Structuring effects of mesozooplankton on freshwater and marine microbial food webs

Dissertation

zur Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Christian-Albrechts-Universität

zu Kiel

vorgelegt von

Eckart Zöllner

Kiel, März 2004

Referent: Prof. Dr. K. Jürgens
Korreferent: Prof. Dr. W. Lampert .....
Tag der mündlichen Prüfung: 28.04.2004.....
Zum Druck genehmigt: Kiel, den.....

Der Dekan

TABLE OF CONTENTS

Page

GENERAL INTRODUCTION AND OUTLINE..... 3

1. Prokaryotic and eukaryotic microorganisms in aquatic systems..... 3
2. Microbial diversity and function..... 7
3. Linking classical and microbial food web models..... 10
4. Thesis outline..... 14

CHAPTER I..... 17

Cascading predation effects of *Daphnia* and copepods on microbial food web components

Abstract..... 17
Introduction..... 18
Methods..... 19
Results..... 24
Discussion..... 35

CHAPTER II..... 41

Effects of zooplankton regime on spring microbial community dynamics and a bloom of filamentous bacteria

Abstract..... 41
Introduction..... 42
Methods..... 44
Results..... 47
Discussion..... 57

CHAPTER III..... 62

Mesozooplankton (copepods, appendicularians) impact on microbial food web structure in a coastal marine system

- I. Cascading predation effects on protists and bacteria

Abstract..... 62
Introduction..... 63
Methods..... 64
Results..... 68
Discussion..... 81

CHAPTER IV 87

Mesozooplankton (copepods, appendicularians) impact

on microbial food web structure in a coastal marine system

- II. Patterns of bacterial community composition and diversity

Abstract 87
Introduction 88
Methods 89
Results 92
Discussion 108

CHAPTER V 116

Trophic cascades affect aquatic microorganisms – Synoptical remarks

Abstract 116
Introduction 117
Methods 119
Results 119
Discussion 123

SUMMARY 125

ZUSAMMENFASSUNG 129

REFERENCES 134

DANKSAGUNG – ACKNOWLEDGEMENTS 149

CURRICULUM VITAE 150

ERKLÄRUNG

General Introduction
&
Thesis Outline

GENERAL INTRODUCTION AND OUTLINE

1. Prokaryotic and eukaryotic microorganisms in aquatic systems

Bacteria are an important and integral component in all aquatic ecosystems, their metabolism accounts for large proportions of energy flux and matter turnover, they are recycling dissolved chemical components and channel them up to higher trophic levels.

The number of prokaryotes on earth was estimated to range between 4-6x10^{30} cells, thereby forming 350-550 pg of carbon (1pg=10^{15}g) which equals 60-100% of the estimated total carbon in plants (Whitman et al. 1998). This already points at the importance of prokaryotes for the global carbon budget. Bacterial production can often comprise 30-50% of the simultaneous particulate primary production (Ducklow et al. 2002) and their respiration rates can even exceed phytoplankton production in unproductive aquatic systems (Del Giorgio et al. 1997).

Since Pomeroy (1974) recognized the potential importance of microbes in the ocean´s biogeochemical cycling and Azam et al. (1983) first formulated the concept of the "microbial loop", our view and understanding of microbially mediated processes has deepened and many of the complex interactions within the microbial food web have been elucidated. Traditionally, the view of the microbial loop was based on the mediation of biogeochemical pathways (Cho and Azam 1988) and its role in carbon flux and transfer efficiencies. Nowadays, for more and more components black boxes were opened and a variety of microbial key players were identified. However, we are just beginning to understand the shaping forces and regulating factors of bacterial communities and the underlying complexity and mechanisms of temporal and spatial dynamics within the microbial food web. Being able to consume dissolved organic (and inorganic) substrates, bacteria are the central microbial food web component for the energy and matter transfer to higher trophic levels. The extraordinary diversity of metabolic pathways found in prokaryotic organisms suggest that their abundance and composition has profound implication for matter fluxes and ecosystem functioning.

Aquatic microbial food webs can be characterized by a variety of functional compartments, whereof each meanwhile has been further elucidated and consists of a large number of species, ecological niches and evolutionary strategies. Despite the numerical importance and enormous species richness, bacterial abundances in most aquatic natural habitat are astonishingly constant and show an overall amplitude of approximately 0.1-10x10^{6} cells per ml, not exceeding one order of magnitude within a given system (Jürgens and Güde 1994). Besides heterotrophic bacteria (see also 2.), autotrophic taxa can be of cardinal importance for energy and matter fluxes mainly in oceanic food webs (e.g. Ting et al. 2002). The most dominant cyanobacterial taxa are *Synechococcus* and *Prochlorococcus*, of

which the former is also a common freshwater component (Vörös et al. 1998). Usually, autotrophic forms are around ten times less abundant than heterotrophic bacteria, but, in turn, numerically more important than eukaryotic ultraphytoplankton. Nevertheless, the latter group often dominates in terms of primary production (Li 1995). Recent studies discovered that ocean cyanobacteria (*Prochlorococcus* and *Synechococcus*) exhibit distinct niche adaptations (Ferris and Palenik 1998), utilize different nitrogen sources (Moore et al. 2002) and possess very different photosynthetic light-harvesting antennas (Ting et al. 2002). Similar as their heterotrophic relatives, they are subject to viral infection (Suttle and Chan 1993) and grazing by heterotrophic nanoflagellates (Dolan and Simek 1998) and ciliates (Christaki et al. 1999).

In general, auto- and heterotrophic bacteria are embedded in a complex web of interactions with numerous taxa of ciliated and flagellated protists of the micro- and nano-sized fraction as well as with phytoplankton and viruses (see Fig. 1).

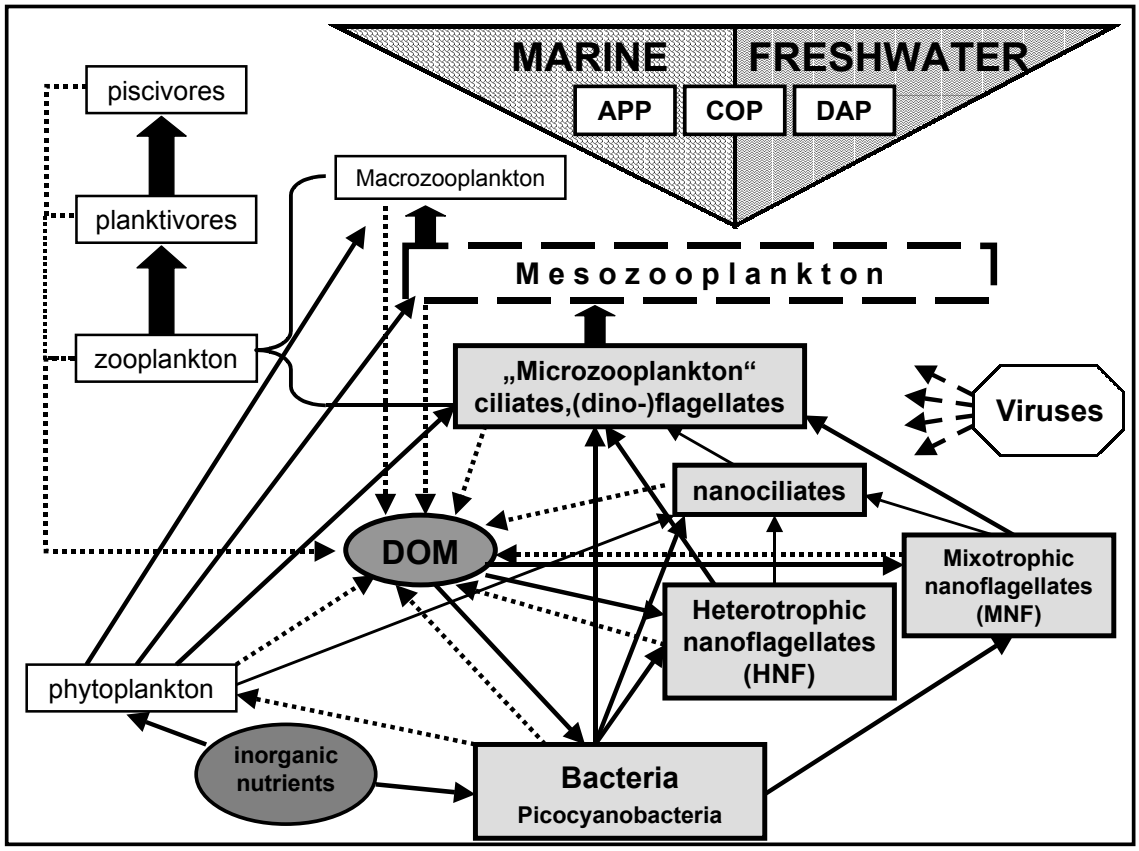


Figure 1:
Simplified scheme of a pelagic microbial food web. The experimental manipulation of mesozooplankton communities (APP=appendicularians, COP=copepods, DAP=*Daphnia*) is shown as triangles. Components coloured light grey were analyzed in this thesis, arrow means “C-transfer by uptake”, dotted arrow means “releases”.

Global protist biodiversity is almost impossible to assess with the present knowledge (Foissner 1999) and is still a matter of debate. Foissner (2000) points out that local ciliate

diversity can not be used for estimating global diversity, as many regions are only poorly investigated and many taxa are still undescribed, whereas Fenchel et al. (1997) as well as Finlay (2002) have a contrasting point of view. Due to nearly unlimited protist dispersal and the existence of many ubiquitous species they assume global species number to be relatively low and local species richness to be always sufficient to drive ecosystem functions.

Nevertheless, ciliates are an abundant and species-rich component of most microbial food webs. Diversity of free-living ciliates is still intensely debated and estimates vary between approximately 3000 (Finlay et al. 1998) and probably about 30000 morphospecies (Foissner 1999). Doubtlessly, they occupy important positions in most freshwater and marine pelagic habitats, where they can exhibit conspicuous seasonal successions (Müller et al. 1991) or distinct spatial patches in their fine- to mesoscale (m-km) distribution (Montagnes et al. 1999). Aquatic ciliates form a functionally and ecologically diverse assemblage as different ciliate species may be autotrophic, mixotrophic, or primary and/or secondary consumers (Pierce and Turner 1992). They can be the dominant group (up to 100 %) of microzooplankton in temperate coastal waters (Pierce and Turner 1992) and act as a link between small phytoplankton and larger zooplankton (Reid et al. 1991). Ciliates are able to graze large proportions of primary production in many aquatic systems. For example, 32-55% and 20-60% of the annual primary production was consumed by ciliates in Disko Bay and the Kattegat, respectively (Levinsen and Nielsen 2002). In the Baltic Sea small planktonic ciliates (less or equal to 30 µm) cleared 50-125% of the water volume per day in summer (Setälä and Kivi 2003). The trophic role of ciliates can be further extended from algivory to bacterivory. Pelagic ciliates from marine and freshwater systems were observed to feed on bacteria at high rates (Sherr and Sherr 1987) and are sometimes even the dominant bacterial grazers (Kisand and Zingel 2000, Šimek et al. 2000).

Oligotrichida probably constitute the numerically most important group within the pelagic ciliate community. These filter-feeders mostly belong to the microzooplankton and are well known as voracious feeders on nanoplanktonic but also on picoplanktonic organisms (Šimek et al. 2000). A mass occurrence of planktonic oligotrichous ciliates, for example, was reported from a bay in southern Norway (Dale and Dahl 1987).

Flagellate protozoa, in particular nanoflagellates, are ubiquitous in all aquatic environments (in Laybourn-Parry and Parry 2000) and by feeding on heterotrophic and phototrophic bacteria (phagotrophy) they play a cardinal role in planktonic carbon and nutrient fluxes. This importance further increases as they can also take up dissolved and particulate organic carbon (osmotrophy) of mainly colloidal sizes (Christoffersen et al. 1996). Presently, there is no consensus on the geographic distribution and the overall diversity of HNF. Patterson and Lee (2000) estimated no more than 3000 mostly cosmopolitan species. As HNF are morphologically difficult to distinguish and due to methodological constraints

many studies show a lack of taxonomic resolution (Arndt et al. 2000). Recent application of molecular tools like 18S-rDNA-targeted fingerprinting (Moon-van der Staay et al. 2000, 2001, Massana et al. 2002) have revealed an enormous marine eukaryotic diversity even among picoplankton, a size-fraction (2-3 µm) which can be a main contributor to both primary production and biomass in open oceanic regions. Most newly found sequences were related to important marine phyla such as prasinophytes, haptophytes, dinoflagellates, stramenopiles (chromista, heterokonta), choanoflagellates and acantharians (Moon-van der Staay et al. 2001). In freshwater dominant HNF groups are heterokont taxa like chrysomonads and bicosoecids as well as choanoflagellates, kathablepharids and a significant proportion of Protista *incertae sedis* (Arndt et al. 2000). Besides taxonomical there is also a high functional diversity among HNF. They have developed diverse feeding strategies and behaviors (attached, free-living, selective interception feeding etc., e.g. Sanders 1991, Arndt et al. 2000), and exhibit nutrition modes ranging from pure heterotrophy through various degrees of mixotrophy to pure autotrophy (Laybourn-Parry and Parry 2000).

Abundances in different pelagic habitats can vary from roughly about 20 to 20000 cells per ml and are mostly related to the abundance of bacteria (for review see Sanders 1992). Their potential to grow at high rates (e.g. up to 1.76 d^{-1} in Lake Constance, Weisse 1997) and thus follow bacterial growth, enables them to exert strong trophic impact on bacterial communities in freshwater and marine systems (Jürgens and Güde 1991, Strom 2000, Massana and Jürgens 2003). Many flagellate taxa show a distinct feeding selectivity in terms of bacterial phenotypic traits such as swimming speed, biochemical surface composition (Matz et al. 2002) and, last not least, size as probably the most important trait. Although the particle spectrum ingestible by various groups of flagellates can be large (Arndt et al. 2000), most heterotrophic nanoflagellates preferentially feed on bacteria of 1-3 µm in length (Šimek and Chrzanowski 1992). Due to their selective feeding characteristics, HNF can impose strong structuring impacts on planktonic bacterial assemblages (González et al. 1993, Jürgens et al. 2000, Beardsley et al. 2003), cause community shifts and trigger bacterial feed-back mechanisms, e.g. such as the formation of morphotypes with reduced protist grazing susceptibility (e.g. Jürgens and Güde 1994).

Viruses have long been regarded as a negligible component in aquatic microbial food webs. Since about one decade, there is increasing evidence that virally induced lysis can be a significant factor in freshwater and marine populations of auto- as well as heterotrophic microbes (Suttle and Chan 1993, Thingstad et al. 1993a, Sommaruga et al. 1995, Sime-Ngando 1997), but results are not consistent and the degree of relevance seems to be rather variable. For example, Maranger and Bird (1995) found the virus-to-bacteria ratio to be significantly higher in freshwater (22.5:1) than marine environments (2.5:), but from many systems this ratio is approximately 10:1 and viral lysis accounts for approximately 10-50% of

bacterial mortality (Guixa-Boixereu et al. 1999, Thingstad 2000b). The relative importance of protozoan predation and viral lysis as causes for bacterial mortality has been examined in several studies (e.g. Weinbauer and Höfle 1998, Tuomi and Kuuppo 1999, Šimek et al. 2001) and the constraints and implications evaluated by models (e.g. Thingstad 2000b).

Differential viral impact was observed at different spatial and temporal scales as well as in terms of bacterial community composition. Weinbauer and Höfle (1998) found depth-dependent changes in the partitioning of virus- and grazing-induced bacterial mortality and Šimek et al. (2001) even assumed protistan grazing to stimulate viral activity. Apparently, the overall density of bacteria was either not affected by phages (Middelboe et al. 2001) or their quantitative impact was much smaller than their functional importance (Sime-Ngando 1997). At the level of individual bacterial host species or strains, viruses often show temporary effects and can strongly influence the clonal diversity (Middelboe et al. 2001). Recent investigations using pulsed-field gel electrophoresis fingerprinting showed some annual viral community changes (Wommack et al. 1999), but a general community stability over shorter time periods of days to months (Riemann and Middelboe 2002). Other studies proved virus dynamics in the NW Mediterranean Sea to be more closely related to *Synechococcus* than to heterotrophic bacteria. Viral-induced bacterial mortality turned out to be rather low (approx. 10% of production) (Bettarel et al. 2002) and flagellates were ascribed a larger importance in consuming bacterial production (Bettarel et al. 2003).

2. Microbial diversity and function

Understanding microbial diversity, the number of species, their patterns of substrate utilization and the underlying mechanisms driving population dynamics constitute a main objective in microbial ecology. However, the lack of diverse external characters and difficulties in classifying microorganisms has hampered our understanding for a long time.

