and *Condylostoma arenarium*) and other ciliates (d); and meiofauna abundance (e) in the *Arenicola* experiment in the second sampling (Jul 1; 2003) at the low intertidal area. Abbreviations and error bars as in Fig. 7.

Meiofauna

Copepods were the predominant meiofaunal group at the mid intertidal area but nematodes were most abundant at the low intertidal area; additionally; ostracods were also a frequent meiofaunal taxon but with low abundance at both areas (Fig. 6e; 7e). Mean abundances of copepods; nematodes and ostracods at the mid intertidal area were 84 ind. ml^{-1}; 24 ind. ml^{-1}; and 2 ind. ml^{-1}; respectively; which were all significantly lower than those at the low intertidal area; viz. 188 ind. ml^{-1}; 262 ind. ml^{-1} and 22 ind. ml^{-1}; respectively (Fig. 6e; 7e).

Meiofauna showed significant but different responses to *Arenicola* exclusion and dredging at the two areas (Fig. 6e; 7e; Table 6). *Arenicola* exclusion resulted in a significant increase of meiofauna at the mid intertidal area; and a significant decrease at the low intertidal area. In contrast; dredging led to a decrease of meiofauna at the mid intertidal area; and a significant increase at the low intertidal area. Therefore; the effects of Grazer × Area interactions were significant for all meiofaunal groups (Table 6).

4. Discussion

Effects of macrograzers addition in laboratory experiments

Many studies have been carried out to test the effects of grazers on planktonic or epibenthic ciliates in laboratory or field conditions. Sanders & Wickham (1993) reviewed data for the planktonic microbial loop indicating that marine and freshwater zooplankton species may include ciliated protozoa as an important part of their diets and many metazoans grow and/or reproduce when fed ciliates. Further; metazoan predation can alter the abundance and species composition of protozoa. Much less; however; is known about the grazing effects on benthic ciliates. Taylor (1980) observed that ciliate abundance was significantly reduced by the addition of oligochaetes in a laboratory experiment. Further studies also showed that with the addition of micrometazoa (rotifers and rotifers, cladocerans, copepods) or ostracods or with the elevated densities of macrograzers (snails and mayfly nymphs) ciliate abundance was significantly decreased (McCormick 1991; McCormick & Cairns 1991; Wickham et al. 2000). Thus it was unexpected that there were no significant grazing effects for macrograzers on ciliates (abundance; biomass; species richness and diversity) and meiofauna (abundance) within our laboratory experiments.

In contrast; Wickham et al. (2004) found that the abundances of both meiofauna and ciliates were distinctly decreased when macrozoobenthos were removed; and ciliate biomass tended to increase with macrozoobenthos presence; indicating an indirect (predation) effect of the macrozoobenthos on ciliates. Our laboratory experiment results showed a nonsignificant trend toward higher ciliate biomass with grazer addition; thus agree with this point of view.

Previous studies suggested that many factors; including food limitation and food quality; species composition and life history (e.g. cystformation) of ciliates and meiofauna and their community production; etc. can influence the effect of predation (e.g. McCormick & Cairns 1991; Coull 1999). However; there was no predictable pattern to explain the mute grazing effects in our laboratory experiments. Food-limiting pressures might be a factor that reduced the effects of predation in Potsdam Lake experiment; as indicated by the significant decline of ciliates and meiofauna due to incubation. Further; the low densities of macrograzers used in our laboratory incubations may have affected the out outcome of the experiments. McCormick (1991) found a decrease of benthic ciliate abundances by addition of elevated densities (about 3 to 4 ind. ml^{-1}) of snails and mayfly nymphs. In our laboratory experiments; the densities of macrograzers were only 1 to 2 ind. ml^{-1}. Considering the large quantities of meiofauna (23 to 386 ind. ml^{-1}) and ciliates (64 to 677 ind. ml^{-1}); the grazing effects of the

few macrograzers might be weak. On the other hand; the significantly positive correlations between ciliate and meiofaunal groups in Dorum and Potsdam Lake experiments indicated a weak predation pressure between meiofauna and ciliates.

Effects of *Arenicola* exclusion in the field experiment

Meiofauna taxa were significantly impacted by the *Arenicola* exclusion in the second sampling; that is; a significant increase of meiofauna abundance was observed at the mid intertidal area while a significant decrease was revealed at the low intertidal area. However; there were no significant effects on ciliate abundance and biomass; but there was a general trend towards higher ciliate biomass (Fig 6-9; Table 4). On the other hand; it is not surprising that exclusion of predators did not always have strong effects on total the ciliate community (abundance and biomass) in the field experiments; because a total exclusion of grazer functional types is impossible. Coull (1999) suggested that when prey has the ability to rapidly reproduce; predator effects on the demographics of the populations are not longlasting. Many ciliates have life-history characteristics that allow rapid replenishing of the prey population. As a result; meiofauna may make have little impact on ciliate prey populations if ciliates reproduce so rapidly and are so abundant that predators cannot significantly reduce the population size. Thus; although predation effects for macrograzers on meiofauna were significant; the same effects on ciliates might be minor.

The influence of top-down and bottom-up control: Do carnivorous ciliates play a role in regulating the system?

If attention is concentrated on direct and indirect grazing interactions within the ciliate community and between ciliates and meiofauna; ciliates should be divided into two functional groups: carnivorous and non-carnivorous species (Fig. 5d; 6d; 7d). Interestingly; despite the fact that there were no consistent patterns of variations between meiofauna abundance and total ciliate biomass; there was a tendency for the decrease of meiofauna when carnivorous ciliates increased or dominated; and *vice versa* (Fig. 8). This poses the question whether carnivorous ciliates play a large role in regulating the top-down and bottom-up control in the system.

In the first sampling at the mid intertidal area; meiofauna abundance decreased in the Exclusion treatment; where the carnivorous ciliate; *Condylostoma arenarium* dominated the community (Fig; 5b; d; 8a; Table 3). In the second sampling at the mid intertidal area; both the ciliate biomass and meiofauna abundance increased in the Exclusion treatment (where

Trachelocerca sp. was decreased) and decreased in the Control treatment (where *Trachelocerca* sp. dominated the ciliate community). A potential bottom-up control was revealed at this area (Fig. 6b; e; 8b; e; Table 3). At the low intertidal area; meiofauna abundance significantly decreased while ciliate biomass increased in the Exclusion treatment (where the ciliate community was dominated by *Condylostoma arenarium* and *Trachelocerca* sp); and there was a reverse situation for the meiofauna abundance and ciliate biomass in the Control treatment. A top-down control was shown in this area (Fig. 7b; e; 8c; d; Table 3).

It is commonly known that macrograzers eat meiofauna; and meiofauna in turn are known to prey on ciliates (McCormick & Cairns 1991; Epstein & Gallagher 1992; Wickham et al. 2000; Hamels 2001; Zöllner et al. 2003). However; there is evidence (mostly qualitative) that these trophic roles are sometimes reversed. Senders & Wickham (1993) reviewed that some protozoa are able to prey on metazoan; while they thought it is probably qualitatively insignificant to metazoan population in planktonic microbial loop. Further; Arndt (1993) found that a planktonic rotifer was a common food source for *Condylostoma vortivella* in the field; and supposed a 'reversed loop' existed; though of little quantitative importance in the planktonic food web. We also observed that *Condylostoma magnum* (ca. 700 µm in length) frequently effectively engulfed even 1000 µm-long nematodes in surface waters of marine sediment (Fig. 8f). Thus it is very probably that *Condylostoma arenarium* also can prey on meiofauna. We assume that this predacious ciliates may play a role in obscuring the effects of macrograzer predation on both ciliates and meiofauna. The carnivorous ciliates (*Condylostoma arenarium* and *Trachelocerca* sp.) were frequently dominant; occupying on average 42% of total ciliate biomass in the *Arenicola* field experiment. Although we can not demonstrate that *Trachelocerca* sp. also feeds on meiofauna; the large proportion of carnivorous species to the total ciliate biomass was at least an indication of great quantitative importance in regulating the microbial loop in the sediment environment within our study. If there are numerous trophic steps between bacteria and macrograzers; then the microbial loop is a respiratory energy sink (Wikner & Hagström 1988; Sanders & Wickham 1993). Possibly; more attention should be paid to the carnivorous functional groups and the 'reversed loop'. The negative relationship between the dominant carnivorous ciliates and meiofauna in sediments due to competition or predation is obviously an attractive question which has to be answered by further studies.