Early attempts to enumerate and cultivate heterotrophic microorganisms (Jannasch and Jones 1959, Kogure et al. 1979) yielded large discrepancies between numbers countable by microscopy and those producing colonies by standard plating techniques (only 0.01 to 0.1% of oceanic marine bacterial cells) and led Staley and Konopka (1985) to coin the expression of “the great plate count anomaly". Moreover, the imitation of optimal growth conditions in vitro remained difficult and conflicted for a long time with the complex bacterial demands in terms of substrates, temperature and other biotic and abiotic factors. Of course, the development of new techniques and high-throughput cultivation approaches have considerably improved bacterial culturability and increased the number of cultured species (e.g. in Connon and Giovannoni 2002).

Within the last two decades a variety of new invaluable tools and methodological approaches based on bacterial 16S-RNA genes have been developed and applied in order to

unveil the diversity of complex natural microbial communities not yet able to be cultured. These new molecular methods are generally culture-independent, have allowed for remarkable progress and have fundamentally impacted the emerging phylogenetic view of bacterial diversity (Hugenholtz et al. 1998).

The most revealing methods have been cloning and sequencing of 16S rRNA genes, community fingerprinting and fluorescent in situ hybridization (FISH) (Fuhrman et al. 2002). The latter technique even allows for relating phenotype with genotype (Amann et al. 1995). While cloning and sequencing approaches normally yield the maximal number of taxa per investigated unit, fingerprinting methods are comparably fast tools which are more appropriate to monitor community changes, e.g. due to environmental perturbations. DNA fingerprinting techniques which were applied to investigate bacterial community composition were Amplified Ribosomal DNA Restriction Analysis (ARDRA, e.g. Tiedje et al. 1999, Denaturing Gradient Gel Electrophoresis (DGGE, e.g. Muyzer et al. 1993, Muyzer and Smalla 1998), Automated Ribosomal Intergenic Spacer Analysis (ARISA, e.g. Yu and Mohn 2001), Single Strand Conformation Polymorphism (SSCP, e.g. Schwieger and Tebbe 1998) and Terminal Restriction Fragment Length Polymorphism (T-RFLP, e.g. Liu et al. 1997, Moeseneder et al. 1999, Dollhopf et al. 2001).

Amongst these, DGGE is probably the most widespread and most often used approach of genetic fingerprinting of microbial assemblages. Genomic DNA is extracted from a complex microbial assemblage and PCR-amplified with primers specific for a molecular marker (e.g. 16S rRNA gene). The obtained PCR product is separated in a polyacrylamide gel containing a linear gradient of DNA denaturants (Urea, Formamide). Sequence variation among the different DNA molecules influences the melting behaviour, and therefore molecules with different sequences will stop migrating at different positions in the gel.

Since Woese (1987) presented a new phylogenetic classification based on rRNA and containing the 3 domains Bacteria, Archaea and Eukarya, an enormous number of new taxa and lineages has been described, of which a vast majority still remains uncultivable. In addition to the 12 bacterial phyla distinguished in 1987, another 14 phyla with cultivated representatives and another 26 phyla not containing any known cultivated representative have been recognized (Rappe and Giovannoni 2003).

The magnitude of prokaryotic species richness (domains Bacteria and Archaea) is still intensely discussed and also a question of scales and the respective methods applied in diversity analysis (e.g. Curtis et al. 2002). Maximal diversity estimates peaked in the range of millions of species (Torsvik et al. 2002). However, prokaryotic diversity can range over four orders of magnitude among habitats (Nee 2003) and thereby is orders of magnitude less in aquatic environments than in sediments and soils (Torsvik et al. 2002). Although maximal numbers of bacterioplankton taxa in one water sample measures by T-RFLP can reach up to

about 100 (Philippine coral reef, Fuhrman et al. 2002) and would have been likely higher when including molecular cloning, a dereplication of GenBank sequences yielded “only” 1,117 unique ribotypes of marine bacteria and led Hagström et al. (2002) to the conclusion that the apparent marine bacterioplankton species richness is relatively low. These statements are consistent with the finding of often very widespread taxa (Fuhrman et al. 2002) and the global distribution of several marine planktonic prokaryote SSU rRNA gene phylotypes (Rappé et al. 2000).

Although some subgroups seemed to be mostly restricted to freshwater systems (e.g. Beta-proteobacteria) and others dominate in marine habitats (Glöckner et al. 1999), there were also numerous overlaps, where e.g. coastal bacterioplankton communities contained SSU rRNA gene lineages shown previously to be prevalent in freshwater habitats (Rappé et al. 2000). Nevertheless, Zwart et al. (2002) found specific planktonic bacterial communities in rivers and lakes distinct from bacteria in neighbouring environments such as soil and sediments and pointed out that these planktonic bacteria are distributed in diverse freshwater ecosystems around the world (Zwart et al. 1998).

Amongst heterotrophic marine bacteria, alpha-Proteobacteria comprise one of the largest fractions (e.g. Hagström et al. 2002) and include two clades, SAR11 and *Roseobacter*, which account for 26 and 16% of 16S ribosomal RNA gene clones retrieved from marine bacterioplankton (Giovannoni and Rappé 2000). 16S rRNA gene clones The related to the SAR11 clade were among the first groups organisms to be identified by cultivation-independent approaches based on rRNA gene cloning and sequencing (Giovannoni et al. 1990). They can represent as much as 50% of subtropical surface bacterioplankton communities and among the most successful organisms on Earth (Morris et al. 2002) and Rappé et al. (2002) recently succeeded in cultivating members of this clade.

Also just lately, a cluster affiliated to the *Roseobacter* clade was newly discovered and turned out to be numerically very important from temperate to polar regions (Selje et al. 2004).

The relationship between diversity and ecosystem functioning is still far from being understood (e.g. Finlay et al. 1997, Hulot et al. 2000). Bacterial biodiversity can potentially impact on ecosystem stability, food web structure and biogeochemical dynamics in terms of process magnitude and maintenance. However, at least for soil microbial communities there is evidence of a high functional redundancy, and evidence for low impact of bacterial diversity on the magnitude of ecosystem processes came from studies by Yin et al. (2000), but this topic should be also addressed in aquatic microbes. The development of recent promising and successful techniques to link between bacterial function and diversity (MICROFISH: Ouverney 1999, STARFISH: Fuhrman 2002), should enable to approach this question, but respective methods are rather difficult to apply and data basis is still scarce.

3. Linking classical and microbial food web models

3.1 The importance of biotic interactions

A variety of environmental factors is known to influence and regulate bacterial communities (e.g. Øvreås 2000) with regard to temporal and spatial changes in biomass, activity as well as quantitative and qualitative composition. Among these governing parameters, the availability of nutrients and dissolved organic carbon are important determinants (e.g. Lindström 2000, Crump et al. 2003). Other factors affecting bacterial growth conditions like salinity (Langenheder et al. 2003, Crump et al. 2004) were also found to affect community composition. The relative importance of bottom-up and top-down forces in structuring and regulating (microbial) food webs has extensively been discussed. Studies still yield conflicting results and often point at interactions between both aspects. Various food web compartments were investigated for relevant regulating factors, such as the fish-zooplankton-phytoplankton-nutrients cascade (Brett and Goldman 1997) or heterotrophic nanoflagellates (Gasol 1994). Moreover, the regulation of bacterial growth (Pace and Cole 1994, Pace and Cole 1996, Gasol et al. 2002) and bacterial community composition (Muylaert et al. 2002) were subject for bottom-up versus top-down studies.

In recent years, a number of direct and indirect biotic and trophic interactions turned out to significantly affect bacterial communities. These interactions take place between species, functional groups and trophic levels. The whole aspect of competitive interactions I will mention only briefly in this chapter. Bacteria and phytoplankton partly compete for the same inorganic nutrients (Thingstad et al. 1993b, Havskum et al. 2003), but bacteria are also favored by exudates of algae and sometimes even closely linked to the occurrence of certain algal species or blooms (Zubkov et al. 2001). Moreover, bacterioplankton species are competitors for the same substrates and sometimes even interact antagonistically (Long and Azam 2001). The dominant compartments of the microbial food web specified above are at the same time potential causes for bacterial mortality.

Besides viral infection and competitive interactions, predation by numerous taxa of protists is a major selective force shaping and controlling aquatic microbe communities (Sherr and Sherr 2002). The general significance, distribution and diversity of interactions in the microbial world have been extensively discussed in the dissertation of Matz 2001. Amongst these, the interaction between protists (mainly ciliates and nanoflagellates) and bacteria constitute a core component of microbial food webs and is characterized by a huge variety of adaptations, foraging strategies and physiological as well as behavioural and phenotypic traits (e.g. Jürgens and DeMott 1995, Jürgens and Matz 2002). Diverse morphological and non-morphological mechanisms are responsible for bacterial resistance traits against protist grazing (mainly HNF). Size is probably the most important factor affecting the susceptibility to HNF predation (Gonzalez et al. 1990, Pernthaler et al. 1996).

When bacterial cells are below or above a certain threshold characterizing the optimal prey item size for grazing-selective flagellates, they become less vulnerable due to increasing handling time or ingestion being impossible.

In recent years, other modes of grazing resistance and size-independent resistance traits such as cell surface properties, motility, capsules and exopolymers received more attention (Matz and Jürgens 2001, Matz et al. 2002) and even differential digestion and toxicity (Boenigk et al. 2001, Matz 2001) turned out to be relevant determinants for the coexistence of bacteria and their predators. The regulation of grazing resistance is still extensively discussed, such as the impact of resources on resistance traits (Matz and Jürgens 2003) and the relative contribution of polymorphism and induction to the adaptive responses of bacterial communities towards protist grazing (Šimek et al. 1997, Hahn et al. 1999, Hahn and Höfle 2001).

Differential feed-back mechanisms at the level of bacteria thus further complicate the patterns of community dynamics and the response to top-down effects of other planktonic organisms belonging to higher trophic levels. These patterns can contribute to explain why plankton communities do not settle to equilibrium in a rather constant environment. Apart from the fact that even apparently homogeneous environments like the pelagic habitat show some spatial complexity, various competition and predation models suggest that interactions between multiple species will give rise to oscillations and chaotic population dynamics (Huisman and Weissing 1999, Huisman and Weissing 2001, Scheffer et al. 2003).

In many studies of planktonic food webs bacteria are still treated as a “black box”, not accounting for the heterogeneous structure and dynamics within the bacterial assemblage. Among the current concepts and models dealing with aquatic pelagic food webs, the focus often was on production mechanisms of dissolved organic carbon and the carbon flow through food webs (Nagata 2000). For such approaches, it was mostly inevitable to integrate over major food web components as “total bacteria” or “total protozoa”. This bottom-up perspective in the conceptual view of food web control is more common (Thingstad 2000a), for example in order to estimate how much of the available carbon pool would be transferred to fish production. In general, top-down approaches have rarely been applied with respect to marine planktonic systems (Verity and Smetacek 1996) and, for example, marine ecologists have almost never attempted top-down explanations of phytoplankton size structure (Sommer and Stibor 2002). The same holds for heterotrophic paths of the (especially marine) pelagic food web, where cascading top-down impact of higher trophic level organisms such as mesozooplankton has rarely been investigated. This could be taken as an example for advances made in freshwater aquatic ecology and a probably more progressive view on mechanisms of food web structuring when being compared with the marine counterpart of this discipline.

3.2 The role of higher trophic levels – mesozooplankton as a pelagic interface

Zooplankton organisms of the size-fraction 0.2 - 2 mm are referred to as mesozooplankton and constitute a major component of all aquatic pelagic systems. While copepods and cladocerans occur in freshwater as well as marine habitats, tunicates such as appendicularians and salps are restricted to marine waters. These organisms directly link phytoplankton with larger zooplankton and fish and thus have long been regarded as exclusively herbivorous. So far, many studies focussed on zooplankton-phytoplankton-interactions (Calbet 2001), which formed the basis for most food web concepts from early food chain models (Steele 1974) to the extended view including the microbial loop (Azam 1983). In order to further characterise and compare the trophic impact of dominant mesozooplankton groups on lower trophic levels, their ecophysiological peculiarities and feeding characteristics should be of central interest. Key features of these dominant zooplankton groups are summarised more in detail in Sommer and Stibor (2002).

Planktonic copepods are primary consumers in the ocean and are perhaps the most numerous metazoans on earth, directly affecting pelagic fish populations and the biological pump of carbon into the deep ocean (Ohman and Hirche 2001). Also in freshwater systems they can temporarily contribute significantly to mesozooplankton. The two most important subgroups, calanoids and cyclopoids copepods, are characterized by a complicated life-cycle (obligate sexuality, larval nauplius stages, subadult copepodid stages), slow somatic growth and distinct feeding modes. In marine systems, calanoid copepods normally constitute the majority within the copepod assemblage. Despite differences in foraging strategies with respect to swimming behaviour (Tiselius and Jonsson 1990), copepods prey on larger particles and thereby show conspicuous feeding selectivity in terms of size and chemical properties. All copepod groups can strongly affect lower trophic levels (e.g. Kiørboe 1997), on one hand by producing substrates (sloppy feeding, excretion, defecation) (Møller and Nielsen 2001, Møller et al. 2003), but mainly by direct predation. There is more and more evidence that copepods, previously regarded as herbivorous, can be rather considered omnivorous (Kleppel 1993) and that there exists a strong coupling between copepods and protists in fresh waters (Wickham 1995, Burns and Schallenberg 2001b, Yoshida et al. 2001) as well as in marine systems (Hansen et al. 2000, Levinsen et al. 2000).

Cladocerans are likely the most well-studied group of zooplankton. They are characterized by high metabolic rates (sexual maturity within a few days under optimal conditions) and simple life cycles without larval stages and with parthenogenetic reproduction through most of the year (Sommer and Stibor 2002). Freshwater cladocerans of the dominant genus *Daphnia* are unselective filter-feeders (DeMott 1982, Brendelberger et al. 1986), which feed on prey items ranging from ~1 to 30 µm in size (e.g. Geller and Müller 1981). Daphnids performs diel vertical migrations (Lampert and Taylor 1985) and can efficiently control

phytoplankton (Lampert et al. 1986). Furthermore, *Daphnia* can be the main reason for mortality of protozoans and rotifers in lakes (Jack and Gilbert 1994, Pace and Vaqué 1994) and even impact bacterial communities (e.g. for review see Jürgens 1994, Jürgens et al. 1994b, Degans et al. 2002) phytoplankton species composition (Sommer et al. 2003b).
There are no real counterparts of *Daphnia* in marine systems, and much less is known about the only three marine cladoceran genera *Evadne*, *Podon* and *Penilia*, of which only the last one resembles *Daphnia* in terms of nutrition. Slow escape reaction from visually oriented predators is suggested to explain why cladocerans in general are of minor importance in marine food webs (Verity and Smetacek 1996).

Pelagic tunicates (salps, appendicularians, pyrosomas and doliolids) are restricted to marine systems and are referred to as gelatinous zooplankton (e.g. Sommer and Stibor 2002). They are ubiquitous filter-feeders (wide range of particle sizes, depending on body size, filtering structure and taxonomic group, Acuña 2001) and are generally characterized by a complex life cycle and very high potential growth rates (e.g. Nakamura et al. 1997). The knowledge on the importance of appendicularians in marine planktonic food webs is still limited (Calbet 2001, Lopez-Urrutia et al. 2003) and more measurements of their feeding at different temporal and spatial scales are required to ascertain their role as grazers in pelagic food webs (Lopez-Urrutia et al. 2003).

However, by high clearance rates at maximal population densities, appendicularians like *Oikopleura dioica* can exert significant grazing pressure (Alldredge 1981). Microphageous appendicularians revealed high retention efficiencies for very small particles (0.2 – 6 µm) (Fernandez et al. 2004), thus, by feeding on prokaryotes and colloidal particles, suggesting a crucial role in marine carbon fluxes. Interestingly, recent studies found calanoid copepods to prey directly on *Oikopleura dioica* (Lopez-Urrutia et al. 2004), indicating further complicated direct and indirect interactions between these two mesozooplankton groups and their microbial prey organisms. For future marine zooplankton research, Bathmann et al. (2001) emphasised the importance to focus on elucidating the trophic role of certain key species like *Calanus finmarchicus* and *Oikopleura dioica.*

Interactions of producers and consumers can have profound consequences for the biogeochemistry of the oceans and also of lakes. Hence, numerous studies have discussed the underlying mechanisms structuring food webs and have compared the relative importance of top-down vs. bottom-up forces and factors (e.g. Brett and Goldman 1997, Gasol et al. 2002, Muylaert et al. 2002). As the central role of protozoa in the aquatic food chain not only consists of being a predator of bacteria and other protists, but also a prey item for larger zooplankton, one should assume qualitative and quantify alterations at higher trophic levels to cascade down to lower food web components.