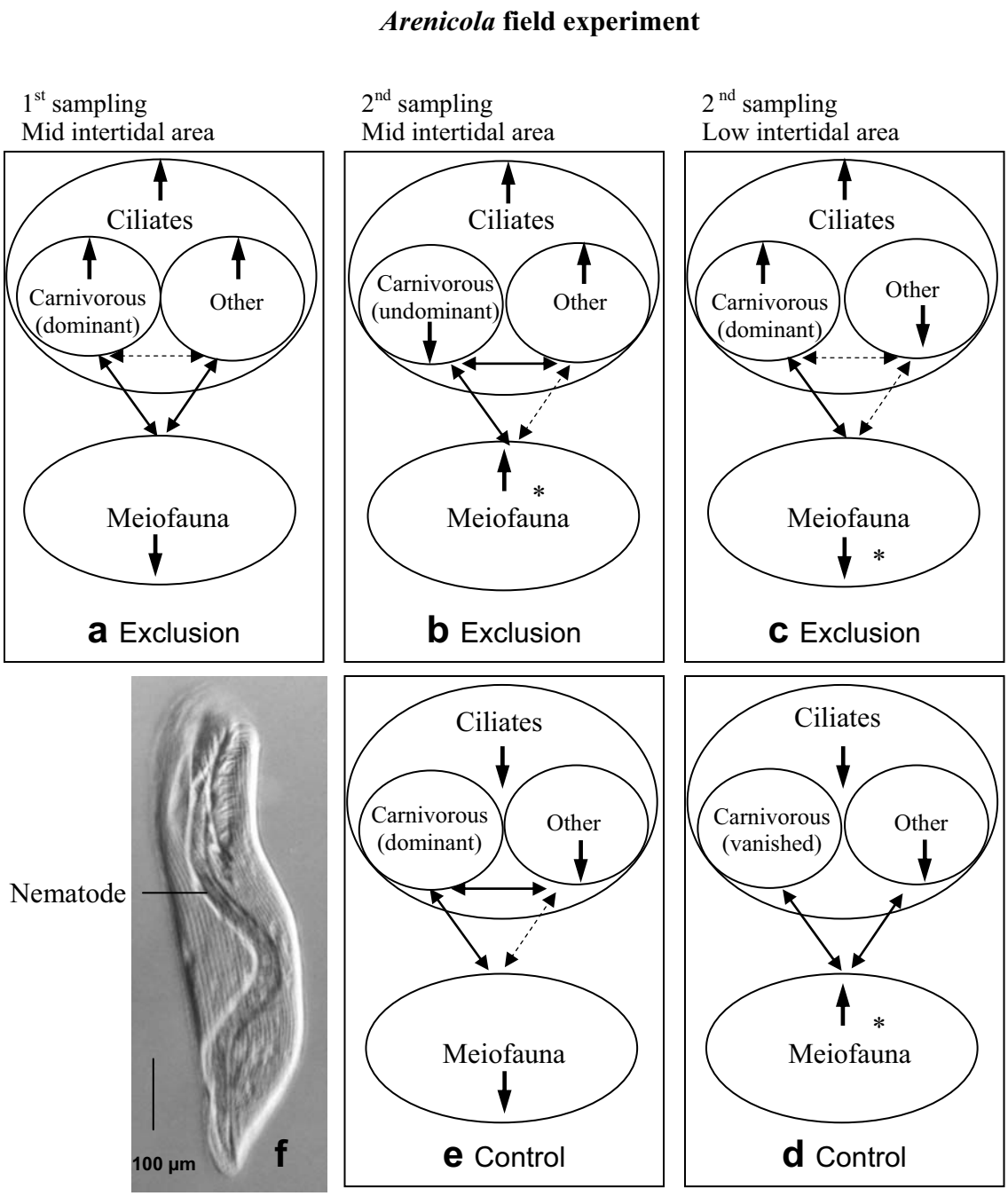


Fig. 8 a-e: Summary of effects of *Arenicola* exclusion (a-c) and digging event (d; e) on meiofauna abundance and ciliate biomass (carnivorous and the others) in the *Arenicola* field experiment in the first sampling at the mid intertidal area (a); and in the second sampling at the mid (b; e) and low intertidal areas (c; d). (f) A live *Condylostma magnum* (about 700 µm long) engulfing a long (ca. 1000 µm) nematode from surface water of a marine sediment. The upward and downward arrows denote tendencies of increase and decrease; respectively. Asterisks mark significant effects on meiofauna by *Arenicola* exclusion; as detected by ANOVA. Solid and broken double arrows imply; respectively; potentially strong and less strong interactions between groups. Despite there were no consistent relationships between meiofauna abundance and total ciliate biomass; there were tendencies that when the carnivorous ciliates increase or dominate; meiofauna decrease; and vice versa.

Impact of *Arenicola* exclusion on ciliate community structure

The ciliate species composition was distinctly altered by exclusion of *Arenicola*; as indicated by the relatively low community similarity indices between the Exclusion treatment and other treatments in both the samplings and the areas (Table 7). Strikingly; the exclusion of *Arenicola* resulted not only in a structural but also in a functional shift for ciliate communities (Table 3): in the first sampling; the large carnivorous ciliate; *Condylostoma arenarium* increased and dominated the community; and as a results; led to a functional shift from mixed omnivorous and carnivorous dominance to only carnivorous dominance (Fig. 5d; Table 3); in the second sampling; another large carnivorous ciliate *Trachelocerca* sp. was distinctly reduced by *Arenicola* exclusion at the mid intertidal area; and as a result; there was a shift from carnivorous dominance to omnivorous dominance in this area (Fig. 6d; Table 3). At the low intertidal area both the *Condylostoma arenarium* and the *Trachelocerca* sp. were enhanced and dominated the community; and as a result; there was a shift from herbivorous and/or omnivorous dominance to only carnivorous dominance at this area (Fig. 7d; Table 3).

Some dominant species of ciliates were significantly affected by *Arenicola* exclusion; but different species showed unequal responses at the two areas. For example; *Clamydonellopsis* sp. was significantly increased at the mid intertidal area but completely vanished at the low intertidal area; *Aspidisca fusca* was distinctly reduced at the both areas; and *Frontonia marina* was not influenced at all. Similar results were shown in previous studies. Epstein & Gallagher (1992) also documented that increases in meio- and macrobenthic densities did not influence total ciliate abundance; but they did strongly affect the densities of individual ciliate species. The negative and positive influence of metazoans on ciliates were large but species-specific. Wickham et al. (2004) also found that predation effects of macrozoobenthos on ciliates were ciliate group specific. Thus our results agreed with this.

There were no significant effects of *Arenicola* exclusion on ciliate species richness and diversity; which; however; was shown to be more influenced by dredging than *Arenicola* exclusion in the second sampling (Fig. 6; 7; Table 6). Previous studies documented significant changes in benthic algal diversity with changing productivity and predation regimes (Paine & Vadas 1969; Kassen et al. 2000; Sommer 2000; Worm et al. 2002). Further; ciliate diversity was found to be altered by the addition of ostracods in a deeper layer of the sediment (Wickham et al. 2000). However; this result was in accordance with Wickham et al. (2004) that removal of macrozoobenthos did not change species richness and diversity of epibenthic ciliate community in the littoral zone of a lake and brackish water.

Effects of dredging on meiofauna and ciliates

Three lines of evidence suggest that dredging also can exert influence on both meiofauna and ciliates in the second sampling (Fig. 6-9; Table 6): 1) meiofauna abundance were significantly increased at the low intertidal area in the Control treatment; 2) two species of ciliates exhibited significant but different responses to dredging (*Clamydonellopsis* sp. was enhanced while *Aspidisca fusca* was reduced; and 3) ciliate diversity was significantly decreased in the Control treatment at the mid intertidal area.

Numerous studies on the effects of disturbance were conducted on diverse organisms (e.g. phytoplankton; protozoa and zooplankton) in many habitats (Eckert & Walz 1998; Barbiero et al. 1999; Kassen et al 2000; Weithoff et al. 2001; Thomson et al. 2002). Kneitel & Chase (2004) found that protozoan and rotifer abundance were significantly decreased by both disturbance and predation in a container community of a forest system. Thomson et al. (2002) studied the effect of hydrological disturbance on the impact of a benthic invertebrate predator and found that the impact of a stonefly predation on its mayfly prey was either unchanged or increased by disturbance. However; we still have no good explanation for the differences caused by dredging in the *Arenicola* experiment since it was done 14 months before. We agree with Thomson et al. (2002) that predation effects and their potentially complex interaction with other biotic and/or abiotic factors may be important during disturbance.

Effects of Area and Grazer × Area interactions on ciliates and meiofauna

Analysing of the parallel experimental treatments conducted in the two different intertidal areas revealed more pronounced significant effects due to Area or Grazer × Area interaction than Grazer. Both ciliate community structure (including species composition) and meiofauna were significantly influenced by Area or Grazer × Area interaction (Table 6; 7).

Several reasons may result in “Area” or “Grazer × Area” becoming more significant factors that impact on benthic ciliates and meiofauna in sediments. For example; the different grain size of sediment and the extent of wave action and tidal disturbance on the two study areas may greatly influence the composition of the fauna (Carey 1992). In this study; the low intertidal area with fine sediment is flooded 9-10 h per day; the mid intertidal area with coarse sediment is flooded 6-7 h per day. Further; distribution strategies of adult and juvenile *Arenicola* are different in the two tidal areas (Flach & Beukema 1994; Reise et al 2001; Volkenborn 2005). Usually adult *Arenicola* distributes seaward (low intertidal area); while juvenile colonize the upper tidal zone above the range of the adults (mid intertidal area). Only

at low densities of adults (< 20 ind. m^{-2}) may juveniles also settle in between the adults (Flach & Beukema 1994; Reise et al 2001). Flach & Beukema (1994) assume high bioturbation activity or food limitation as possible mechanisms that keep juveniles away from adults. The different bioactivities caused by adults and juveniles that resulted in the difference of bottom-up (at mid intertidal area) and top-down (at low intertidal area) controls in the second sampling is a substantial possibility. This biological disturbance as a major factor was documented by Paine & Vadas (1969) that depending on tidal height; urchin removal leads to significant changes in algal species composition within periods of less than a year. The present result suggested that the "Area" is actually a biotic-abiotic factor complex. The potential interactions between biotic and abiotic factors (Grazer × Area interaction) are complex; and may sometimes play a more important role than a single factor (e.g. predation) in the system; and should be paid more attention in future food web research programmes (Berlow et al. 2004).

Conclusion

Laboratory experiments showed that there were no significant grazing effects of individual macrograzers on ciliates (abundance; biomass; species richness and diversity) and meiofauna abundance. Ciliate species richness and diversity were marginally significantly increased by grazer addition in the Potsdam Lake experiment; and several dominant ciliate species marginally significantly increased or decreased depending on different grazers in the Sylt and Dorum experiment. However; incubation resulted in significant decreases of ciliate abundance and biomass; and meiofauna abundance in the Potsdam Lake experiment.

The field experiment revealed that ciliate abundance; biomass; species richness; and diversity were not significantly affected by the exclusion of *Arenicola*. However; the species composition was distinctly changed; and there were functional shifts between carnivorous dominance and omnivorous/herbivorous dominance due to the shifts of dominant species. In contrast; meiofauna abundance was significantly enhanced or decreased by *Arenicola* exclusion; depending on sampling dates and sites. Further; effects of dredging on ciliate diversity and several dominate species; and meiofauna abundance were sometimes significant. Moreover; both ciliates (abundance; species richness and diversity; and dominant species) and meiofauna abundance were more affected by Area or Area × Grazer interactions than by Grazer (*Arenicola* exclusion and dredging); indicating an important and complex abiotic-biotic interaction in regulating the system.

Despite the fact that there were no consistent patterns of variation between meiofauna abundance and total ciliate biomass; there was a tendency for the decrease of meiofauna when carnivorous ciliates increase or dominated; and *vice versa* (Fig. 8). Since the predacious ciliates were frequently predominant; they probably obscure the effects of macrograzer predation on both meiofauna and ciliates. This functional group of ciliates might play a great quantitative importance in regulating the microbial loop in sediment environments within our study. Thus; more attention should be paid to the carnivorous functional groups and the 'reversed loop' in sediment environments.

This study suggests that ciliate community composition (especially occurrence of the dominant carnivorous functional group) sometimes might influence the effects of macrograzer predation on both meiofauna and ciliates; therefore impact the trophic relationships in microbial loop of sediments. Further; the potential nontrophic interactions between biotic and abiotic factors (i.e. Area × Grazer interaction) may sometimes play a more important role than a single factor (e.g. predation) in the system. Simple trophic cascades were not sufficient to explain the complexity in the benthic microbial food web within our study.

Acknowledgements

The authors thank the Dr. J. Matthiessen for assistance in measuring environmental parameters; Dr. N. Volkenborn for precious discussions and providing some physicochemical data and Dr. Kuidong Xu for valuable discussions. The present work has been financed by the DFG (Deutsche Forschungs Gemeinschaft) grant number BE 2279/3-1; by the Alfred-Wegener-Institute for Polar and Marine Research.

References

Alongi; D.M.; 1986. Quantitative estimates of benthic protozoa in tropical marine systems using silica gel: a comparison of methods. Estuar. Coast. Shelf. Sci. 23; 443-450.

Arndt; H.; 1993. Rotifers as predators on components of the microbial web (bacteria; heterotrophic flagellates; ciliates)-a review. Hydrobiologia. 255/256; 231-246.

Barbiero; R.P.; James; W.F.; Barko; J.W.; 1999. The effects of disturbance events on phytoplankton community structure in a small temperate reservoir. Freshwater. Biol. 42; 503-512.

Berlow; E.; Neutel; A.-M.; Cohen; J.E.; De Ruiter; P.C.; Ebenman; B.; Emmerson; M.; Fox; J.W.; Jansen; V.A.A.; Jones; J.I.; Kokkoris; G.D.; Logofet; D.O.; Mckane; A.J.; Montoya; J.M.; Petchey; O.; 2004. Interaction strengths in food webs: issues and opportunities. J. Anim. Ecol. 73; 585–598.

Berninger; U.-G.; Epstein; S.S.; 1995. Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea sediment. Aquat. Microb. Ecol. 9; 229-236.

Blumenshine; S.C.; Hambright; K.D.; 2003. Top-down control in pelagic systems: a role for invertebrate predation. Hydrobiologia. 491; 347–356.

Böttcher; M. E.; Hespenheide; B.; Llobet-Brossa; E.; Beardsley; C.; Larsen; O.; Schramm; A.; Wieland; A.; Böttcher; G.; Berninger; U.-G.; Amann; R.; 2000. The biogeochemistry; stable isotope geochemistry; and microbial community structure of a temperate intertidal mudflat: an integrated study. Cont. Shelf. Res. 20; 1749-1769.

Burgess; R.; 2001. An improved protocol for separating meiofauna from sediments using colloidal silica sols. Mar. Ecol. Prog. Ser. 214; 161–165.

Carey; P.G.; 1992. Marine interstitial ciliates: an illustrated key. Chapman & Hall; London. 351 pp.

Coull B.C.; 1999. Role of meiofauna in estuarine soft-bottom habitats. Aust J Ecol 24: 327–343.

Eckert; B.; Walz; N.; 1998. Zooplankton succession and thermal stratification in the polymictic shallow Müggelsee (Berlin; Germany): a case for the intermediate disturbance hypothesis? Hydrobiologia. 387/388; 199–206.

Epstein; S.S.; Gallagher; E.D.; 1992. Evidence for facilitation and inhibition of ciliate population growth by meiofauna and macrofauna on a temperate zone sandflat. J. Exp. Mar. Biol. Ecol. 155; 27-39.

Epstein; S.S.; 1995. Simultaneous enumeration of protozoa and micrometazoa from marine sandy sediments. Aquat. Microb. Ecol. 9; 219-227.

Epstein; S.S.; 1997. Microbial food webs in marine sediments. II. Seasonal changes in trophic interactions in a sandy tidal flat community. Microb. Ecol. 34; 199–209.

Fenchel; T.; 1967. The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. Ophelia. 4; 37-121.

Fenchel; T.; 1968. The ecology of marine microbenthos II. The food of marine benthic ciliates. Ophelia. 5; 73-121.

Fenchel; T.; 1969. The ecology of marine microbenthos. IV. Structure and function of the benthic ecosystem; its chemical and physical factors and the mcirofauna communities with special reference to the ciliated protozoa. Ophelia. 6; 1-182.