Describing a special case of indirect biotic interactions with important implication at the ecosystem level, the trophic cascades concept (Paine 1980, Carpenter et al. 1985, Shurin et al. 2002) gained substantial attention and proved to hold true for diverse but mainly aquatic food webs (Pace et al. 1999, see also Chapter V). This indirect trophic interaction can be regarded as the inverse patterns of abundance or biomass across more than one trophic link in a food web (Pace et al. 1999). There is some experimental evidence that complex cascading influences of higher-order consumers can elicit indirect responses of lower trophic levels, e.g. fish affected phytoplankton via zooplankton (Brett and Goldman 1996). However, there were also strong indications that mesozooplankton impact on microbial food webs and bacteria via predatory cascades (e.g. Calbet and Landry 1999, Katechakis et al. 2002). Interestingly, various factors can prevent or modify trophic cascades, such as high trophic level heterogeneity and the substitutability of species (Strong 1992, Persson 1999) or cause effects on composition rather than on biomass (Tessier and Woodruff 2002). Consequently, one could expect various possible species and community level scenarios depending on food web structure, the degree of omnivory, feeding selectivity and potential feed-back mechanisms (e.g. reduced grazing susceptibility).

4. Thesis outline

Only few studies exist from marine habitats conducting experimental food web manipulations on a larger scale (Koshikawa et al. 1999, Graneli and Turner 2002), mostly dealing with changes in the nutrient regime (Lebaron et al. 2001b, Schäfer et al. 2001, Jacquet et al. 2002 Joint et al. 2002). Up to now there has been no study directly comparing freshwater and marine microbial food webs in response to mesozooplankton grazing impact using the same basic experimental setup and methodological approaches. Thus, one major aim of this thesis is to gain general evidence from freshwater and marine systems on the structuring effects of different mesozooplankton groups on population dynamics, activity and diversity of lower trophic levels and functional components. In order to elucidate general unifying or differing patterns in mesozooplankton impact, large-scale mesocosm experiments were performed in selected marine and freshwater habitats using the same experimental design, manipulations of mesozooplankton composition and density, as well as the same set of methods. In these experiments, ecologically very relevant and abundant taxa were included, such as *Daphnia* and *Eudiaptomus* in freshwater and key species like *Calanus finmarchicus* and *Oikopleura dioica* in marine systems.

One major emphasis was put on the comparison of mesozooplankton groups with differing feeding selectivity and foraging strategies (e.g. filter feeder versus selective predator). According to these differences, one would expect differently cascading direct and

indirect impacts on functional components of the respective microbial food webs. Extending this hypothesis further, one would assume mesozooplankton with similar nutrition strategies, like filter-feeding *Daphnia* sp. in lakes and appendicularians in the marine habitat or omnivorous copepods in both systems, to be comparable in terms of their trophic effects.

The main focus within the presented mesocosm studies I put on temporal responses as well as changes along gradients of zooplankton density. Thereby, by performing experiments in spring and summer food web scenarios, the aspect of seasonal differences and plankton peculiarities within the same system was accounted for. For reasons of clarity, several additional experiments and mesocosm treatments were not included in this thesis or were only partly touched, such as the impact of nutrient additions, the shift of nutrient ratios (N:P, marine summer experiment) or the impact of food web alterations on dynamics of DOC (dissolved organic carbon) and DOC degradation. Furthermore, some functional groups within the microbial food webs were either of minor numerical importance within the studied systems at the time of investigation (like heterotrophic dinoflagellates and sarcodines), were not sampled (viruses) or counted, but not included for reasons of a reduction of food web complexity (eukaryotic picoplankton).

The chosen systems and study sites (mesotrophic Schöhsee and a fully marine Norwegian fjord, for closer descriptions and images, see chapters I and III) provided very different constellations of nutrient regime and plankton composition. Figure 1 displays a simplified scheme of the main functional microbial food web compartments with those components marked in light grey whose response to mesozooplankton constituted the central issue of this thesis. My aim in this thesis was to comprehensively illustrate the impact modes of different mesozooplankton groups on freshwater and marine microbial food webs and to characterise their complex interactions and response characteristics to alterations at higher trophic levels. In a variety of evaluation approaches and by applying diverse analyses on a broad methodological basis, I will elucidate patterns of microbial food web dynamics ranging from single-taxon to community level responses. The first two chapters will deal with freshwater experiments performed in summer and spring, while the next two will present results obtained from marine experiments.

In **chapter I**, I will demonstrate major outcomes of a detailed enclosure study in mesotrophic Schöhsee, comparing the contrasting effects of *Daphnia* and copepods on the microbial food web during the summer plankton situation. Besides a more detailed view on the ciliate community, this investigation focussed on the density-dependent effects of filter-feeding versus selective particle-catching mesozooplankton and attempted to include not only quantitative variations in bacterioplankton, but also bacterial function, activity and community composition.

Chapter II deals with a mesocosm experiment in the same lake (Schöhsee) in spring and puts the aspect of temporal microbial food web succession and the phenomenon of a mass development of grazing-resistant heterotrophic filamentous bacteria in the focus of investigation. The unmanipulated lake as well as selected zooplankton treatments with experimentally induced *Daphnia* and protozoan dominance were monitored over a period of 25 days and under phosphorus-depleted conditions. Besides tracing filament formation at the general level of bacterial morphotypes, the application of a newly-designed taxon-specific oligonucleotide probe made it possible to better resolve the heterogeneity inherent in filamentous bacterial communities. The emergence of potentially grazing-resistant bacterial filaments will be discussed in the light of predation and abiotic factors.

In **chapter III**, I seek to demonstrate temporal and density-dependent dynamics within marine microbial food webs due to mesozooplankton manipulation in a set of 3 independent large-scale mesocosm experiments (5 copepod density gradients) in spring and summer, thereby including functional groups of protists, the abundance of auto- and heterotrophic picoplankton as well as bacterial activity. The cascading trophic impact of copepod communities composed of small and large (*Calanus finmarchicus*) will be shown as well as the aspect of dominance of either filter-feeding *Oikopleura dioica* or copepods. Finally, as a side aspect, effects of enrichment with inorganic nutrients will be discussed in the context of food web constellation.

In **chapter IV**, I will further extend the view on marine microbial food web responses and extensively analyse qualitative and quantitative changes in marine bacterial community composition. Moreover, I will elucidate patterns of bacterial diversity and community similarity in relation to the observed food web responses described in chapter III, at the level of both community subgroups and single taxonomic units.

Finally, **chapter V** synoptically summarises the importance of copepod-mediated trophic cascades for composition and function of marine and freshwater microbial food webs.

I

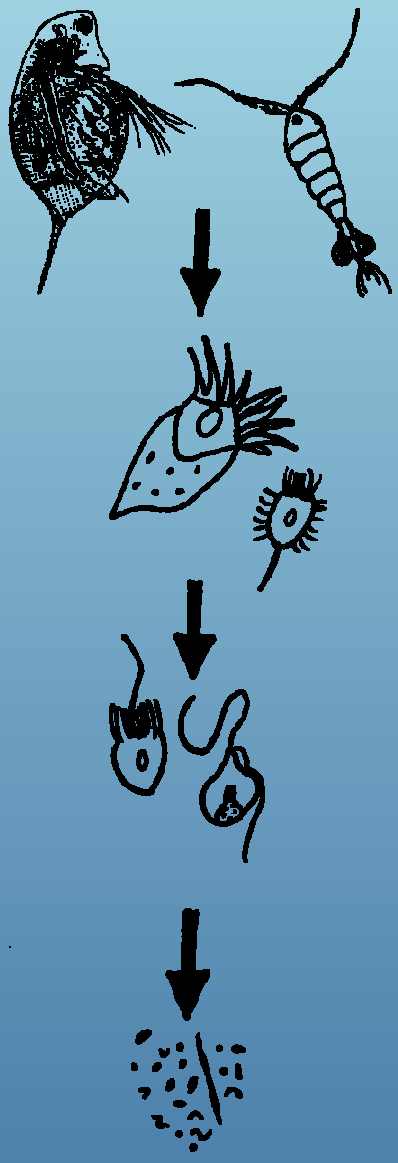
Chapter 1

Lake

– microbial food

web in summer

Daphnia vs. copepods



Cascading predation effects of *Daphnia* and copepods on microbial food web components

ABSTRACT

We performed a mesocosm experiment to investigate the structuring and cascading effects of two predominant crustacean mesozooplankton groups on microbial food web components. The natural summer plankton community of a mesotrophic lake was exposed to density gradients of *Daphnia* and copepods. Regression analysis was used to reveal top-down impacts of mesozooplankton on protists and bacteria after days 9 and 15.

Selective grazing by copepods caused a clear trophic cascade via ciliates to nanoplankton. Medium-sized (20-40 µm) ciliates (mainly *Oligotrichida*) were particularly negatively affected by copepods whereas nanociliates (mainly *Prostomatida*) became more abundant. Phototrophic and heterotrophic nanoflagellates increased significantly with increasing copepod biomass, which we interpreted as an indirect response to reduced grazing pressure from the medium-sized ciliates.

In *Daphnia*-treatments ciliates of all size classes as well as nanoflagellates were reduced directly but the overall predation effect became most strongly visible after 15 days at higher *Daphnia* biomass.

The response in bacterioplankton involved only modest changes in bacterial biomass and cell-size distribution along the zooplankton gradients. Increasing zooplankton biomass resulted either in a reduction (with *Daphnia*) or in an increase (with copepods) of bacterial biovolume, activity and production. Patterns of bacterial diversity as measured by PCR-DGGE (Denaturing Gradient Gel Electrophoresis) showed no distinct grouping after 9 days, whereas a clear treatment-coupled similarity clustering occurred after 15 days.

The experiment demonstrated that zooplankton-mediated predatory interactions cascade down to the bacterial level but also revealed that changes occurred rather slowly in this summer plankton community and were most pronounced with respect to bacterial activity and composition.

INTRODUCTION

Planktonic bacteria are regulated by the availability of inorganic and organic nutrients ("bottom-up-control"), by bacterivorous protists ("top-down-control"), and by viral lysis (Thingstad and Lignell 1997). In recent years it has become evident that the classical grazer-food chain and the microbial food web are linked by several direct and indirect interactions. Most planktonic protists are within the prey size spectrum of different metazooplankton groups (Carrick et al. 1991, Arndt 1993, Sanders and Wickham 1993). Moreover, cladocerans, especially *Daphnia* spp., can sometimes replace protozoans as the major bacterial consumer in freshwater lakes (Pace 1990, Jürgens 1994). Because bacteria mediate key pathways in global biogeochemical cycles we should assume that factors controlling biomass, production and turnover rates of microorganisms are also factors that regulate key functions at the ecosystem level (Cotner and Biddanda 2002).

Grazing by different mesozooplankton groups (zooplankton with a body size of 0.2-2 mm), consisting of crustacean taxa like cladocerans and copepods, is an important factor shaping the autotrophic and heterotrophic communities at lower trophic levels. In particular, filter-feeding cladocerans (e.g. *Daphnia* spp.) can cause order-of-magnitude biomass reductions in phytoplankton (Lampert et al. 1986) and both can affect phytoplankton species composition (Vanni and Temte 1990, Sarnelle 1993).

Although a wide overlap in the food spectrum of cladocerans and copepods is generally found, the minimal sizes for food particles ingested by most copepods are clearly larger than for cladocerans (Gliwicz 1980) and copepods can select easily between food items (DeMott 1988). Consequently, these mesozooplankton guilds are expected to exert different predation impacts on heterotrophic microorganisms. Although both copepods and cladocerans occur in most lakes, there have been only a few direct comparisons of their consumer effects on microbial food webs (Wickham 1998, Burns and Schallenberg 2001a; Yoshida et al. 2001). Because of the overwhelming predation effects of large-sized cladocerans, particularly daphnids, *Daphnia*-based studies constitute the basis of most current models of metazoan effects on microorganisms in freshwater systems (see reviews in Porter et al. 1988, Riemann and Christoffersen 1993, Jürgens 1994). Being a relatively unselective filter feeder, *Daphnia* can exert strong top-down impact on phytoplankton as well as on the protozoan and bacterial assemblages. In contrast, relatively little is known about the potential cascading trophic interactions proceeding, via direct and indirect effects, from copepods to bacteria. The selective predatory impact of cyclopoid and calanoid copepods has been demonstrated with ciliates as prey (e.g. Burns and Gilbert 1993, Wickham 1995), but few investigations have also examined possible cascading trophic effects from copepod-dominated zooplankton down to the bacterial community (e.g. Burns and Schallenberg 1996, Burns and Schallenberg 1998, Burns and Schallenberg 2001b, Yoshida et al. 2001).

However, whereas experimental manipulations of crustacean zooplankton generally resulted in clear predation-mediated effects on planktonic protists, changes in bacterial biomass have only rarely been detected (Pace 1991, Wickham 1998, Adrian et al. 2001) and studies in oligo- to mesotrophic lakes generated somewhat conflicting results. This is possibly due to the importance of the actual zooplankton densities used in the experiments and also due to the fact that previous studies have focussed almost exclusively on the numerical response of the bacterioplankton to the different zooplankton treatments. Therefore, in this mesocosm study we set out to assess and refine the differential effects of cladocerans and copepods on microbial food web components, while other information on the response of the plankton community, derived from the same study, can be obtained from Sommer et al. (2001, 2003b). In this mesocosm experiment, we used gradients of realistic cladoceran and copepod densities in order to account for zooplankton density effects. We measured the response of bacterial biomass, but also considered changes in bacterial activity, morphology and diversity.

METHODS

Experimental design— The mesocosm experiment (7 - 28 August 2000) was performed in Schöhsee, a mesotrophic lake in northern Germany (mean depth 13 m, max. depth 30 m, area 82 ha). Natural phytoplankton and microzooplankton were exposed to density gradients of cladocerans (*Daphnia hyalina* x *galeata*) and copepods (consisting of approximately 50% *Eudiaptomus* spp. and 50% copepodite stages of cyclopoid copepods).

Image 1: Mesocosms in Schöhsee.

24 transparent polyethylene bags (2.5 m³ in volume, 3.2 m in depth, see image 1) were filled with lake water from which mesozooplankton was removed by sieving through 50 µm-plankton gauze. To ensure a balanced total N:total P ratio (Redfield ratio 16:1, here 34.86 µM N, 2.18 µM P), used for studying the impact of both zooplankton groups on the stoichiometry of N and P recycling (Sommer et al. 2003b), the bags were fertilized by adding phosphorus (1.53 µM NaH_2PO_4).

After two days, cladocerans from stock cultures of the Max Planck Institute for Limnology (Plön, Germany) were added to the cladoceran treatments (“dap”) to achieve approximately logarithmically scaled seeding densities of 1.25, 2.5, 5, 10, 20 and 40 individuals per liter. More details on size structure and pre-treatment of mesozooplankton can

be found in Sommer et al. 2001, Sommer et al. 2003b). Copepods derived from wild catches from the same lake 2 days prior to the experiment were added in logarithmically scaled seeding densities of approximately 5, 10, 20, 40, 80 and 160 individuals per liter to the copepod treatments ("cop"). Apart from lowest density enclosures (dap 1.25, cop 5) all mesozooplankton seeding density treatments were replicated. Two mesocosms without the addition of zooplankton served as controls. For data evaluation we used the actual zooplankton biomass in each bag, so that each gradient consisted of 13 data points.

Throughout the course of the experiment, we measured chlorophyll a daily, determined zooplankton densities every three to four days, and used these data to select appropriate dates for our in-depth analysis of the microbial communities. Days 9 and 15 were chosen according to the development of the zooplankton gradient: after 9 days with rather low numbers and a narrow biomass range, and after 15 days with a wider range and higher maximal biomass values. On days 9 and 15 we determined the concentrations of protists and bacteria, evaluated bacterial diversity using DGGE, and measured bacterial activity using ^{3}H-thymidine and ^{3}H-leucine incorporation as well as exoenzyme (protease) hydrolysis measurements (see below).

Sampling and enumeration of organisms. Sampling of the mesocosms for microbial components (picoplankton, protozoans) was done using a 10 L-can after gently mixing the water column up to 2 m depth with a Secchi disc. Zooplankton sampling was carried out every 3rd to 4th day by towing a 50 µm-mesh plankton net with a reducing cone diameter of 9 cm from a depth of 3 m to the surface. Zooplankton was fixed with formaldehyde (4 % final concentration) and counted and sized under a dissecting microscope. Zooplankton biomass was calculated from length measurements using published length-biomass-regressions (Bottrell et al. 1976). Chlorophyll a measurements were performed with a submersible fluorometer (Fluoroprobe, BBE Moldaenke, Kiel, Germany) that is able to register in vivo fluorescence of phytoplankton (e.g. Beutler et al. 2002).