Fenchel; T.; 1975. The quantitative importance of the benthic microfauna of an arctic tundra pond. Hydrobiologia. 46; 64-445.

Fenchel; T.; 1978. The ecology of micro and meiobenthos. Ann. Rev. Ecol. Syst. 9; 99-121.

Flach; E.C.; Beukema; J.J.; 1994. Density-governing mechanisms in populations of the lugworm *Arenicola marina* on tidal flats. Mar. Ecol. Prog. Ser. 115; 139-149.

Foissner; W.; Berger; H.; Schaumburg; J.; 1999. Identification and ecology of limnetic plankton ciliates. - Bayer. Landesamt für Wasserwirtschaft; Heft 3/99. Infomationsberichte des Bayerischen Landesamtes für Wasserwirtschaft; Munich.

Garstecki; T.; Verhoeven; R.; Wickham; S.A.; Arndt; H.; 2000. Benthic-pelagic coupling: a comparison of the community structure of benthic and planktonic heterotrophic protists in shallow inlets of the southern Baltic. Freshwater. Biol. 45; 147-167.

Giere; O.; 1993. Meiobenthology. The Microscopic Fauna in Aquatic Sediments. Springer-Verlag; Berlin; Heidelberg; New York. 328 pp.

Hamels; I.; Moens; T.; Muylaert; K.; Vyverman; W.; 2001. Trophic interactions between ciliates and nematodes from an intertidal flat. Aquat. Microb. Ecol. 26; 61-72.

Hartwig; E.; 1973. Die Ciliaten des Gezeiten-Sandstrandes der Nordseeinsel Sylt II. Ökologie. Mikrofauna Meeresboden. 18; 385-453.

Hartwig; E.; 1977. On the interstitial ciliate fauna of Bermuda. Cah. Biol. Mar. 18; 26-113.

Hausmann; K.; Hülsmann; N.; Polianski; I.; Schade S.; Weitere; M.; 2002. Composition of benthic protozoan communities along a depth transect in the eastern Mediterranean Sea. Deep-Sea Res. PT I 49; 1959-1970.

Hayward; B.H.; Droste; R.; Epstein; S.S.; 2003. Interstitial ciliates: benthic microaerophiles or planktonic anaerobes? J. Eukaryot. Microbiol. 50; 356-359.

Hillebrand; H.; Kahlert; M.; Haglund; A.-L.; Berninger; U.-G.; Nagel; S.; Wickham S.; 2002. Control of microbenthic communities by grazing and nutrient supply. Ecology 83; 2205-2219.

Kahl; A.; 1930-1935. Urtiere oder Protozoa: I: Wimpertiere oder Ciliata (Infusoria); eine Bearbeitung der freilebenden und ectocomensalen Infusorien der Erde; under Ausschluß der marinen Tintinnidae. In Dahl F (ed) Die Tierwelt Deutchlands. Fishcher Verlag; Jena p 1-886.

Kassen; R.; Buckling; A.; Bell; G.; Rainey; P.B.; 2000. Diversity peaks at intermediate productivity in a laboratorymicrocosm. Nature. 406; 508-512.

Kneitel; J.M.; Chase; J.M.; 2004. Disturbance; predator; and resource interactions alter container community composition. Ecology. 85; 2088–2093.

Krogh; A.; Spärck; K.; 1936. On a new bottom-sampler for investigation of the microfauna of the sea bottom with remarks on the quantity and significance of the benthic microfauna. Biol. Medd. K. Dan. Vidensk. Selsk. 13; 1-12.

Lei; Y.; Choi; J.K.; Xu; K.; Petz; W.; 2005. Morphology and infraciliature of three species of Metaurostylopsis (Ciliophora; Stichotrichia): *M. songi* n. sp.; *M. salina* n. sp.; and *M. marina* (Kahl; 1932) from sediments; saline ponds and coastal waters. J. Eukaryot. Microbiol. (in press).

Lynn; D.H.; Small; E.B.; 2002. Phylum Ciliophora. In: An illustrated guide to the protozoa. Lee; J.J.; Bradbury; P.C.; Leedale; G.F. (eds.). Society of Protozoologists; Lawrence; Kansas. pp 371-656.

Magurran; A.E.; 1988. Ecological diversity and its measurement. Princeton University Press; Princeton; NJ.

Mare; M.F.; 1942. A study of marine benthic community with special reference to the microorganisms. J. Mar. Biol. Ass. UK. 25; 54-517.

McCormick; P.V.; 1991. Lotic protistan herbivore selectivity and its potential impact on benthic algal assemblages. J. N. Am. Benthol. Soc. 10; 238-250.

McCormick; P.V.; Cairns; J.J.; 1991. Effects of micrometazoa on the protistan assemblage of a littoral food web. Freshwater Biol. 26; 111-119.

Montagana; P.A.; 1984. In situ measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. Mar. Ecol. Prog. Ser. 18; 119-130.

Montagnes; D.J.S.; Lynn; D.H.; 1993. A quantitative protargol stain (QPS) for ciliates and other protists. In: Handbook of Methods in Aquatic Microbial Ecology; Lewis Publishers; Chapter 28; 229-240.

Nielsen; K. J.; 2001. Bottom-up and top-down forces in tide pools: test of a food chain model in an intertidal community. Ecol. Monogr. 71; 187–217.

Paine; R.T.; Vadas; R.L.; 1969. The effects of grazing by sea urchins; *Strongylocentrotus* spp.; on benthic algal populations. Limnol. Oceanogr. 14; 710-719.

Patterson; D.J.; Larsen; J.; Corliss; J.O.; 1989. The ecology of heterotrophic flagellates and ciliates living in marine sediments. Progr. Protistol. 3; 185-277.

Posey; M.H.; Alphin; T.D.; Cahoon; L.; Lindquist; D.; Becker; M.E.; 1999. Interactive effects of nutrient additions and predation on infaunal communities. Estuaries. 22; 785-792.

Putt; M.; Stoecker; D.K.; 1989. An experimentally determined carbon:volume ratio for marine “oligotrichous” ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34; 1097-1103.

Reise; K.; Simon; M.; Herre; E.; 2001. Density-dependent recruitment after winter disturbance on tidal flats by the lugworm *Arenicola marina*. Helgol. Mar. Res. 55; 161–165.

Sanders; R.W.; Wickham; S.A.; 1993. Planktonic protozoa and metazoa: predation; food quality and population control. Mar. Microb. Food Webs. 7; 197-223.

Shimeta; J.; Starczak; V.R.; Ashiru; O.M.; Zimmer; C.A. 2001. Influences of benthic boundary-layer flow on feeding rates of ciliates and flagellates at the sediment-water interface. Limnol. Oceanogr. 46; 1709-1719.

Skibbe; O.; 1994. An improved quantitative protargol stain for ciliates and other planktonic protests. Arch. Hydrobiol. 130; 339-347.

Sommer; U.; 2000. Benthic microalgal diversity enhanced by spatial heterogeneity of grazing. Oecologia. 122; 284-287.

Starink; M.; Bar-Gilissen; M.-J.; Bak; R.P.M.; Cappenberg; T.E.; 1994. Quantitative centrifugation to extract benthic protozoa from freshwater sediments. Appl. Environ. Microbiol. 60; 167-173.

Taylor; W.D.; 1980. Observations on the feeding and growth of the predacious oligochate *Chaetogaster langi* on ciliated protozoa. Trans. Amer. Microsc. Soc. 99; 360-368.

Thomson; J.R.; Lake; P.S; Downes; B.J.; 2002. The effect of hyderological disturbance on the impact of a benthic invertebrate predator. Ecology. 83; 628–642.

Timmermann; K.; Andersen; O.; 2003. Bioavailability of pyrene to the deposit-feeding polychaete A*renicola marina*: importance of sediment versus water uptake routes. Mar. Ecol. Prog. Ser. 246; 163–172.

Volkenborn; N.; 2005.Ecosystem engineering in intertidal sand bi tue lugworm Arenicola marina. PhD Thesis; University of Bremen.

Weithoff; G.; Walz; N.; Gaedke; U.; 2001. The intermediate disturbance hypothesis - species diversity or functional diversity? J. Plankton. Res. 23; 117-1155.

Wickham; S.; Gieseke; A.; Berninger; U.-G.; 2000. Benthic ciliate identification and enumeration: an improved methodology and its application. Aquat. Microbial. Ecol. 22; 79-91.