Ciliates were fixed with acid Lugol´s solution (final concentration 1%) and counted and sized in sedimenting chambers with an inverted microscope (Zeiss Axiovert 35). Ciliate groups were distinguished according to Foissner and Berger 1996. Three size classes of ciliates were differentiated (largest dimension): ciliates < 20 µm (referred to as nanociliates), 20-40 µm and > 40 µm. Samples for enumeration of bacteria, of chroococcal cyanobacteria (autotrophic picoplankton, APP, mainly *Synechococcus*-like cells) and nanoflagellates were preserved in formalin (final concentration 2%) and stored at 4°C until further processing (usually within the next 24 h). Subsamples were filtered onto black polycarbonate filters, 1 ml for counting of picoplankton (25 mm, pore size 0.2 µm), 5 ml for enumeration of nanoflagellates (25 mm, pore size 0.8 µm), and stained with DAPI (4´,6-Diamidino-2-

Phenylindole, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at a final concentration of 4 µg ml^{-1} and stored at -20°C until microscopical enumeration. DAPI preparations were analyzed in an epifluorescence microscope (Zeiss Axiophot, Carl Zeiss Jena GmbH, Jena, Germany) at 1250x magnification. APP were enumerated under green light excitation. Heterotrophic flagellates were distinguished from phototrophic (auto and mixotrophic nanoflagellates, AMNF) ones by checking for chlorophyll a autofluorescence under blue light excitation.

Bacterial abundances and biovolumes were determined by using an automated imaging system (analySIS 3.0, Soft Imaging Systems GmbH, Münster, Germany). Epifluorescence images (10 per sample with a total of 500-700 cells) were digitized and automatically processed including filtering, threshold setting and binarization as described in Massana et al. 1997). Cell volumes (V) were calculated according to: $V = 8.5 \times A^{2.5} \times P^{-2}$, where A is the area and P the perimeter of the cell (Björnsen 1986).

Bacterial diversity. For the extraction of bacterial DNA, 100 ml of samples fixed with formalin for up to 12 h were filtered onto Durapore filters (47 mm, pore size 0.2 µm, Millipore) and stored in Petri-dishes at –80°C until DNA extraction. Extraction of DNA followed the protocol reported by (Schauer et al. 2000), which includes the addition of lysozyme, sodium dodecyl sulfate and proteinase K, followed by extraction with phenol-chloroform-isoamyl alcohol (25:24:1, pH 8) and chloroform-isoamyl alcohol (24:1) and subsequent concentration and rinsing steps. After quantifying spectrofluorometrically the extracted microbial DNA with a Hoechst fluorescence assay (Paul and Myers 1982), 1-8 ng of DNA was used as template for PCR (polymerase chain reaction) amplification of bacterial 16S rDNA. The reactions (50 µl volume) contained 200 µM of each of the desoxynucleoside triphosphates, 0.3 µM of each of the primers, 1.5 mM $MgCl_2$, 10x PCR buffer and 1.25 U *Taq* DNA polymerase (Biozym Diagnostik GmbH, Hessisch Oldendorf, Germany).

We used the bacterial specific primer 358f, with a 40-bp GC clamp, and the universal primer 907r, which amplifies a 550-bp DNA fragment of bacterial 16S rDNA (Muyzer et al. 1998). The PCR was performed with an *iCycler* (Bio-Rad Laboratories GmbH, München, Germany) thermal cycler using the following program: initial denaturation at 94°C for 5 min; 10 touchdown cycles of denaturation (at 94 °C for 1 min), annealing (at 65-55 °C for 1 min, decreasing 1 °C each cycle) and extension (at 72 °C for 3 min); 20 standard cycles of denaturation (at 94 °C for 1 min), annealing (at 55°C for 1 min) and extension (at 72 °C for 3 min) and a final extension at 72 °C for 7 min. PCR products were verified by agarose gel electrophoresis with a standard in the gel (Low DNA Mass Ladder, Gibco BRL) and quantified again (see above).

DGGE was carried out with a D-Code system (Biorad) as described in Muyzer et al. 1998. A 6% polyacrylamide gel with a gradient of a DNA-denaturing agent was cast by

mixing solutions of 40% and 80% denaturant agent (100% denaturant agent is 7 M urea and 40% deionised formamide) with a gradient former (Biorad). Equal amounts of PCR product were loaded and the gel was run at 100 V for 16 h at 60°C in 1x TAE buffer (40 mM Tris, pH 7.4, 20 mM sodium acetate, 1 mM EDTA). The gel was stained with the nucleic acid stain SYBR Gold (Molecular Probes) for 30 min, rinsed with Milli-Q water, removed from the glass plate to a UV-transparent gel scoop and visualised with UV. By using the software GeneSnap 4.00. (SynGene, Cambridge, England) high-resolution images were saved as computer files. To obtain quantitative information from DGGE fingerprints, digitized DGGE images were analyzed with the software GeneTools 3.00.22 (SynGene, Cambridge, England). The software performs a density profile through each lane, detects the bands (regarded as operational taxonomic units, OTUs) and calculates the relative contribution of each band to the total band signal in the lane after applying a rolling disk as background subtraction. Bands with a relative intensity of less than 0.2 % of the total intensity of the lane were disregarded. The bands occupying the same position in the different lanes of the gel were identified by visual inspection. A matrix was compiled based upon the presence or absence of individual bands in each lane and the relative contribution of the band to the total band signal.

Bacterial production and physiological activity. On days 8 and 15, production of heterotrophic bacterioplankton was measured as ^{3}H-thymidine incorporation into cold-TCA-insoluble material following a slightly modified protocol given by Bell 1993. ^{3}H-thymidine (specific activity 87 or 90 Ci/mmol, Amersham Biosciences Europe GmbH, Freiburg, Germany) was added at a saturating concentration (40 nmol L^{-1} final concentration). As a measure of metabolic activity and protein synthesis, incorporation of ^{3}H-labeled leucine (80 nmol L^{-1} final concentration, 145 Ci/mmol, Amersham) into cold-TCA-insoluble material was registered according to a slightly modified protocol described by Kirchman 1993. All samples were incubated for 1 h at in-situ-temperature. Formalin-killed controls were used to correct for background absorption of radioactivity. For an estimation of bacterial doubling times we used the empirical conversion factors of 2 x 10^{18} cells mol^{-1} thymidine (Smits and Riemann 1988) and 0.18 x 10^{18} cells mol^{-1} leucine (Ducklow et al. 1992).

To determine shifts in size distribution of bacterial activity, fractionated ^{3}H-thymidine uptake was measured for selected enclosures on days 10 and 15. Samples were incubated with ^{3}H-thymidine (20 nmol L^{-1} final concentration) for 1h at in situ temperature. Subsamples were filtered with low pressure (gravity filtration) on 0.2 µm, 1 µm and 3 µm filters. Filters were rinsed with MQ-water only to avoid disruption of cells, and then they were put into scintillation cocktail and radioassayed.

Enzymatic protease hydrolysis was determined on days 8 and 15 by using Leu-MCA (Leucine-methylcoumarinyl-amide) as a fluorogenic substrate analogue (Hoppe 1993). The

concentrations of added substrates were 1 µmol L^{-1} for determining the turnover rate (TR) and 250 µmol L^{-1} for determining the maximal velocity of hydrolysis (Vmax). These concentrations were successfully applied in gradients of eutrophication (Hoppe et al. 1998). This approach was adopted from the two concentration methods used in the determination of turnover time and Vmax in substrate uptake studies (Gocke 1977). Samples were incubated in microtiterplates (four parallels for each concentration). Readings of fluorescence at the beginning and end of the incubation period (3h) were made in an automatic Titertek Fluoroskan II fluorometer. The factor necessary for converting fluorescence units to units of µmol L^{-1} h^{-1} (µmol of substrate hydrolyzed per unit of volume and time) was derived from standard additions of the fluorescing compound AMC (Amidomethylcoumarin).

Statistical analysis. The binary matrix (presence/absence of bands) obtained from the DGGE gel was used to calculate a distance matrix using Euclidean distances (root-mean-squared differences). A dendrogram comparing samples was obtained by UPGMA (Unweighted Pair-Group Method with Arithmetic averages) in cluster analysis. To analyse the effects of mesozooplankton on the abundances, biovolumes and incorporation rates of pico- and nanoplankton, regression analyses were performed. Since the dependent parameters at the sampling dates are a time integrated response of the period prior to sampling, time averaged zooplankton densities (geometric means of day 6 and 9 for day 9, and of day 13 and 16 for day 15) were multiplied with a mean biomass value for each enclosure obtained from length measurements (see above). Frequency data were arcsin-transformed before regression analysis. In order to test if the same regression relationship might fit data from both days, we combined the data from days 9 and 15, and conducted multiple linear regression (MLR) with zooplankton biomass (geometric means), a binary dummy variable for time and an interaction term for these two as independent variables. This last term was tested for significance to see if there really was a time-related change in the quantitative response to zooplankton density between days 9 and 15.

RESULTS

General development of zooplankton and chlorophyll a—

During the course of the experiment, mesozooplankton gradients were established with abundances mostly lower than the calculated seeding densities. *Daphnia* densities showed a distinct gradient with a strong population increase in the higher density treatments in the second half of the experiment (Fig. 1A). The *Daphnia* biomass gradient after 9 days ranged between 0 and 155 µg DW L^{-1} (here and thereafter as geometric means, see above), while that after 15 days varied between 0.3 and 489 µg DW L^{-1}. The copepod gradient persisted more constantly in the course of the experimental period, with a less intense population increase in the highest density treatment (Fig. 1B). Here analogous biomass values were 0 to 584 µg DW L^{-1} on day 9 and 2.4 to 683 µg DW L^{-1} on day 15, respectively.

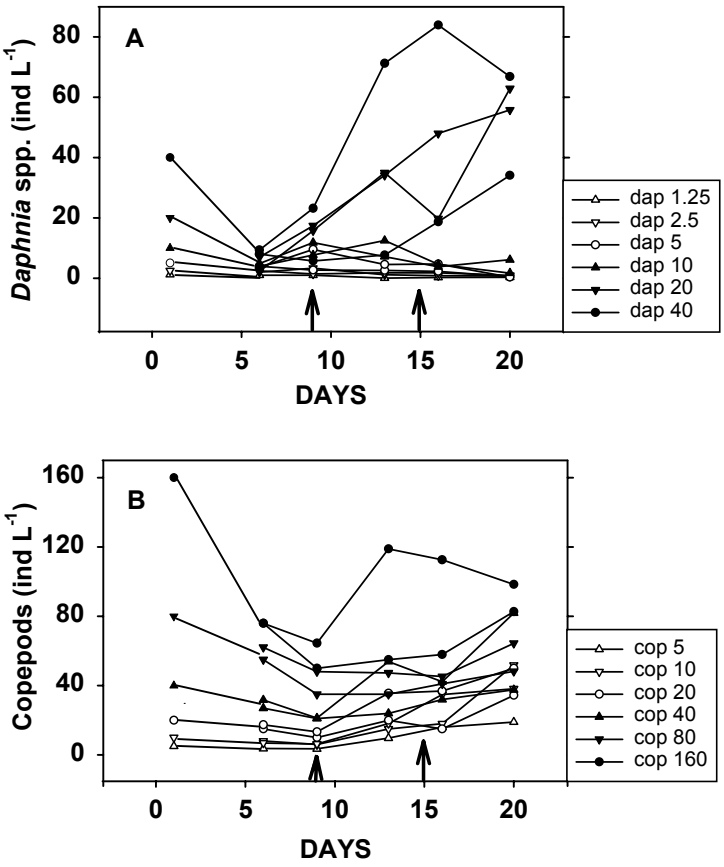


Figure 1:
Zooplankton development [ind L^{-1}] in *Daphnia* enclosures (A, upper graph) and copepod enclosures (B) in the course of the experiment. The first data point of each plot represents the calculated nominal seeding density of the different treatments and was not determined by sample counts. The two selected sampling dates are marked by arrows.

The date for the first in-depth analysis, performed at day 9, was chosen according to the analysis reported by Sommer *et al*. (2001, 2003b) and was mainly based on the zooplankton development**.** Both zooplankton gradients were well established and showed an overlap in their biomass range. However, the population densities, particularly in the *Daphnia* enclosures, were lower than expected from the calculated seeding densities (mean 9 ind L^{-1}, range 1-23 ind L^{-1}). Therefore, day 15 was chosen as a second time point for in-depth analysis, where zooplankton gradients were still distinct with increased zooplankton numbers

in highest density treatments (*Daphnia*: mean 16.9 ind L^{-1}, range 0.2-80.8 ind L^{-1}) and thus a wider biomass range. Sommer et al. (2001, 2003b) reported that contamination of copepod enclosures with *Daphnia* occurred towards the end of the experiment. Detailed zooplankton analysis revealed a marked increase of *Daphnia* in copepod enclosures after day 16. Up to then, contaminating *Daphnia* were mostly small juvenile stages. *Daphnia* biomass calculated from length measurements of selected samples using a length-biomass-regression in Bottrell *et al*. (1976) indicated a comparably small biomass contribution of *Daphnia* to total mesozooplankton biomass in the copepod enclosures with a mean value of 11.2%.

As zooplankton were sampled with a 50 µm net, rotifers (*Keratella* sp.) were not quantitatively measured. Rotifers show generally low abundances in summer (Fussmann 1996) and were monitored roughly together with zooplankton counts and Utermöhl counts of Lugol samples. Only in control enclosures (after 15 days) without *Daphnia* or copepods were estimated rotifer densities high, ranging from 600 to 1200 ind L^{-1}. Rotifers like *Keratella* sp. can strongly reduce ciliate growth rate due to direct predation and mechanical interference (Arndt 1993; Weisse and Frahm 2002). Therefore, those two mesocosms were not included in the regression analysis for day 15.

Chlorophyll-a values in the enclosures (Fig. 2) were around 1 µg L^{-1} on day 1 and reached maxima of around 6.5 µg L^{-1} (cop40 treatment). Compared to copepod treatments, chlorophyll values were consistently lower in *Daphnia* treatments throughout the experimental period and varied between 0.5 and 2.5 µg L^{-1}.

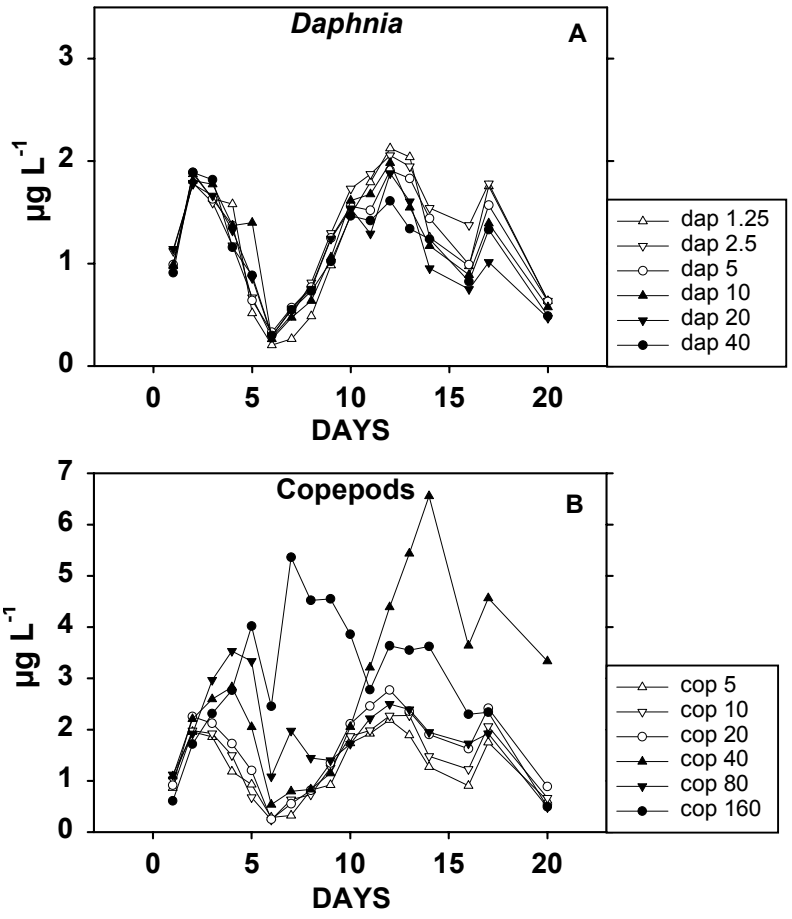


Figure 2:

Chlorophyll-a concentrations [µg L^{-1}] shown in *Daphnia* enclosures (A, upper graph) and copepod enclosures (B) in the course of the experiment as mean of 2 enclosures per treatment.

Impact of mesozooplankton on populations of protists and bacteria

Daphnia treatments

Four main ciliate groups (Oligotrichida, Prostomatida, Scuticociliatida, Haptorida) were discriminated besides a small proportion (on average 3.2-5.4%) of other and undetermined forms. Nanociliates < 20 µm mainly comprised small prostomatids like *Urotricha* and *Balanion*, while the medium-sized ciliates (20-40 µm) were found to be mainly oligotrich forms such as *Halteria* spp., but also *Urotricha* spp., scuticociliates like *Histiobalantium* and haptorids like *Askenasia*. Ciliates > 40 µm consisted mainly of large oligotrichs (e.g. *Rimostrombidium lacustris*), large prostomatids and raptorial forms like *Lagynophrya* sp..

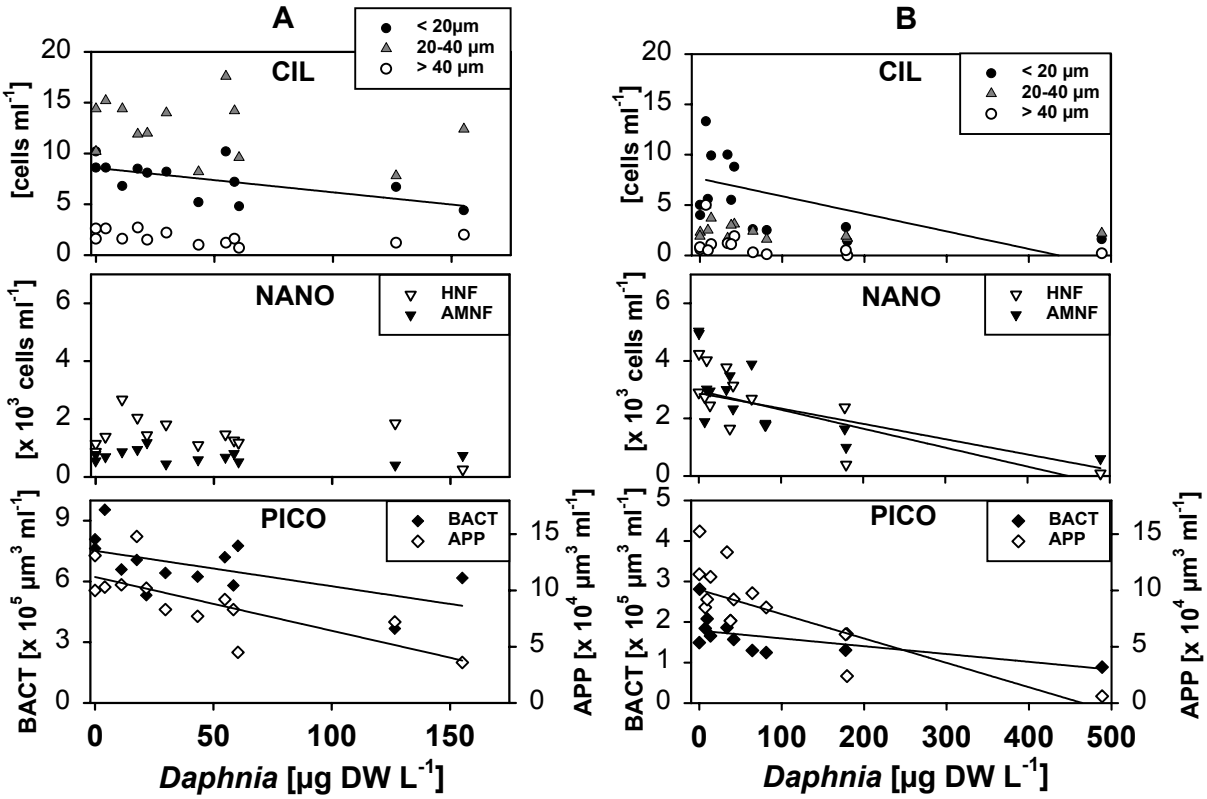


Figure 3:
Impact of *Daphnia* biomass [geometric mean, µg DW L^{-1}] on abundances of ciliates (CIL, upper graph), hetero- and auto-/mixotrophic nanoflagellates (HNF, AMNF, middle) and on biovolumes of bacteria (BACT) and autotrophic picoplankton (APP, *Synechococcus*) for days 9 (A) and 15 (B). Significant regressions equations can be obtained from Table 1. In (B) controls were excluded from regression analysis.

On day 9 a slight decrease in all size classes of ciliates with increasing *Daphnia* abundance was registered, but only the reduction of nanociliates was statistically significant (Fig. 3A, Table 1). A more pronounced impact became apparent on day 15 (Fig. 3B), when *Daphnia* densities had also reached higher values. However, multiple linear regression analysis (MLR) revealed that only the absolute level of values (intercept) changed significantly due to higher *Daphnia* biomass (Table 1), indicating no real time-related effect

(regression slope not significantly different) of *Daphnia* grazing impact on ciliates. The abundance of ciliates had strongly decreased compared to day 9 and declined fivefold from lowest to highest *Daphnia* biomass. Only in low-density *Daphnia* enclosures (dap 1.25, 2.5 and 5) did higher ciliate numbers remain (in total 9-23 cells ml^{-1}) which consisted mainly of species < 20 µm (57-78%). These significantly declined with increasing *Daphnia* biomass ($p < 0.05$, see Table 1).

Table 1:
Results of regression analysis of *Daphnia* impact (biomass, geometric mean) on different parameters are given. Only significant regression equations (p<0.05, see figures) are shown together with the net impact (negative or positive). Significant (p<0.05) multiple regression results with time (+) as independent variable are indicated (i.e. significantly different y-intercept). There was no significant result with an interaction term of time and biomass as independent variable (i.e. significantly different slope, #, see Table 2).

		Daphnia							
	MLR	day 9				day 15			
Dependent variable (Y)		r^2	*p*	regression equation	impact	r^2	*p*	regression equation	impact
Ciliates, < 20 µm		0.38	0.025	y=8.579-0.024x	neg.	0.37	0.048	y=7.618 -0.018x	neg.
Ciliates, > 20-40 µm	+		n.s.				n.s.		
Ciliates, total	+		n.s.				n.s.		
% prostomatid ciliates		0.58	0.003	y=0.730-0.001x	neg.		n.s.		
% oligotrich ciliates		0.45	0.013	y=0.914+0.0004x	pos.		n.s.		
HNF	+		n.s.			0.56	0.008	y=2.960-0.007x	neg.
AMNF	+		n.s.			0.53	0.011	y=2.877-0.005x	neg.
APP biovolume		0.57	0.003	y=11.120-0.048x	neg.	0.69	0.002	y=10.068-0.022x	neg.
Bacterial biovolume	+	0.34	0.036	y=7.514-0.018x	neg.	0.54	0.010	y=1.788-0.002x	neg.
specific ^{3}H-thy incorporation	+		n.s.				n.s.		
total ^{3}H-thy incorporation	+		n.s.			0.59	0.009	y=28.623-0.033x	neg.
specific protease turnover rate			n.s.				n.s.		
total protease turnover rate		0.67	0.044	y=8.009-0.010x	neg.		n.s.		
specific protease, Vmax	+		n.s.				n.s.		
total protease, Vmax			n.s.				n.s.		

There was a decreasing but not significant trend in HNF abundances (2.7 x 10^3 in dap2.5 to 0.3 x 10^3 cells ml^{-1} in dap40) on day 9 (Fig. 3A). Phototrophic nanoflagellates remained mostly < 1 x 10^3 cells ml^{-1}. On day 15 (Fig. 3B), due to increased *Daphnia* biomass and not to time-related grazing effects (time x biomass not significant in MLR), the decline of HNF and AMNF was significant ($p < 0.05$) and nanoflagellate concentrations were reduced below 1 x 10^3 ml^{-1} at highest *Daphnia* densities. Phototrophic nanoflagellates consisted mainly of cells < 5 µm with *Chrysochromulina* sp. and cryptomonads as the dominant taxa. A *Rhodomonas* sp. of around 10 µm in length was the predominant autotrophic nanoflagellate under most intense *Daphnia* grazing.

Autotrophic picoplankton (APP), mainly *Synechococcus*-like cells with a mean length of 1.42 µm and a mean cell volume of 0.65 $µm^3$, showed a significantly linear biovolume decrease ($p < 0.05$) with increasing *Daphnia* density at both sampling dates (Fig. 3). Compared to this, the decrease in biovolume of heterotrophic bacteria along the zooplankton gradient was less intense but significant on both days. Both bacteria and APP decreased in

abundance from the first to the second sampling date (average concentrations of bacteria declined from 5.70 $x10^6$ ml^{-1} to 1.84 $x10^6$ ml^{-1}, those of APP from 1.39 to 1.21 $x10^5$ ml^{-1}). Maximal picoplankton values were measured in the controls, where ciliate numbers were very low but HNF abundances relatively high. Furthermore, compared to enclosures with highest copepod densities (mean cell volume of bacteria 0.12 µm³, not shown in graph), *Daphnia* grazing resulted in a reduced mean bacterial cell volume of 0.08 µm³.

Summarising the observed effects of *Daphnia* treatments, it became apparent that a higher *Daphnia* biomass was necessary to cause strong reductions in micro-, nano- and picoplankton. This reduction along the biomass gradient was more pronounced for ciliates and nanoflagellates than for bacteria.

Copepod treatments

In copepod enclosures the ciliate community showed a differential response to exposure to copepod grazing as was apparent in the analyses of day 9 (Fig. 4 A). The total number of ciliates was highest without and with low zooplankton densities (19-28 ciliates ml^{-1}) but then decreased strongly with increasing copepod density. Intermediate-sized ciliates (20-40 µm) were especially affected and declined significantly ($p < 0.05$) with increasing copepod density. Nanociliates, on the other hand, increased and reached abundances of up to 16 cells ml^{-1} under high copepod abundance. These shifts in size distribution of the ciliate community were accompanied by a shift in the taxonomic composition from oligotrichs to small prostomatids (mainly *Urotricha*, *Balanion*) (Table 2).

On day 15 (Fig. 4B), ciliate numbers had already declined in all copepod bags and only nanociliates showed a marginally significant ($0.05 < p < 0.10$) relationship with the copepod gradient. In treatments with lowest copepod density did high abundances of ciliates (22 cells ml^{-1}) remain, consisting mainly of nanociliates. In contrast to *Daphnia* treatments, MLR analysis suggested a true dynamical time-related grazing effect of copepods on nanociliates and ciliates 20-40 µm in size (Table 2, significant time x biomass interaction term).

Heterotrophic and phototrophic nanoflagellates showed a strong and significant ($p < 0.001$) increase in abundance along the copepod gradient on day 9 (Fig. 4A). The population incline was more than twofold in case of HNF whereas phototrophic nanoflagellates increased more than twentyfold at highest copepod densities, with *Chrysochromulina* sp. as the dominant taxon. On day 15, high concentrations of HNF (up to 6.2 x 10^3 cells ml^{-1}) were found in most copepod treatments with no significant relationship to zooplankton biomass (Fig. 4B). MLR analysis yielded significant time-scale grazing effects on both HNF and AMNF with respect to y-intercept and also for the regression slope in case of AMNF (Table 2).

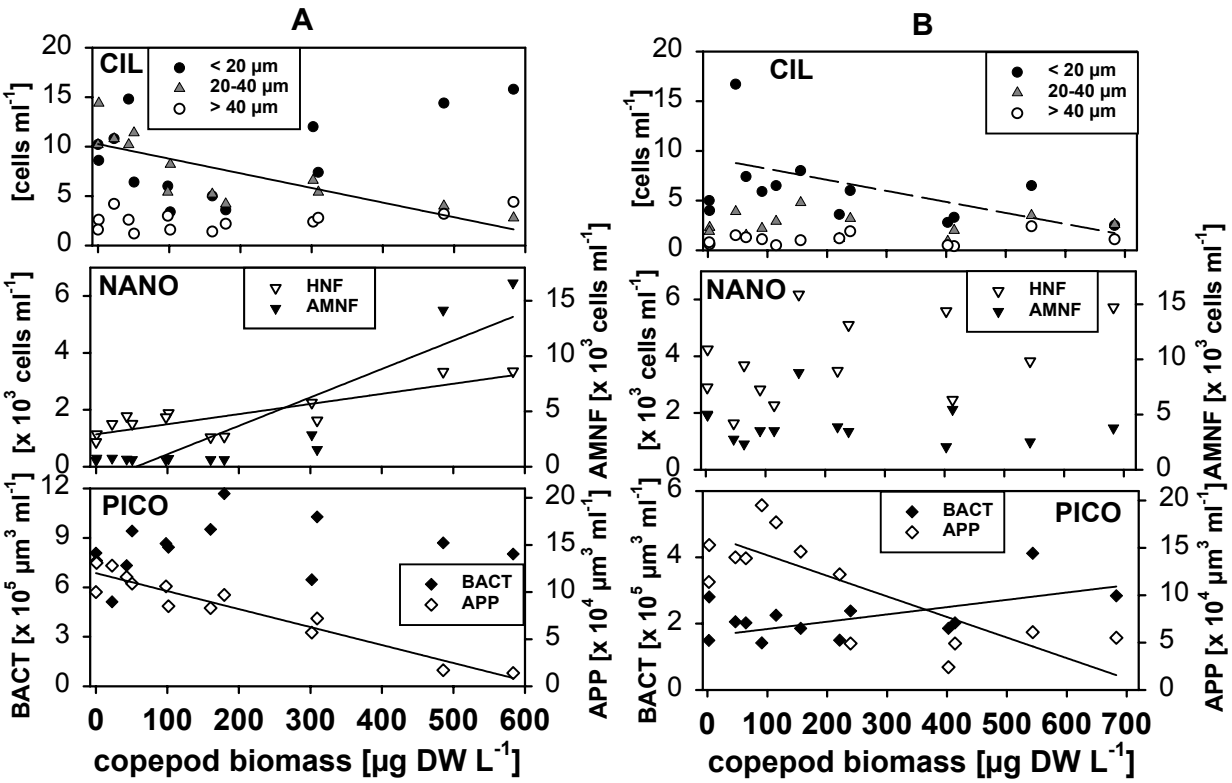


Figure 4:
Impact of copepod biomass [geometric mean µg DW L^{-1}] on abundances of ciliates (CIL, upper graph), hetero- and auto-/mixotrophic nanoflagellates (HNF, AMNF, middle) and on biovolumes of bacteria (BACT) and autotrophic picoplankton (APP, *Synechococcus*) for days 9 (A) and 15 (B). Significant regressions equations can be obtained from Table 2. In (B) controls were excluded from regression analysis.

Table 2:
Results of regression analysis of copepod impact (biomass, geometric mean) on different parameters are given. Only significant regression equations ($p<0.05$, see figures) are shown together with the net impact (negative or positive). Significant ($p<0.05$) multiple regression results with time (+) and an interaction term of time and biomass (#) as independent variables are indicated.

		Copepods							
	MLR	day 9				day 15			
Dependent variable (Y)		r^2	p	regression equation	impact	r^2	p	regression equation	impact
Ciliates, < 20 µm	#		n.s.			0.35	0.053	y=9.288-0.011x	neg.
Ciliates, > 20-40 µm	+ #	0.63	0.001	y=10.258-0.015x	neg.		n.s.		
Ciliates, total			n.s.				n.s.		
% prostomatid ciliates			n.s.				n.s.		
% oligotrich ciliates	+ #	0.33	0.040	y=0.916-0.0001x	neg.		n.s.		
HNF	+	0.71	0.000	y=1.136+0.004x	pos.		n.s.		
AMNF	+ #	0.78	<0.0001	y=-1.451+0.026x	pos.		n.s.		
APP biovolume	+	0.92	<0.0001	y=12.010-0.019x	neg.	0.61	0.005	y=16.390- 0.022x	neg.
Bacterial biovolume	+		n.s.			0.39	0.040	y=1.617+ 0.002x	pos.
specific ^{3}H-thy incorporation	+		n.s.				n.s.		
total ^{3}H-thy incorporation			n.s.			0.51	0.014	y= 23.992+0.035x	pos.
specific protease turnover rate	+	0.82	0.012	y=1.128+0.003x	pos.		n.s.		
total protease turnover rate		0.89	0.005	y=7.432+0.014x	pos.		n.s.		
specific protease, Vmax		0.79	0.017	y=26.415+0.065x	pos.		n.s.		
total protease, Vmax		0.85	0.010	y=173.836+0.302x	pos.		n.s.		

Within the picoplankton fraction, APP and heterotrophic bacteria responded differently on day 9 (Fig. 4A). Biovolume of APP decreased significantly with increasing copepod density ($p < 0.001$), whereas bacteria remained relatively constant. Bacterial biovolume varied between 5.1 and 11.7 $x10^5$ μm^3 ml^{-1} with a peak in intermediate copepod density treatments.

A differential response in picoplankton, including a decrease in APP and an increase in heterotrophic bacteria ($p < 0.05$), along the zooplankton density gradient was noted also on day 15 (Fig. 4B). Biovolume of APP ranged between 2.4 and 19.5 $x10^4$ μm^3 ml^{-1} and decreased significantly with increasing copepod density ($p < 0.05$). The range of bacterial biovolume (1.4-4.1 $x10^5$ μm^3 ml^{-1}) on day 15 was much lower compared to day 9, but still above values found in *Daphnia* treatments and with highest values at maximal copepod densities. On day 15, also mean volume of bacterial cells increased significantly (not shown in graph). Here, the biovolume of cells >2 µm in length ranged from 18 to 33 $x10^3$ μm^3 ml^{-1} in cop160 treatments, thereby differing considerably from values found in dap40 treatments (0-6 $x10^3$ μm^3 ml^{-1}). Filamentous bacteria (>5 µm) generally were of minor importance (around 6 $x10^3$ cells ml^{-1} in cop160, not shown in graph), but still almost twice as high as in enclosures with highest *Daphnia* biomass.