Wickham; S.; Nagel; S.; Hillebrand; H.; 2004. Control of epibenthic ciliate communities by grazers and nutrients. Aquat. Microbial. Ecol. 35; 153-162.

Wieltschnig; C.; Fischer; U.R.; Kirschner; A.K.T.; Velimirov B.; 2003. Benthic bacterial production and protozoan predation in a silty freshwater environment. Microb. Ecol. 46; 62–72.

Wikner; J.; Hagström; Å.; 1988. Evidence for a tightly coupled nanoplanktonic predator-prey link regulating the bacterivores in the marine environment. Mar. Ecol. Prog. Ser. 50; 137-145.

Worm; B.; Lotze; H.K.; Hillebrand; H.; Sommer; U.; 2002. Consumer versus resource control of species diversity and ecosystem functioning. Nature. 417; 848-851.

Zöllner; E.; Santer; B.; Boersma; M.; Hoppe; H.-G.; Jürgens; K.2003. Cascading predation effects of Daphnia and copepods on microbial food web components. Freshwater. Biol. 48; 2174–2193.

General discussion

Studies on food webs in a specific environment often deal with the investigation of factors controlling only the local community. The comparison of results originating from studies of communities in different specific environments are then used to derive general relationships and controlling mechanisms. In this thesis small benthic communities in different soft sediment habitats were investigated in a series of studies. The abundance and biomass of diatoms, cyanobacteria, bacteria, nanoflagellates (phototrophic and heterotrophic), ciliates and meiofauna were determined in freshwater and marine soft sediments of Germany, North America and Greenland. This approach was used to investigate the interactions between the organisms in a community as well as the general influence of salinity, climate and geographic region on the structure of small benthic communities. The conclusions of the five studies in this thesis were integrated into a proposal for a comprehensive model of matter and energy flow in small benthic communities.

Influence of environmental attributes

Light, temperature, the presence of liquid water and the concentration of gas and ions are considered among others to be the most important abiotic factors governing the distribution of organisms (Odum 1971; Begon et al. 1996). The seasonal dynamics of a small benthic community in a temperate intertidal mudflat in Dorum were investigated in Studies 1 and 3. The microphytobenthic components of the community exhibited a seasonal cycle with blooms during spring and autumn. Bacteria, HNF and ciliates followed these microphytobenthic blooms with high abundance and biomass during spring and autumn. The ciliate species richness and diversity however did not reveal seasonal patterns. Meiofauna exhibited the highest abundance during summer. Several studies describe a rise of microphytobenthic production from spring to summer, when insolation and temperature increase (Cadée & Hegeman 1977; Asmus & Asmus 1985). The relative minimum of microphytobenthic abundance identified in Dorum during this period could be explained by an increase of grazing pressure during summertime. We assumed that grazing pressure of the meiofauna and ciliates, which were present in high abundance and biomass, increased during summer and thus controlled effectively the microphytobenthic biomass. During spring, autumn and winter the meiofauna biomass was low and the grazing of meiofauna did not seem to be high enough to control the microphytobenthos. Even if the ciliate species were mainly herbivorous, their

grazing also did not seem to be high enough to control the microphytobenthos during spring and autumn.

The question arose as to whether the structure of the small benthic food web found in Dorum held true basically for communities in other shallow sediment habitats like in freshwater or in arctic climate as well. Salinity and climate are known to exert distinct influences on organisms. The "latitude diversity gradient" is one of the most striking statements in biogeography**,** predicting that species diversity and latitude are inversely related. Furthermore the biomass of terrestrial organisms are obviously much smaller in an arctic climate than in temperate regions, mainly caused by the short vegetation period in polar regions and the low temperatures and light intensity during the winter period, when the sea and lakes are frozen, at least in shallow regions and active life is impossible there. The biogeography of microbes, however, part of the focus of this thesis, is different compared to the biogeography of most metazoans. Three major characteristics fundamentally distinguish microbes from most multicellular organisms: (1) large absolute population sizes, (2) short generation times and (3) high dispersal capabilities. With regard to these attributes microbes fit perfectly into the hypothesis that "everything is potentially everywhere, the environment selects" (Fenchel 1978). As to the general influence of salinity Odum (1971) emphasises the richness of marine biota compared to that of freshwater.

Considerable differences in the biomass of the small benthic components as well as in ciliate species composition and richness were identified in the analysis of soft sediment communities from freshwater and marine sites in Germany, North America (USA; Pennsylvania, New Jersey) and in the North East of Greenland. However, differences in biomass, abundance of the small benthic components as well as in ciliate species composition and richness could not be attributed to the measured differences in carbon content, salinity or climatic parameters. The feeding type of the dominant ciliates indicated that herbivory was the most common and important feeding strategy. The importance of bacterivory however varied significantly between the different sites. The mean percentage of total heterotrophic biomass (bacteria, HNF, ciliates and meiofauna) on the total biomass was found to be smaller in freshwater than in marine sites (Study 2). According to literature the organic matter of marine and freshwater sediments can be qualitatively different (Capone & Kiene 1988). In freshwater systems complex structural polysaccharides and phenolic polymers (as ligno-cellulose) represent a larger fraction of the organic input than in marine systems. This higher fraction of humic substances, which can only be used by detritus feeding heterotrophs with difficulty, might

keep the heterotrophic biomass in freshwater systems on a lower level. It is assumed in literature that the composition of easily available carbon (protein, carbohydrates, lipids, biopolymeric carbon) has a major influence on the structure and biomass of microbial communities in shallow sediments (Manini et al. 2003). The biomass measured in neighbouring lakes and beach systems in Greenland and in USA supported the assumption, that neither simply latitude nor climate and geographic position could explain the differences. Furthermore the investigation of neighbouring areas within a mudflat (mid an low intertidal areas) on Sylt revealed significant differences in small benthic organisms' biomass and ciliate species composition. However, these results also provided evidence that the position of the habitat in the mudflat and thus the exposure time to air as well as geochemical characteristics of the sediment exerted strong influences on microbial biomass and species composition. The common parameters such as total and organic carbon content measured during all studies did not prove to be suited for the prediction of microbial benthic community biomass and structure. We found that the amount of chlorophyll-*a* appeared to be of major importance for microbenthic community structure and biomass. However, the measured values of chlorophyll-*a* did not represent the evaluated microphytobenthic biomass. Chlorophyll-*a* in sediment habitats can originate from sedimented phytoplankton, shreddered macroalgae, fecal pellets, shreddered plant material. Consequently the amount and the sources of chlorophyll-*a* should be regarded as a component of the detrital organic matter of different availability and thus as an indicator for the easy availability of organic carbon.

Relationships between organisms

Organisms can interact with each other either via direct mechanisms such as grazing and prey availability or via indirect ones as stimulation and inhibition by bioturbation, exudation of inhibitory substances, sloppy feeding or competition. Correlations between organisms' abundance were used to find relationships between the different groups of organisms. The different studies revealed that the investigated groups of HNF in different size classes feed more or less on everything which is smaller than themselves. In contrast to plankton habitats, a considerable portion of nano- and micro organisms in sediments live attached to particles which can easily be ingested by larger organisms such as small meio- and macrofauna. Hence meio- and macrofauna do not invest much more energy in feeding on smaller prey than in hunting prey of larger size. Predators in sediments are therefore able to feed effectively on much smaller prey than organisms in plankton communities can do. In benthic habitats detritus is known to be an important energy source and many meiofauna species are known to

feed on detritus or at least on the micro- and nanoorganisms attached to the detritus particles (Fenchel & Jorgensen 1977). DOM (dissolved organic matter) and POM (particulate organic matter) are parts of detritus. Mainly bacteria use DOM and POM in the plankton microbial food web model. Several studies report on a size-dependent uptake of fluorescent labelled detritus by some ciliates and flagellates (Sherr 1988; Posch & Arndt 1996). These results prove that ciliates and flagellates can use POM and DOM at least as additional food source. Correlations between the abundance of diatoms and small HNF (2-5 µm) found in the studies of this thesis were interpreted as showing that small HNF compete with bacteria as for polysaccharides (as from exudates), but they can also feed on bacteria. Medium HNF (5-10 µm) are thought to feed on bacteria, small phototrophic and heterotrophic nanoflagellates. Furthermore large HNF (>10 µm) are thought to feed on bacteria, small and medium PNF and HNF, cyanobacteria and small diatoms. The analysis of ciliate species and feeding types revealed that herbivory was the main feeding preference in most investigated sites. In only a few sites bacterivory was the dominant ciliate's feeding type. On the one hand it is assumed that in sediments flagellates and ciliates also use POM and DOM and so compete with bacteria such as HNF (Montagna 1984). On the other hand, nanoflagellates are known to feed on viruses and particles in the size of viruses (10 – 400 nm), similar to the sizes of DOM (<0,45µm) (González & Suttle 1993). According to Mei & Danovaro (2004) viruses are abundant and productive in sediments (abundance 10^8 to 10^9 virus ml^{-1}; production of 0,13-1,6*10^8 viruses ml^{-1} h^{-1}). It might be interesting to investigate the grazing pressure on viruses in comparison to the grazing pressure on DOM and POM in sediments.