To summarise, selective copepod grazing resulted in a reduction of intermediate-sized ciliates and in a strong increase in nanoflagellates accompanied by a significant decrease in APP, but no significant response in heterotrophic bacteria. Persistent copepod grazing further reduced ciliate densities, raised the level of nanoplankton and caused an increasing trend in bacterial biovolume and cell size along the copepod gradient.

Impact of mesozooplankton on bacterial community composition

The DGGE profile of enclosures with *Daphnia* and copepods revealed 20 different OTUs of bacteria on day 9 (Fig. 5A). The highest number of OTUs (19) was found in treatments without zooplankton (0 ZP), in the others the number of OTUs varied between 8 and 16. The enclosures deviated slightly from the lake situation on day 9 with respect to number, position and intensity of bands. Results obtained from a UPGMA cluster analysis of a distance matrix using only the presence and absence of bands did not show any distinct grouping patterns or separation of *Daphnia* and copepod treatments on day 9 (Fig. 5B). Also when including relative DGGE band intensities, no treatment coupled clustering of OTU composition could be observed (not shown).

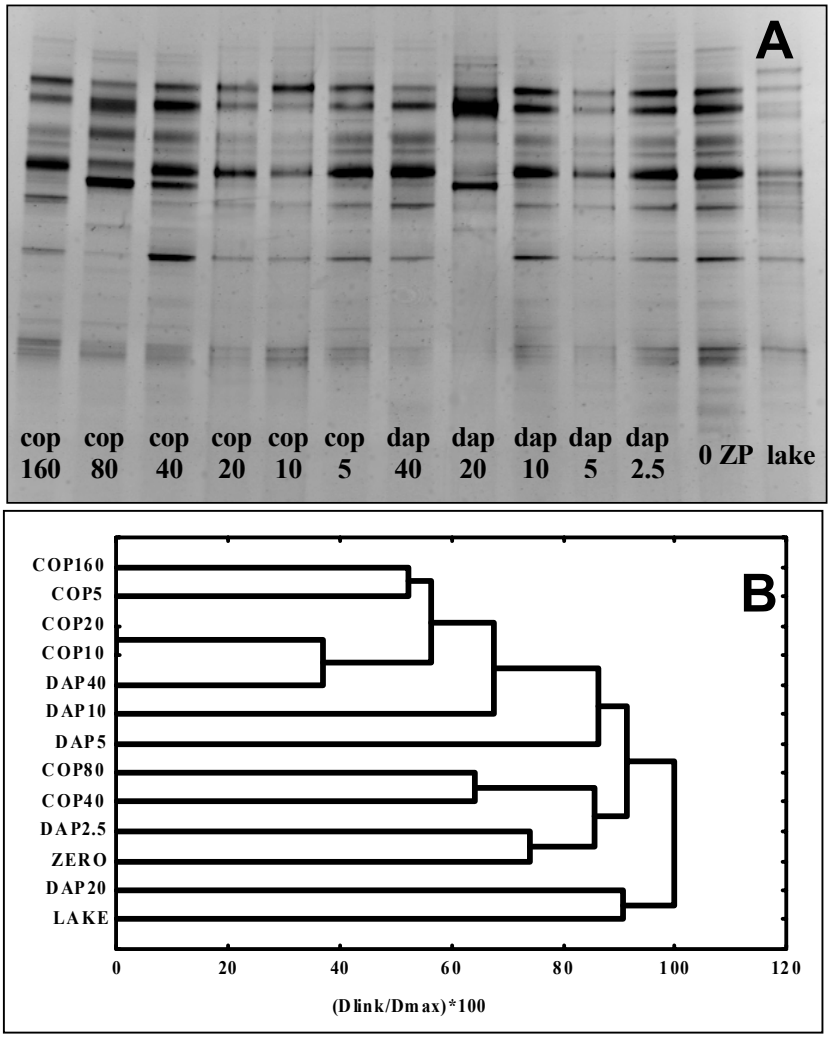


Figure 5 A,B:
DGGE gel image obtained for day 9 (A) and dendrograms (B) derived from UPGMA cluster analysis of a matrix that takes into account the presence and absence of bands. Clustering is expressed as relative dissimilarity of a linkage compared to the maximal dissimilarity (Dlink/Dmax x 100).

After 15 days the situation had changed and an analysis of the DGGE profiles using the presence and absence of bands clearly revealed a clustering of OTUs according to the zooplankton treatments (Fig.5C, D). Even within the *Daphnia* and copepod treatments the DGGE patterns of low and high zooplankton density treatments were clustering together. When taking band intensity into account, an even stronger grouping and less similarity of treatments were observed (not shown). Still 20 different OTUs were found in total, 13 in *Daphnia* and 15 OTUs in copepod treatments. Three of them appeared exclusively with *Daphnia* whereas 5 OTUs were copepod-specific. The DGGE patterns of the lake sample as well as the 0 ZP enclosure were more closely linked to the *Daphnia* treatments

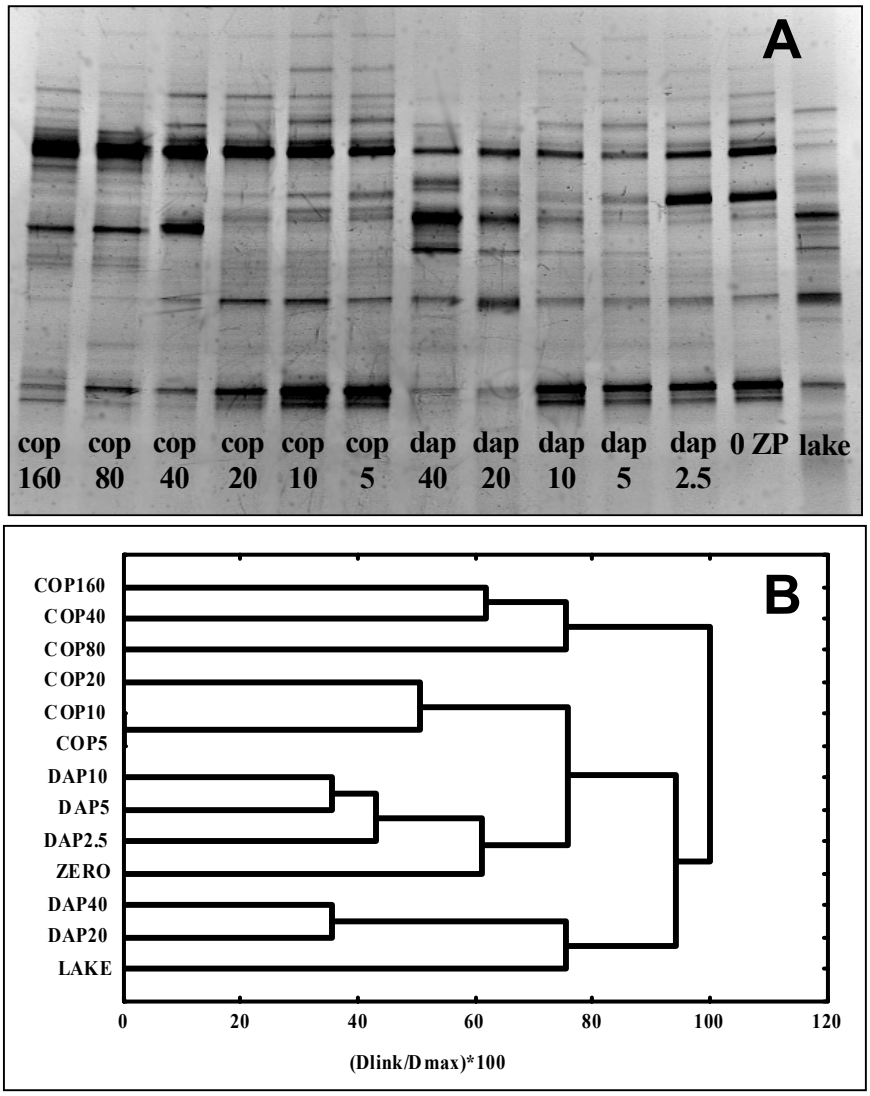


Figure 5 C,D:
DGGE gel image obtained for day 15 (C) and dendrograms (D) derived from UPGMA cluster analysis of a matrix that takes into account the presence and absence of bands.
Clustering is expressed as relative dissimilarity of a linkage compared to the maximal dissimilarity (Dlink/Dmax x 100).

Mesozooplankton impact on bacterial production and activity

Bacterial production showed a strong scatter and a significant correlation was found neither in total nor in cell-specific incorporation (data not shown) along the *Daphnia* biomass gradient on day 8 (Fig. 6A). Maximal values of ^{3}H-thymidine (50-56 pmol L^{-1} h^{-1}) and leucine (0.54-0.58 nmol L^{-1} h^{-1}) incorporation corresponded to bacterial doubling times of 36 to 45 hours. Nevertheless, the activity patterns of protease revealed a significant trend along the *Daphnia* gradient (Fig. 6A), but only with respect to substrate turnover rates (TR, range 5.2-6.3 nmol L^{-1} h^{-1}) and not to the maximal substrate hydrolysis rates (Vmax, range 124-140 nmol L^{-1} h^{-1}).

On day 15, bacterial production was still high at low *Daphnia* biomass values (0-80 µg DW L^{-1}) but then clearly declined along the *Daphnia* gradient (p<0.05 for ^{3}H-thymidine), which resembles the observed pattern in bacterial biovolume (Fig. 6B). Exoenzyme activity on day 15 showed a strong decrease in both the turnover rate and maximal hydrolysis velocity of protease with increasing *Daphnia* density and thus was congruent with the decline in bacterial biovolume and ^{3}H-thymidine incorporation (Fig. 6B).

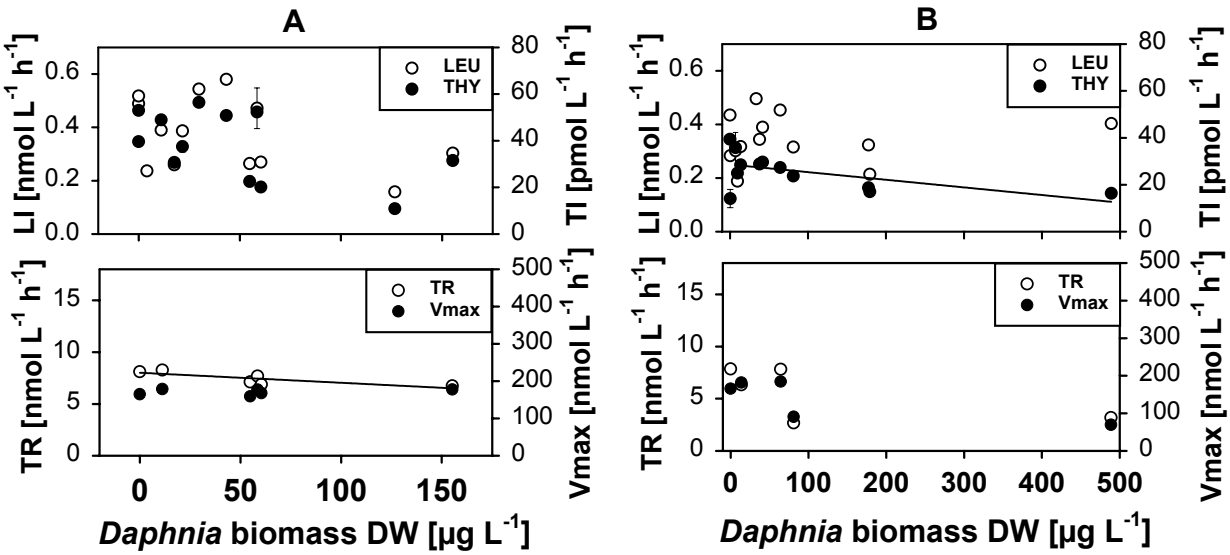


Figure 6:
Impact of *Daphnia* biomass [geometric mean, µg DW L^{-1}] on total bacterial production (upper graphs) measured as ³H-thymidine [pmol L^{-1} h^{-1}] and ³H-leucine incorporation [nmol L^{-1} h^{-1}] and on substrate hydrolysis rates of the bacterial exoenzyme protease (lower graphs) shown as turnover rates (TR) and maximal velocities (Vmax) of hydrolysis [nmol L^{-1} h^{-1}] on days 8 (A) and 15 (B). Significant regressions equations can be obtained from Table 1. In (B) controls were excluded from regression analysis.

Bacterial production in copepod enclosures did not correlate significantly with mesozooplankton density on day 8 (Fig. 7A). Maximal values of ³H-thymidine incorporation (up to 60 pmol L^{-1} h^{-1}) and ³H-leucine incorporation (up to 0.60 nmol L^{-1} h^{-1}) suggest bacterial doubling times of 34 and 37 hours, respectively. In contrast, protease activity showed a significant increase in Vmax ($p < 0.05$) and turnover rate ($p<0.05$) along the copepod gradient on day 9. Six days later, the patterns of bacterial production and activity clearly followed the trend in bacterial biovolume with a significant ($p< 0.05$) increase in ³H-thymidine incorporation along the copepod gradient (Fig. 7B). Protease Vmax was within the same range as on day 9 and still increased with copepod density ($p= 0.066$), the increase in turnover rate was not significant anymore ($p = 0.52$; Fig.7B). In general, mean enzyme activity values for protease were found to be 20-62 % higher in copepod compared to *Daphnia* treatments.

Parallel to the increase in total bacterial biovolume the proportion of cells >1µm increased from 32% in controls to 52% in the enclosures with highest copepod biomass (data not shown in graph). This shift in bacterial size distribution was also evident from size-fractionated ³H-thymidine uptake (Fig. 8). It revealed that in high-density copepod enclosures (cop80, cop160 on day 10, cop160 on day 15) as well as in control enclosures without metazooplankton large bacteria (>3 µm) contributed with a relatively high proportion (up to 22 % in 0 ZP, Fig. 8B) to total ³H-thymidine uptake. In contrast, in *Daphnia* enclosures the smallest bacterial size fraction > 0.2 - <1 µm was responsible for 84 to 91 % of the total bacterial uptake.

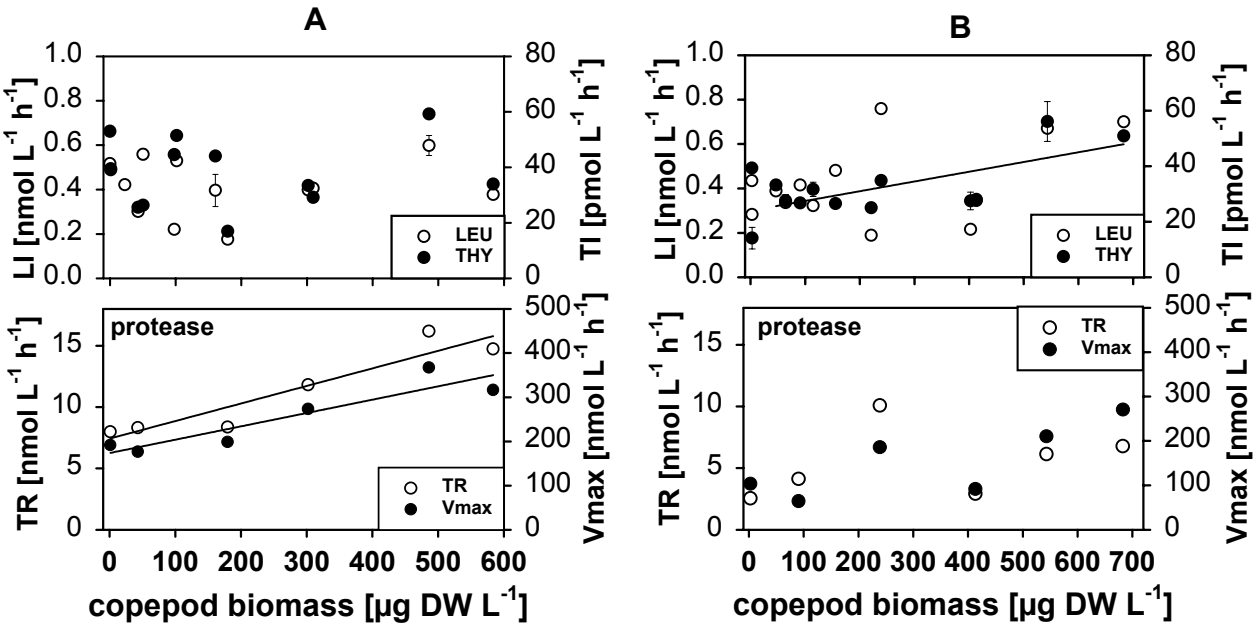


Figure 7:
Impact of copepod biomass [geometric mean, µg DW L^{-1}] on total bacterial production (upper graphs) measured as ^{3}H-thymidine [pmol L^{-1} h^{-1}] and ^{3}H-leucine incorporation [nmol L^{-1} h^{-1}] and on substrate hydrolysis rates of the bacterial exoenzyme protease (lower graphs) shown as turnover rates (TR) and maximal velocities (Vmax) of hydrolysis [nmol L^{-1} h^{-1}] on days 8 (A) and 15 (B). Significant regressions equations can be obtained from Table 2. In (B) controls were excluded from regression analysis.

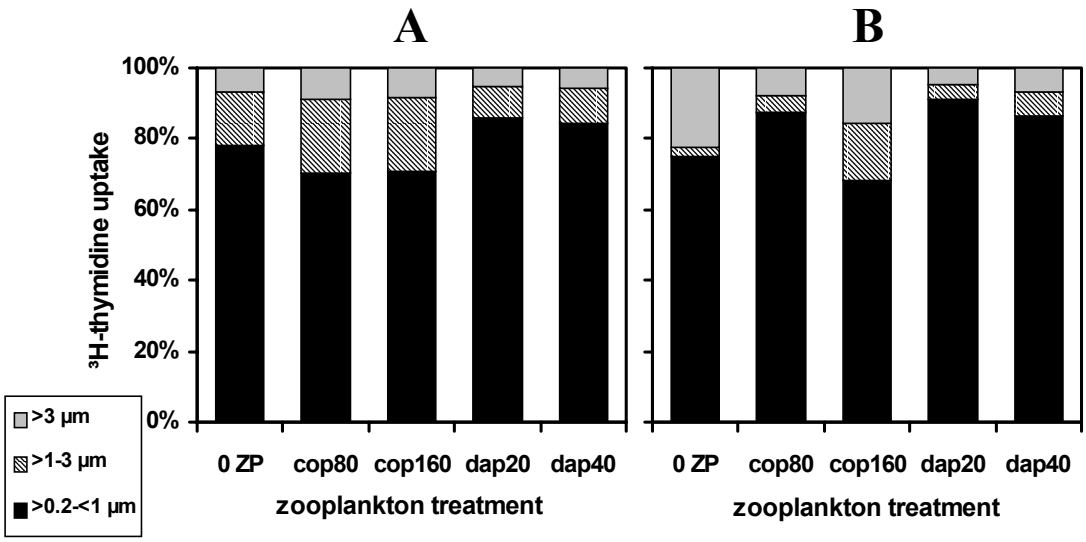


Figure 8:
Size-fractionated ^{3}H-thymidine uptake in five enclosure treatments (0ZP, cop80, cop160, dap20, dap40) at days 10 (A) and 15 (B). Shown are the relative contributions of three bacterial size classes (>0.2-<1 µm, 1-3 µm, >3 µm) to total ^{3}H-thymidine uptake. Values are means of three replicates. The mean coefficients of variation of each fraction were 0.10 (>0.2-<1 µm), 0.06 (1-3 µm) and 0.13 (>3 µm).

DISCUSSION

While many previous studies used small-scale microcosms for food web experiments, here the effects of different mesozooplankton guilds were analyzed on a larger scale by using enclosures of a sufficiently large volume to provide more reliable predictions of whole-lake phenomena (Sarnelle 1997). Furthermore, this mesocosm experiment was based on a gradient design of mesozooplankton concentrations and so allowed a finer resolution of critical densities, which are able to cause significant responses in the microbial components. The zooplankton concentrations which developed in the enclosures were, with the exception of the highest densities at the end of the experiment, within the range reported from the study lake (Fussmann 1996). Also, the observed protozoan and bacterial concentrations in the different enclosures, as well as bacterial growth rates, were within the range found normally in this mesotrophic lake (Jürgens et al. 1994a). Therefore, the extent of the observed responses can be considered realistic for the lake situation. The chosen time-points for a detailed analysis of the microbial community structure (9 and 15 days of experimental duration) corresponded to approximately 3-8 generations of bacteria and of the different functional groups of protozoans. Previous experiments at this study site suggest that this scale is appropriate to demonstrate both direct short-term predation impacts of the metazooplankton, cascading effects via protozoans to bacteria as well as bacterial adaptations towards changes in predation pressure (Jürgens et al. 1994a).

Contrasting effects of *Daphnia* and copepods on protozooplankton

Studies on the effect of cladocerans and copepods on populations of protozoa in lakes have mostly shown significant impacts of both crustacean groups on ciliates and other microplankton (Adrian and Schneider-Olt 1999; Burns and Schallenberg 2001a; Yoshida et al. 2001). Our experiment also found that both zooplankton guilds affected the ciliate community. By the second time point higher *Daphnia* densities had strongly reduced the total ciliate abundance, thereby confirming other studies, which have proved that *Daphnia* exerts efficient top-down control on ciliate assemblages (Wickham 1998, Burns & Schallenberg, 2001a). For ciliates it is known that their species and size composition influences their vulnerability to *Daphnia* and calanoid copepods (Burns and Gilbert 1993, Jack and Gilbert 1993, Wiackowski et al. 1994). This is mainly due to the fact that a number of species have jumping behavior that reduces their vulnerability to predators (Gilbert 1994). In our experiments medium-sized Oligotrichida with bristles for jumping, such as *Halteria* spp. (or *Pelagohalteria* spp.), also remained in highest *Daphnia* density treatments.

In contrast to *Daphnia*, differential and selective grazing of copepods induced a shift in size and composition of the ciliate assemblage. The ciliate size-fraction 20-40 µm was most efficiently reduced by copepod predation. This fraction comprised important oligotrich

taxa such as *Strobilidium*, *Halteria* and *Pelagohalteria*, which are voracious grazers on pico- and nanoplankton (Šimek et al. 2000). In contrast, small prostomatid ciliates (*Urotricha* spp*., Balanion* sp.) increased in abundance. Similar shifts towards prostomatid nanociliates were also found in a hypertrophic lake during high abundance of cyclopoid copepods (Jürgens et al. 1999b; Hansen 2000). Yoshida *et al*. (2001) also found copepod size selectivity with *Eudiaptomus* feeding mainly on larger algae (>20 µm) and microzooplankton in mesotrophic Lake Biwa.

Whereas the overall impact on ciliates differed only in qualitative aspects between daphnids and copepods, the mesozooplankton groups showed clearly opposite effects on the abundance of nanoflagellates: increasing *Daphnia* biomass resulted in a significant reduction of HNF abundance but an increase in copepods lead to an increase in HNF density. A likely explanation is that copepods impact nanoplankton only indirectly via ciliates whereas daphnids exert a well-known direct grazing pressure on nanoplankton (Jürgens 1994). It is probable that a combination of ciliate and *Daphnia* grazing accounted for low abundance levels of nanoflagellates along the *Daphnia* gradient on day 9, whereas six days later, in spite of strongly reduced ciliate grazing, higher *Daphnia* densities caused a significant reduction of nanoflagellates. This would be consistent with previous studies showing that HNF are within the optimal prey size spectrum of filter-feeding cladocerans, and that HNF populations are suppressed when *Daphnia* are abundant (e.g. Porter et al. 1988; Jürgens 1994; Pace and Vaqué 1994).

Nanoflagellates showed a remarkable two- (HNF) to twentyfold (AMNF) increase with increasing copepod biomass. This is most probably due to the fact that ciliates, particularly oligotrichs, are highly efficient feeders on nanoplankton (Cleven 1996; Šimek et al. 2000) and HNF are increasingly released from ciliate grazing pressure with the expansion of copepod biomass. Copepods themselves are generally thought to be relatively inefficient feeders on nanoplankton although this seems to be more valid for cyclopoid than for calanoid species (Jürgens et al. 1996; Burns & Schallenberg 2001a). The significant positive relationship between HNF and copepods did not persist until the second time-point of in-depth analysis (Fig. 4B). However, here HNF were already at higher concentrations and might have become increasingly food-limited due to the decline in picoplankton and the development of less edible bacteria (indicated by an increasing proportion of larger bacteria).

The contrasting feeding modes of *Daphnia* and copepods thus differentially impacted the protozoan community. *Daphnia* seemed to top-down control nearly the whole protozooplankton, from large ciliates to nanoflagellates. Copepods, instead, efficiently and selectively preyed upon 20-40 µm-sized ciliates, thereby triggering a trophic cascade to the nanoplankton level, enabling high HNF numbers, which potentially affect the picoplankton (see also Sommer et al. 2003b). Similar to other studies (Burns & Schallenberg, 2001a),

ciliates turned out to be key links in the copepod-mediated trophic cascade down to nano- and possibly picoplankton, thereby linking the microbial and the classic food chain in lakes.

Direct and indirect mesozooplankton effects on planktonic bacteria

We focused in this study on short-term reactions of bacterial biomass, activity and genetic diversity to an altered zooplankton-grazing regime. Within the picoplankton, heterotrophic bacteria and autotrophic picoplankton (mainly *Synechococcus* cells) reacted differently. APP strongly decreased in both gradients of crustacean mesozooplankton, in *Daphnia* enclosures presumably caused by direct *Daphnia* grazing, in copepod treatments likely due to high protist grazing pressure. The larger cell volume of APP (mean 0.65 µm³) compared to heterotrophic bacteria (range 0.08-0.16 µm³) probably makes them vulnerable to different grazers.

Compared to micro- and nanoplankton, the biomass of heterotrophic bacteria showed a less pronounced response to mesozooplankton manipulation in this experiment and appeared to be buffered against zooplankton density alteration. The mean total bacterial biovolume over all *Daphnia* enclosures was slightly lower than in control and copepod enclosures. While a *Daphnia* biomass gradient between 0 and 155 µg DW L^{-1} apparently caused a significant decrease in total bacterial biovolume (day 9, Fig. 3A), here a comparable biomass range of copepods had only little impact (Fig. 4). Higher *Daphnia* densities, however, caused a strong reduction of bacterial biovolume (Fig. 3B), thereby supporting other studies that demonstrated control of bacterial populations in lakes by *Daphnia* and other filter-feeding cladocerans (e.g. Riemann 1985 ; Pace 1990 ; Vaqué and Pace 1992).

Strong top-down control of planktonic bacteria by *Daphnia* seems to prevail when they achieve higher densities in meso- to eutrophic lakes and ponds whereas comparable studies in more oligotrophic systems with lower *Daphnia* densities revealed no effect or only weak effects on bacterioplankton (Pace 1991, Brett et al. 1994). Apart from differences in absolute *Daphnia* densities, a very weak impact of *Daphnia* on bacterial communities in oligotrophic systems can be explained by the fact, that the small and often inactive bacteria normally predominating in these systems are not affected, since the filter mesh-size of cladocerans retains bacteria above a certain size limit (approximately > 0.5-0.7 µm, Brendelberger 1991). This means that larger, actively growing bacteria are particularly influenced. This size-selective bacterial grazing by daphnids was probably also responsible for the observed decrease in mean bacterial size and the decline in bacterial production and activity with increasing *Daphnia* biomass (Fig. 6). Similar observations were reported from extended periods of *Daphnia* maxima (“clear-water phase”) in lakes (Güde 1988, Jeppesen et al. 1992, Christoffersen et al. 1993).

Estimated bacterial grazing mortality was in the same order of magnitude on both ends of the gradient, under strong HNF (dap1.25, dap2.5) and *Daphnia* (dap40) grazing, respectively. The high concentrations of HNF in copepod enclosures resulted in a general decline of bacteria on day 15. There was, however, no significant relationship between HNF and bacteria along the copepod gradient and bacterial biovolume even increased with increasing copepod densities (and hence also increasing HNF concentrations) (Fig. 4B). Previous mesocosm experiments revealed that increased predation pressure by HNF could result in the compensation of grazing mortality by the development of inedible morphotypes of bacteria, e.g. filamentous forms and aggregates (Jürgens et al. 1994, Šimek et al. 1999). Although we did not observe a mass development of resistant filaments, as occurred in a previous experiment in the same lake (Jürgens et al. 1994), the shift towards larger bacterial cells in mesocosms with low ciliate and high HNF abundances (controls, cop160, see also Fig. 8) and the occurrence of a higher proportion of filaments are indications of those grazing-resistant forms.

A positive correlation between copepod populations and the abundance of filamentous bacteria has been observed also in eutrophic shallow lakes (Jürgens and Jeppesen 2000) and interpreted as a trophic cascade in which copepod predation on ciliates enhances HNF grazing pressure on bacteria and thereby the development of resistant morphotypes. However, the extent to which filamentous bacteria can develop seems to be influenced also by other factors such as nutrient availability. Besides morphological resistance other mechanisms of grazing-resistance such as motility and physicochemical surface properties can also be of importance for stabilising bacterial biomass (reviewed by Jürgens and Matz 2002).

The difference in bacterial composition between copepod- and *Daphnia*-dominated treatments confirm other enclosure experiments which showed that a change in bacterivory from *Daphnia* to protist grazing results more in taxonomic shifts rather than in differences in bacterial biomass (Langenheder and Jürgens 2001). Such changes in prey composition without changes in prey biomass, mainly due to differences in predator feeding mode and prey vulnerabilities, were called a “cryptic trophic cascade” by Tessier and Woodruff (2002). However, the taxonomic level response of the bacterial community to an altered grazing regime seemed to take place rather slowly. Only at the second time-point of analysis, did the clustering of bacterial OTUs and also the occurrence and contribution of certain OTUs show a clear grouping according to mesozooplankton treatments (Fig. 5D). High copepod density treatments (cop40, cop80, cop160) clustered together suggesting the existence of a distinct HNF-grazing adapted bacterial community.

The increasing bacterial activity and production, as seen from thymidine and leucine incorporation as well as from exoenzymatic activity (Fig. 7) along the copepod gradient points to stimulation of bacteria by protist grazing. The level of protease activity in copepod treatments was considerably higher than in *Daphnia* treatments. This can be attributed to an increase in bacterial biomass as well as enhanced bacterial substrate demand. Furthermore, protease excretions of HNF and AMNF can contribute to the extracellular enzyme pool (Karner et al. 1994). Aquatic bacteria are subject to a range of potential impact modes, and could also have been affected by varying substrate supply due to differences in algal communities and different metazoan and protozoan DOC (dissolved organic carbon) release (sloppy feeding, excretion, Nagata 2000). Nevertheless, we think that the different predation impacts by metazoans (*Daphnia* bags) and protozoans (copepod bags) were probably major factors shaping the structure of the bacterial assemblages in this experiment.

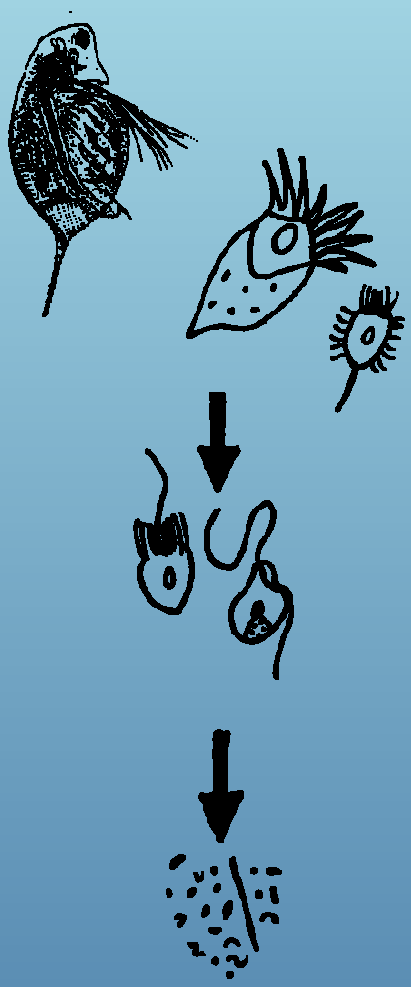
While the existence of clear trophic cascades has been shown between various components in aquatic systems, most often from fish via zooplankton to phytoplankton (Carpenter et al. 1985, Pace et al. 1999), there is considerably less knowledge about the extent of cascading effects on heterotrophic microorganisms, and bacterial assemblages in particular are difficult to integrate into food web patterns. We found clear differences with respect to mesozooplankton effects in *Daphnia* versus copepod-dominated communities on the microbial food web structure. Our results confirm the general concept that microbial food webs differ markedly between systems with and without abundant *Daphnia* populations (Stockner and Porter 1988; Pace 1990; Riemann and Christoffersen 1993). The fate of bacterial production in freshwater systems is determined by the composition of higher trophic levels and the dominant selective pressure can be exerted by protist or *Daphnia* grazing. Our study additionally revealed that copepod-dominated zooplankton can trigger a clear predatory cascade involving strong and opposing fluctuations in micro- and nanoplankton.