The influence of small macrofauna on the dynamics of the small community in different soft sediment systems (Study 4 and 5) was investigated in laboratory experiments. The results of these experiments exhibited that the absence of all small macrograzers initiated a measurable changes of the microbial community biomass, at least in the short term, whereas the presence of one single small macrograzer species and its direct grazing effect were found to be of minor importance. Small macrofauna organisms seemed to influence the community indirectly, mainly by stimulation and inhibition (as bioturbation, fecal pellets recycling, sloppy feeding, competition and excreted inhibitory substances), rather than by direct grazing. A field study, where *Arenicola marina* was excluded in the long term, revealed that after 6 months of exclusion this absence was accompanied by significant increases of bacteria and HNF. However, after 16 month of exclusion the absence or presence of *A. marina* did not make a significant difference any more. It is assumed, that the ecological function of *Arenicola* within the small food web was adopted by other organisms (as other small

macrofauna, meiofauna and ciliates species). This result corresponds to the findings and assumptions of Frank et al. (2005) and Smetacek & Nicol (2005), who report on significant changes in the community structure and production due to the lack of higher trophic levels in large marine food webs in the long term.

Suggested model of matter and energy flow in small benthic communities

The small food web in sediments clearly differs from the models known from plankton systems. In contrast to plankton systems the trophic levels in sediments food webs are blurred and most species rather feed opportunistically on what is most available (Peterson & Howarth 1987; Posey, Alphin, et al. 2002). The dominance of the omnivore feeding type, detritus feeding, stimulation, bioengineering effects and feed back mechanisms create a highly networked food web. A hypothetical model of the matter and energy flow in a coastal small benthic food web summarised the results of this thesis (Fig. 1).

Energy and matter flow in a small benthic food community

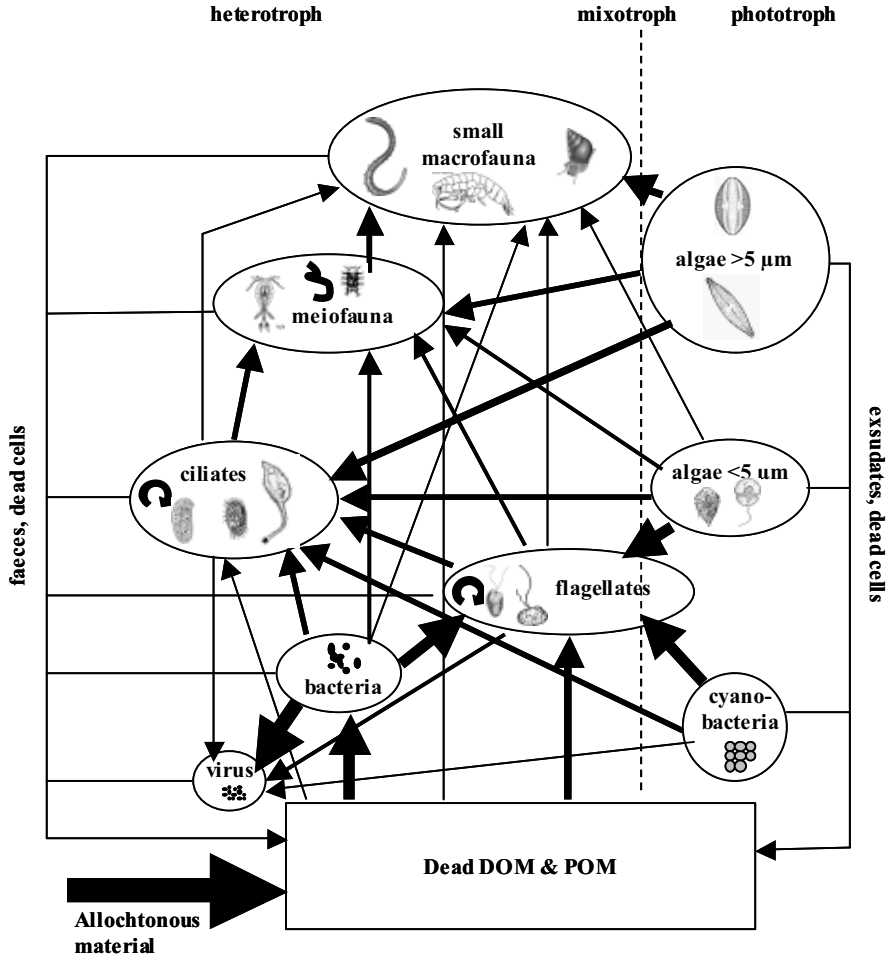


Fig. 1: Energy and matter flow within a generalised small benthic food web. Arrows indicate flow direction and contribution to the food web. The curved arrows in the flagellate and ciliate compartments indicate the predator-prey interactions within the broad classes of these organisms. The width of arrows roughly indicates the extent of energy flow.

Conclusion

The results of this thesis verified the model of Fretwell (1977) for benthic intertidal microbial food webs. It predicts that top-down forces form the trophic structure, but the bottom-up attributes of the ecosystems, such as nutrient availability, temperature and light, determine the fundament of the community as total biomass, abundance and production. The fundamental attributes of microbes such as large absolute population sizes, short generation times and high dispersal capabilities, form a system with fast changes in species composition. According to Odum (1971) such a community can be called a system with pulse stability, where the changing environmental impacts, originating also from indirect effects of large predators, maintain the system in a young, relatively fertile stage. Populations in shallow soft sediments seemed to be rather r-selected and the biocenosis was characterised by low resistance but high resilience.

Subjects of further research

The present studies provided just a narrow insight into the energy and matter flow in benthic systems. Several subjects of further research are presented in the following section.

Energy and matter flow, from the small to the large food web

Coastal areas represent highly productive regions and many larval stages of marine animals which are used by the fish-industry live there. Studies show that omnivory and trophic plasticity are widespread and that many consumers also feed on lower trophic levels of the food web than previously suggested (Pinnegar & Polunin 2000). Many macrofauna organisms, also juvenile stages of fishes, are also known to feed on benthic small organisms. The early life history of marine animals determines the quantity and quality of the adult population, thus the monitoring of the larval stages of fish species or crabs and their food sources might become more relevant for forecasting future crops. The relationships between the small benthic food web and the large benthic and pelagic food web have not been investigated thoroughly.

Coastal regions often are recreational areas of great socio-economic importance such as tourist attractions, for local industry and waste water disposal. The human impact on the beach ecosystem is immense, the impact on the benthic small food web and the connected links to the large food web however has not been studied in depth. The influence of increasing temperature, eutrophication, the entry of heavy metals, pesticides from agriculture and residual drugs in waste water awaits more investigation (Agatz et al. 1999; Riera et al. 2000; Dell'Anno et al. 2002).

Several methods are available for determine the material flow from a small benthic to the large food web in detail. The tracing of radioactively labelled detritus (as glucose, tracer-marked dead bacteria and algae) is a reliable experimental method. The radioactivity in micro- meiofauna and macrofauna can be measured in well-defined time intervals and the material flow can thus be determined (Montagna 1984; Kemp 1987). The measurement of stable isotopes is another powerful tool for the measurement of the accumulation of matter in the form of biomass in the different levels of food webs (Minagawa & Wada 1984; Peterson & Fry 1987; Michner & Schell 1994; Cabana & Rasmussen 1994). Stable isotopes, mainly ^{15}N and ^{13}C, accumulate in tissues of the different trophic levels in certain proportions. The ratio of stable nitrogen isotopes ^{15}N:^{14}N in an organism correlates with its trophic level (Minagawa & Wada 1984). Stable isotopes of carbon (^{13}C) have been very effectively used for the backtracking of transferred carbon sources.

Using these methods, Pinnegar & Polunin (2000) demonstrate that stable isotope data suggest more diverse diets of many species than implied on the base of gut-content analysis of a Mediterranean rocky shore food web. Throp & Delong (1998) demonstrate the importance of DOM, benthic filamentous algae, submerged macroalgae and phytoplankton as carbon sources for plankton fish in a floodplain of a large river, including benthic invertebrates. Studies on the small benthic food web in a coastal marine lagoon and wetlands by stable isotope analysis suggest the importance of the different sources of carbon and nitrogen for the understanding of the food web dynamics (Kwak & Zedler 1997).

Reference of general introduction and general discussion

Agatz, M., Asmus, R.M., Deventer, B. 1999. Structural changes in the benthic diatom community along a eutrophication gradient on a tidal flat. Helgol. Mar Res. 53, 92-101.

Alongi, D.M., 1991. Flagellates of benthic communities: characteristics and methods of study. In: The biology of free-living heterotrophic flagellates. Eds: Patterson DJ, Larsen J. Systematics association by Clarendon press, Oxford, pp 57-75.

Asmus, H., Asmus, R., 1985. The importance of grazing food chain for energy flow and production in three intertidal and sand bottom communities of the northern Wadden Sea. Helgol. Meeresuntersuchungen. 39, 273-302.

Auer, B., Kiertucki, E., Arndt, H., 2003. Distribution of heterotrophic nano- and microflagellates in sediments of two small mesotrophic lakes. Archiv für Hydrobiologie 158 (1), 127-144.

Azam, F., Fenchel, T., Field, J.G., Gray, J.S., Meyer-Reil, L.-A., Thingstad, F., 1983. The Ecological Role of Water-Column Microbes in the Sea. Mar. Ecol. Prog. Ser. 10, 257-263.

Barnes, R.S.K., Hughes, R.N., 1999. An introduction to marine ecology. Blackwell Science Ltd, London, pp286.

Begon, M. E., Harper, J. L., Townsend, C. R. , 1996. Ecology. Oxford, Blackwell Science, pp738.

Berninger, U.-G., Epstein, S.S., 1995. Vertical distribution of benthic ciliates in response to the oxygen concentration in an intertidal North Sea sediment. Aquat. Microb. Ecol. 9, 229-236.

Böttcher, M.E., Hespenheide, B., Llobet-Brossa, E., Beardsley, C., Larsen, O., Schramm, A., Wieland, A., Böttcher, G., Berninger, U.-G., Amann, R., 2000. The biochemistry, stable isotope geochemistry, and microbial community structure of a temperate intertidal mudflat: an integrated study. Cont. Shelf Res. 20, 1749-1769.

Cabana, G.C., Rasmussen, J.B., 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. Letters to Nature. 372, 255-257.

Cadée, G.C., Hegeman, J., 1977. Distribution of primary production of the benthic microflora and accumulation of organic matter on the tidal flat area, Balgzand, Dutch Wadden Sea. Neth. J. Sea Res. 11, 24-41.

Capone, D.G., Kiene, R.P., 1988. Comparison of microbial dynamics in marine and freshwater sediments: Contrasts in anaerobic carbon metabolism. Limnol. Oceanogr. 33, 725-749.

Caron, D.A., Finlay, B.J., 1993. Protozoan links in food webs. Symposium Progress in Protozoology. 125-130

Dell'Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Mar. Pollut. Bull. 44, 611-622

Elton, C.S., 1927. Animal ecology. Methuen, London, pp207.

Fenchel, T., 1969. The ecology of marine Microbenthos IV. Structure and function of the benthic ecosystem, its chemical and physical factors and the microfauna communities with special reference to the ciliated Protozoa. Ophelia. 6, 1-182.

Fenchel, T., 1978. The ecology of micro- and meiobenthos. Ann. Rev. Ecol. Syst. 9, 99-121.

Fenchel, T., 1987. Ecology of Protozoa. The biology of free-living phagotrophic Protists. Science Tech, Wisconsin, pp197.

Fenchel, T., 1988. Marine plankton food chains. Ann. Rev. Ecol. Syst. 19, 19-38.

Fenchel, T., 1990. The role of protozoa in nature in terms in functional properties related to size. Zool. Sci. 7, 51-58.

Fenchel, T., Jorgensen, B.B., 1977. Detritus food chains of aquatic ecosystems: the role of bacteria . Adv. Microb. Ecol. 1, 1-58.

Findlay, R.H., Watling, L., 1998. Seasonal Variation in the Structure of a Marine Benthic Microbial Community. Mar. Ecol. 1, 23-30.

Fogg, G. E., 1998. The biology of polar habitats. Oxford University Press, pp263.

Frank, K.T., Petrie, B., Choi, J.S., Leggett, W.C., 2005. Trophic cascades in a kormerly cod-dominated ecosystem. Science. 308, 1621-1623.

Fretwell, S.D., 1977. The regulation of plant communities by food chains exploiting them. Perspect. Biol. Med. 20, 169-185.

González, J.M., Suttle, C.A., 1993. Grazing by marine nanoflagellates on viruses and virus-sized particles: ingestion and digestion. Mar. Ecol. Prog. Ser. 94, 1-10.

Gray, J.S., 1981. The ecology of marine sediments. Cambridge University Press, Cambridge, pp185.

Hagerthey, S.E., Defew, E.C., Paterson, D.M., 2002. Influence of *Corophium volutator* and *Hydrobia ulvae* on intertidal benthic diatom assemblages under different nutrient and temperature regimes. Mar. Ecol. Prog. Ser. 245, 47-59.

Hausmann, K., Hülsmann, N., 1996. Protozoology. Georg Thieme Verlag, Stuttgart, pp351.

Higgins, R.P., Thiel, H., 1988. Introduction to the study of meiofauna. Smithsonian Institution Press, London, pp488.

Hondeveld, B.J.M., Nieuwland, G., Van Duyl, F.C., Bak, R.P.M., 1994. Temporal and spatial variations in heterotrophic nanoflagellate abundance in North Sea sediments. Mar. Ecol. Prog. Ser. 109, 235-243.

Hubberten, H.-W., 1994. Reports on Polar Research No.174 The Expedition ARKTIS X/2 of the research vessel POLSARSTERN in 1994. Alfred-Wegener-Institute for Polar and Marine Research, Germany, pp186.

Jansson, B.-O., 1967. The significane of grain size and pore water content for the intersitial fauna of sandy beaches. Oikos 18:311-322

Jokat, W., 2004. 'Reports on Polar Research No.475 The Expedition ARKTIS XIX/4 of the research vessel POLSARSTERN in 2003.' Alfred-Wegener-Institute for Polar and Marine Research, Germany.

Kemp, P.F., 1987. Potential impact on bacteria of grazing by a macrofaunal deposit-feeder, and the fate of bacterial production. Mar. Ecol. Prog. Ser. 36, 151-161.

Knox, G.A., 2000. The ecology of seashores. CRC Press, London, pp 557

Kuipers, B.R., De Wilde, P.A.W.J., Creutzberg, F., 1981. Energy Flow in a Tidal Flat Ecosystem. Mar. Ecol. Prog. Ser. 5, 215-221.

Kwak, T.J., Zedler, J.B., 1997. Food web analysis of southern California coastal wetlands using multible stable isotopes. Oecologia. 110, 262-277.

Manini, E., Fiordelmondo, C., Gambi, C., Pusceddu, A., Danovaro, R., 2003. Benthic microbial loop functioning in coastal lagoons: a comparative approach. Oceanol. Acta. 26, 27-38.

McLusky, D.S., McIntyre, A.D., 1988. Characteristics of the benthic fauna. Cont. Shelves. 27, 131-154.

Mei, M.L., Danovaro, R., 2004. Virus production and life strategies in aquatic sediments. Limnol. Oceanogr. 49, 459-470.

Meyer-Reil, L.-A., Köster, M., 1993. Mikrobiologie des Meeresbodens. Fischer, Jena, pp290.

Michner, R.H., Schell, D.M., 1994. Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha K, Michner RH (eds) Stable isotopes in ecology and environmentla sciences. Blackwell scientific publications, Oxford, pp 138-157

Minagawa, M., Wada, E., 1984. Stepwise enrichment of 15N along food chains: Further evidence and the relation between d15N and animal age. Geochim. Cosmochim. Acta. 48, 1135-1140.

Montagna, P.A., 1984. Competition for dissolved glucose between meiobenthos and sediment microbes. J. Exp. Mar. Biol. Ecol. 76, 177-190.

Montagna, P.A., 1984. In situ measurement of meiobenthic grazing rates on sediment bacteria and edaphic diatoms. Mar. Ecol. Prog. Ser. 18, 119-130.

Odum, E.P., 1971. Fundamentals of ecology. Saunders Company, London, pp574.

Patterson, D.J., Larsen, J., 1991. The biology of free-living heterotropic flagellates. The systematics association Special volume. 45, 1-505.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18, 293-320.

Peterson, B.J., Howarth, R.W., 1987. Sulfur, carbon, and nitrogen isotopes used to trace organic matter flow in the salt-marsh estuaries of Sapelo Island, Georgia. Limnol. Oceanogr. 32, 1195-1213.

Pinnegar, J.K., Polunin, N.V.C., 2000. Contributions of stable-isotope data to eluidating food webs of Mediterranean rocky littoral fishes. Oecologia. 122, 399-409.

Pomeroy, L.R., 1974. The Ocean`s food web, a changing paradigm. Bio Science. 24, 499-504.

Posch, T., Arndt, H., 1996. Uptake of sub-micrometre- and micrometre-sized detrital particles by bacterivorous and omnivorous ciliates. Aquat. Microb. Ecol. 10, 45-53.

Posey, M.H., Alphin, T.D., Cahoon, L.B., Lindquist, D.G., Mallin, M.A., Nevers, M.B., 2002. Top-down versus bottom-up limitation in benthic infaunal communities: direct and indirect effects. Estuaries. 25, 999-1014.

Post, D.M., Pace, M.L., Hairston, Jr.N.G., 2000. Ecosystem size determines food-chain length in lakes. Nature. 405, 1047-1049.

Power, M.E., 1992. Top-down and bottom-up forces in food webs: do plants have primacy. Ecology. 73, 733-746.

Reineck, H.-E., 1983. Sediment and dynamical processes. In: Ecology of the Wadden Sea. Eds: Wolff, W.J., A.A. Balkema, Rotterdam, pp 49.

Reise, K., 1985. Tidal flat ecology. Springer-Verlag, Heidelberg, pp191.

Riera, P., Stal, L.J., Nieuwenhuize, J., 2000. Heavy delta15N in intertidal benthic algae and invertebrates in the scheldt estuary (The Netherlands): Effect of river nitrogen inputs. Estuar. Coast. Shelf Sci. 51, 365-372.

Sherr, E., Sherr, B., 1988. Role of microbes in pelagic food webs: A revised concept. Limnol. Oceanogr. 33, 1225-1227.

Sherr, E.B., 1988. Direct use of high molecular weight polysaccharide by heterotrophic flagellates. Nature. 335, 348-351.

Sleigh, M., 1973. The biology of protozoa. Arnold, London, pp315.

Smetacek, V., Nicol, S., 2005. Polar ocean ecosystems in a changing world. Nature, 437, 362-368.

Stonehouse, B., 1989. 'Polar ecology.' Blackie and Son, Glasgow, pp222.

Throp, J.H., Delong, M.D., 1998. Isotopic analysis of three food web theories in constricted and floodplain regions of larger rivers. Oecologia. 117, 551-563.

Tilman, D., 1999. The ecological consequences of changes in biodiversity. A search for general principles. Ecology. 80, 1455-1474.

Wagner, B., 2000. Reports on Polar Research Holovcene encvironmental history of East Greenland - evidence from lake sediments. Germany, Alfred-Wegener-Institute for Polar and Marine Research, pp93.

Wetzel, R.G., 2001. Limnology. Academic Press, London, pp1006.

Wolff, W.J., 1983. Ecology of the Wadden Sea. A.A. Balkema, Rotterdam.

List of abbreviations

ANOVA	analysis of variances
B	Basalt Lake
CON	control area 1 and 4 in Sylt Experiment
D	Duck Lake
d.w.	dry weight
D8, 10, 4, 6	Dorum in August, October, April, June
DAPI	4´,6-Diamino-2-phenylindol
DTAF	5-(4,6-dichlorotriazin-2-yl)aminofluorescein
EXC	gaze area (lugworm free) 1 and 4 in Sylt Experiment
f.c.	final concentration
FLB	fluorescently labelled bacteria
GLL	Green Lane Lake
HL	Lake near Hochstetter Fjord
HNF	heterotrophic nano flagellates
IF	Ice flow
K	Fühlinger See, Cologne
KB	beach on the Islanf of Store Koldewey
M	Melles Lake
N	Nockamixon Lake
O1; O4	dredged area 1 and 4 in Sylt Experiment
P	Schöhsee, Plön
PCA	principal component analysis
Pers. Com.	personal comment
PNF	phototrophic nano flagellates
PO	Potsdam Lake
PPI	Tetrasodiumpyrophosphate
PSU	practical salinity units
QPS	quantitative protagol staining
RDS	redundance analysis
S	Königshafen, Sylt
SB	beach on Shannon Island
sd	standard deviation
SR	Shark River Bay
TC	total carbon
TN	total nitrogen
TOC	total organic carbon
w.w.	wet weight
wc	water content

Danksagung – Acknowledgements

Bei allen die zum Gelingen dieser Arbeit beigetragen haben möchte ich mich herzlich bedanken:
Prof. Dr. Victor Smetacek, meinem Doktorvater, für die Übernahme der Begutachtung.
Prof. Dr. Ulrich Bathmann für die Übernahme des Korreferates und seine Unterstützung in formellen Angelegenheiten.
Prof. Dr. Wilhelm Hagen für sein Interesse an dieser Arbeit.
Prof. Dr. Ulrike Berninger für die Überlassung des Themas und konzeptionelle Hilfe während der Arbeit.

Der Crew der Expedition ARK XIX/4a des Forschungsschiffes „Polarstern“, für Probennahme in Grönland und eine unvergessliche Zeit.
Dr. Eelin Lim, Jonathan Vandergrift, Mark A. Randa, Darryl L'Heureux, meiner Arbeitsgruppe an der Temple University in Philadelphia, dass sie mir molekularbiologischer Methoden beigebracht haben und dafür, dass ich mit „dreckigem“ Sand im sauberen Molekularlabor arbeiten durfte. Dem DAAD für das Stipendium für diesen Aufenthalt.
Helga Schwarz, Erika Allhusen, Christiane Lorenzen, Dr. Jens Matthiessen, Dr. Klaus Valentin, Petra Schulz, Tanja Burgmer, Dr. Brigitte Auer, Dr. Nils Volkenborn, Dr. Stefanie Moorthi, Dr. Nicole Aberle-Mahlzahn, Rita Fröhlking, Dr. Tom Brey, Dr. Ingo Schewe, den Mitarbeiterinnen und Mitarbeitern des AWIs für die Hilfe bei Probenahmen, die Erlaubnis zum Arbeiten in „fremden“ Laboren und für Diskussion zu dieser Arbeit.
Mein besondere Dank geht an meine Familie, für die emotionale und finanzielle Unterstützung schon während meines gesamten Studiums, für aufmunternde Worte zu jeder Zeit bis zur letzten Minute und meinem Vater für die physikalisch genaue Überarbeitung meiner Ungenauigkeiten vor allem in der Sprache.
Und an meine Freunde, für interessante Diskussionen und Anregungen zu dieser Doktorarbeit, Korrekturhilfen, Rechenhilfen und Unterstützung in jeder Lebenslage. Insbesondere: Sabine, Bettina, Uta, Frank, Mathias, Joachim, Philipp, Georgia, Claudia, José, Julie, Christian, Carsten, Marlen, Gerald, Mauricio, Albert.
Und vor allem für den Mut, den ihr mir gegeben habt, indem ihr alle mehr an mich geglaubt habt als ich selber.
Danke ☺

Anlage zur Dissertation

Name: Karen Stumm
Anschrift: Muskaustraße 18
27711 Osterholz-Scharmbeck

Ort, Datum: Bremen 10.3.2006

Erklärung

Gem.§6 (5) Nr.1-3 PromO

Ich erkläre, dass ich Karen Stumm

1. **die Arbeit ohne unerlaubte fremde Hilfe angefertigt habe,**
2. **keine anderen, als die von mir angegebenen Quellen und Hilfsmittel benutzt habe**
3. **die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.**

_____

(Unterschrift)