Our results also confirm previous studies that strong effects of planktonic food web structure are mainly found for populations of ciliates and flagellates. The regulation of bacterioplankton seems to function quite differently, judged from the weak effects of an altered grazer community on bacteria in enclosure experiments (Pace 1991; Wickham 1998; Adrian et al. 2001) and whole lake studies (Pace and Cole 1996). Also in this study the heterotrophic bacterial assemblage proved quite stable, reacted slowly towards an altered grazing regime and showed strongest biomass responses in treatments with lowest and highest mesozooplankton biomass. Weak food web effects on planktonic bacteria have been interpreted as truncation of the trophic cascade at the level of protozoans (Pace 1991; Wickham 1998). However, by considering several bacterial response variables, such as bacterial community composition and activity, it became obvious in our experiment that significant effects did occur at the bacterial level. Evidence for trophic effects on bacterial

composition comes also from other studies, which considered bacterial community structure (Cochran-Stafira and von Ende 1998; Šimek et al. 1999; Langenheder and Jürgens 2001). These results confirm the importance of heterogeneity within bacterial communities and imply that true trophic cascades in the community sense (Strong 1992) are the exception rather than the rule for planktonic bacteria. Instead, there presumably occur, in response to altered biological interactions, shifts and adaptations within bacterial assemblages, which involve changes in species composition, phenotypic characters and physiological activities.

II

Chapter 2



Lake

– microbial food

web in spring

Daphnia

Effects of zooplankton regime on spring microbial community dynamics and a bloom of filamentous bacteria

— evidence from a phosphorus-depleted lake food web and manipulated mesocosms —

ABSTRACT

Microbial food web successions were monitored in large-scale mesocosms and a mesotrophic lake in northern Germany during 25 days in May 2001. Mesocosm food webs were experimentally manipulated by removal or addition of *Daphnia*. *Daphnia*-free control enclosures displayed highly dynamic protist successions with highest abundances of first nanoflagellates and subsequently ciliates. An ephemeral bloom of heterotrophic bacterial filaments, probably resistant against protist grazing, was observed under all food web constellations, achieving more than 60% of total bacterial biovolume. In order to identify and quantify bacterial filaments, molecular cloning and fluorescence-in-situ-hybridization (FISH) with some specific oligonucleotide and one newly designed (LD2-739) oligonucleotide probes was applied. Thus, up to 98% of filamentous cells in lake water could be assigned to a clade of almost identical (99% similarity) 16S rRNA gene sequence types, the cosmopolitan freshwater LD2 cluster (*Cytophaga-Flavobacteria*). This is probably the most pronounced case of dominance by a single bacterioplankton species ever observed in natural freshwaters. When following the temporal development and relative importance of these bloom-forming bacteria, members of the LD2 clade turned out to temporarily constitute >40% of total bacterial biomass. Although filaments were reduced in the *Daphnia*-treatments, the LD2 filaments constituted a significantly larger fraction of filaments in enclosures with high *Daphnia* densities compared to *Daphnia*-free controls.

While *Daphnia* turned out to be the main reason for filament elimination, potential underlying mechanisms and factors determining filament formation appeared to be complex. The development of bacterial filaments seemed to be favoured by previous maxima in heterotrophic flagellates and ciliates, but probably also by the severe phosphorus limitation which occurred during that time.

INTRODUCTION

Cladocerans and copepods as the dominant freshwater mesozooplankton groups with differing feeding selectivity can exert contrasting trophic and structuring impact on the autotrophic and heterotrophic communities of lower trophic levels (see chapter I). In the summer experiment, *Daphnia* grazing top-down impacted all microbial food web components and reduced ciliates, nanoplankton as well as auto- and heterotrophic picoplankton.

Among the variety of factors tightly regulating bacterial production and mortality (reviewed by Fuhrman and Noble 1999), predation is a major shaping force for the phenotypic and genotypic composition of bacterial communities (reviewed by Jürgens and Matz 2002). The response mode of bacterioplankton to top-down effects of mesozooplankton and increasing numbers of protozoans can be largely affected by the phenotypic and genotypic composition of the bacterial assemblage and the existence or development of resistance traits against protist grazing (Jürgens and Güde 1994, Hahn and Höfle 2001). Besides motility and physicochemical surface properties (Matz and Jürgens 2001, Matz et al. 2002) the aspect of size, e.g. the development of very small cells (ultramicrobacteria, passive resistance, Torrella and Morita 1981, Hahn et al. 2003) or long filaments and aggregates, is among the most commonly observed morphological adaptations influencing the susceptibility of bacteria mainly to HNF grazing (Jürgens and Güde 1994).

Most small protists preferentially feed on bacteria of 1-3 µm in length Gonzalez et al. 1990, Šimek and Chrzanowski 1992). Heterotrophic nanoflagellates (HNF), the most important bacterivores in plank tonic systems, have an upper prey size limit in the range 4-10 µm (Hahn et al. 1999, Wu et al. 2004). Thus, filamentous bacterioplankton can be considered as protozoan grazing-resistant bacteria and are clearly favoured under intense grazing by hetero- or mixotrophic nanoflagellates (e.g. Jürgens and Güde 1994, Šimek et al. 2001, Jürgens and Matz 2002) or bacterivorous ciliates (Shikano et al. 1990, Posch et al. 2001). Due to their size, on the other hand, filamentous bacteria are susceptible to direct removal by filter-feeding zooplankton (in particular cladocerans) and reduced *Daphnia*-related mortality of small bacterial competitors (Güde 1988, Jürgens 1994).

The occurrence of filamentous or threadlike bacteria as an ecologically distinct and often dominant microbial component is documented mostly from meso- and eutrophic freshwater systems (Jürgens and Güde 1994, Langenheder and Jürgens 2001, Hahn and Höfle 2001). They have also been reported from ultraoligotrophic (Pernthaler et al. 1998), hypertrophic (Sommaruga and Psenner 1995) and acidified (Vrba et al. 2003) freshwater habitats, but have only rarely been found in marine systems (e.g. Caron et al. 1988, Engström-Öst et al. 2002). Phenotypic plasticity can be involved in filament formation (Hahn et al. 1999) but some bacteria also have a permanently filamentous morphotype (e.g. *Ancalomicrobium* sp., Bianchi 1989).

However, the regulating mechanisms of filament formation and the importance of top-down and bottom-up factors remain unclear. Of course, the extent to which trophic impact can cascade down to bacterial level is dependent also on other factors like seasonal differences in food web structure (e.g. predator composition) and abiotic conditions, mainly characterized by different scenarios of nutrient limitation and temperature regime. Recent findings obtained from a chemostat study (Matz and Jürgens 2003) revealed combined effects of nutrient limitation and grazing as major determinants of bacterial community structure. Thereby, filamentous bacterial forms have been observed to dominate in laboratory chemostats under conditions of P-limitation and grazing.

However, high numbers of filamentous bacteria have earlier been observed in Schöhsee in spring (Jürgens, unpubl.), but the overall patterns of their occurrence and their trophic implications are still poorly understood and investigated. In this context, Langenheder and Jürgens (2001) emphasised the need for species-specific probes in order to follow the population dynamics of filament-forming bacteria in situ and to reveal the contribution of phenotypic plasticity of certain taxa and real compositional changes in bacterial morphotype shifts.

To distinguish between key populations of filamentous bacteria in different freshwater systems and to elucidate their ecological role, it is necessary to develop identification systems. Hence, the so-called "full-cycle rRNA approach" (Amann et al. 1995) was applied, a combination of molecular tools including PCR amplification, cloning and sequencing of an environmental sample and subsequent phylogenetic analysis of environmental sequence types (Pernthaler et al. 2004). This procedure is followed by the design of specific rRNA-targeted DNA probes, which can be used for whole-cell fluorescence in situ hybridization (FISH) and microscopic visualization of selected bacterial subgroups, thereby allowing for the distinction of bacterial morphotypes (Pernthaler et al. 1997a, Pernthaler et al. 1998).

In both the lake water column and the mesocosms a conspicuous bloom of these filamentous bacteria was observed. A preliminary FISH analysis revealed that the majority of these filaments were members of the Cytophaga-Flavobacterium cluster of the Bacteroidetes (*Pernthaler et al. 2004*) and by application of the procedures named above dominant filamentous phylotypes within this lineage could be identified and traced in their in situ development under various food web scenarios (Pernthaler et al. 2004).

This study focuses on the structuring impact of cladoceran mesozooplankton on the longer-term (25 days in spring) temporal performance of microbial food web components in mesotrophic Schöhsee and zooplankton-manipulated mesocosms. In particular, patterns and regulating factors of bacterial filament development (single taxon and morphotype level) will be elucidated in the context of these planktonic food web successions and dynamics.

METHODS

Samples were collected in May 2001 from the surface waters of Schöhsee, a mesotrophic dimictic lake in northern Germany (area, 82 ha, maximum depth 30 m). In parallel, the spring mesocosm experiment was maintained in Schöhsee (see chapter I) for 25 days (03.-28.05.2001), again by establishing 24 enclosures with logarithmically scaled zooplankton additions (copepods and cladocerans). This time, mesocosms were slightly smaller (2.5 m length, 1.7 m³ volume) and did not have a conical end with a sediment trap. The filling of the enclosures took place as described in chapter I.

However, in this experiment highest seeding densities of copepods and *Daphnia* were 80 and 20 individuals per liter, respectively. Instead of the cop160 and dap40 treatments (see chapter I), lowest density treatments were replicated and 2 bags with a mixed zooplankton community ("mix-enclosures") consisting of 5 *Daphnia* and 20 copepods per liter (nominal seeding densities) were introduced. Furthermore, there was no addition of phosphorus in the beginning.

This chapter deals with the microbial food web succession in the lake, in *Daphnia*-free controls (with protist grazing only; sometimes abbreviated with E10 and E24) and in mesocosms with a strong development of *Daphnia* (dap5, seeding density 5 ind. L^{-1}; sometimes abbreviated with E12 and E19). Chapter V contains microbial food web responses to copepod density gradients after 1 week.

The sampling of zooplankton and microbial food web organisms as well as the measurement of chlorophyll a took place as described in chapter I. The fixation and enumeration of ciliates and nanoflagellates, the preparation of DAPI slides and the measurements of bacterial production (bacterial thymidine and leucine incorporation and size-fractionated thymidine uptake) also followed the protocols given in chapter I. Respiratory activity of bacteria in all mesocosms was determined two times (day 13 and 19) via the CTC-method as described in chapter III). Abundances of bacteria and autotrophic picoplankton (*Synechococcus,* data not shown in graph for reasons of clarity) were determined by flow cytometry. More detailed information on fixation and measurement settings can be obtained from chapter III.

Mean cell sizes of non-filamentous bacteria were determined for selected samples with a semi-automated image analysis system (analySIS 3.0, Soft Imaging Systems, Münster, Germany) linked to the epifluorescence microscope. Images were captured with a cooled slow-scan charge-coupled device (CCD) camera (resolution, 1276 x 1008 pixel, 1 pixel = 0.05 µm in the microscopic image) at 1250x magnification, and filtered and binarized as described in (Massana et al. 1997). Cell volumes were estimated according to Björnsen (1986). Filamentous bacteria were counted in 4 size classes (<5 µm, 5-10 µm, 10-20 µm, >20 µm) from DAPI-stained slides (0.2 µm, see chapter I) using an ocular micrometer.

Total filament biovolume was estimated by multiplying the cell numbers within each size class with a mean cell biovolume empirically determined by measuring at least 30 cells of each size class at 1250 x magnification with an automated image analysis system (SIS, Münster, Germany; see chapter I) and by assuming cylindrical geometry and a mean cell width of 0.65 µm.

For **fluorescence in situ hybridization** (FISH) subsamples of 10-30 ml were fixed with formaldehyde solution (final concentration, 2%) for <24 h at room temperature, filtered onto white membrane filters (Type GTTP, diameter, 47 mm, pore size 0.2 µm, Millipore, Bedford, Ma), rinsed with sterile Millipore water and stored at –20°C until further processing.

Nutrient analysis and TN:TP measurements were conducted by T. Hansen (IfM Kiel) as described in chapters I and III. For DOC analysis, replicate water samples were filtered through GF/F filters (Whatman, at 550°C for 24 h), collected in acid-rinsed (1M HCl) vials (Packard) and immediately frozen. Measurements were performed at the Institute for Baltic Research (Warnemünde) with a Shimadzu TOC5000 carbon analyser.

16S rRNA gene clone libraries (Pernthaler et al. 2004). For the construction of a 16S rDNA clone library, 100 ml of formalin-fixed, unfiltered lake water obtained on May 19th was filtered onto Durapore membrane filters (pore size, 0.2 µm, Millipore) and stored at -80°C until further processing. DNA was extracted according to previously published protocols (Schauer et al. 2000). Briefly, samples were pre-treated with sodium dodecyl sulfate, lysozyme and proteinase K. Extraction was performed by phenol-chloroform-isoamyl alcohol (25:24:1) followed by chloroform-isoamyl alcohol (24:1) and subsequent concentration and rinsing. DNA concentration was quantified by agarose gel electrophoresis. 2 ng of extracted DNA was used as template for the amplification of environmental 16s rRNA genes by polymerase chain reaction (PCR). For PCR, 5 µl of bovine serum albumin (BSA) (stock concentration, 3 mg ml^{-1}), 5µl 10 x PCR buffer, 2µl dNTPs (stock concentration, 2.5 mM), 0.5 µl of the general bacterial primers GM3F and GM4R (Muyzer et al. 1993) (stock concentration, 15 µM) and 0.25 µl of TaKaRa-Taq DNA polymerase (TaKaRa BIO Inc., Shiga, Japan) (stock concentration, 5 units per reaction) were adjusted to a final volume of 48 µl with sterile water.

The PCR was run on an Eppendorf Mastercycler (Eppendorf, Hamburg, Germany) at the following conditions: 1 cycle at 94°C for 5min; 35 cycles at 94°C for 30 sec.; 47°C for 45 sec; 74°C for 1min 30 sec and 74°C for 10 min. The amplified rDNAs were purified using the QIAquick PCR purification kit (QIAGEN, Hilden, Germany), inserted into the TOPO vector (TOPO TA cloning kit, Invitrogen, Karlsruhe, Germany) and cloned into competent cells of *E. coli* as described by the manufacturer. The transformed cells were plated on LB agar plates containing 50 µg ml^{-1} ampicillin and incubated overnight at 37 ºC. The clones were screened for right-sized inserts and plasmid preparations were done with the QIAprep Spin Miniprep Kit

(QIAGEN). For a first screening the plasmid DNAs were sequenced with the vector primer M13F (5'-GTA AAA CGA CGG CCA G -3'). The obtained partial sequences were submitted to the BLAST queuing system (http://www.ncbi.nlm.nih.gov/blast/blast.cgi) for a tentative phylogenetic positioning. Nearly full-length sequences were obtained from those inserts that were related to the *Bacteroidetes* by additional sequencing with the primers GM1F (Muyzer et al. 1993) and M13R (5'-CAG GAA ACA GCT ATG AC -3').

Phylogenetic analysis and probe design was performed by Jakob Pernthaler (MPI for Marine Microbiology, Bremen, Pernthaler et al. 2004). Partial sequences were assembled manually using the software Sequencher (Gene Codes Corp., Ann Arbor, USA) and tested for chimeras through the Ribosomal Database Project CHIMERA_CHECK program. All sequences were again analyzed via BLAST to identify their closest relatives. Phylogenetic analyses were performed using the ARB software package (www.arb-home.de). The ARB database (release June 2002) was complemented with sequences from GenBank that were related to freshwater lineages of *Cytophaga-Flavobacteria* (Glöckner et al. 2000, Zwart et al. 2002). For the reconstruction of a phylogenetic tree only nearly complete (i.e. longer than 1,300 nucleotides) 16S rRNA gene sequences affiliated with this subphylum were considered. A 50% base frequency filter was applied on these sequences to exclude highly variable positions. The respective ARB tools were used to perform maximum parsimony, neighbour-joining and maximum likelihood analyses. Downloaded partial sequences of closely related sequence types from freshwaters were subsequently added to the consensus tree according to maximum parsimony criteria, without introducing changes in the topology based on the complete sequences. Newly generated sequences that were included in the phylogenetic analysis have been submitted to GenBank (accession numbers: AJ697697-AJ697708, Pernthaler et al. 2004).

Specific oligonucleotide probes for new and available sequences within the freshwater LD2 and CL500-6 clusters as defined by Zwart et al. (2002), as well as for two other groups of newly obtained sequences within the *Cytophaga-Flavobacteria* were designed using the ARB software package and the 16S rRNA accessibility information provided by Fuchs et al. (1998). Stringent conditions for FISH were established by analysis of fluorescence intensities of the target cells from hybridizations at increasing concentrations of formamide in the hybridization buffer (Pernthaler et al. 2001).

Fluorescence in situ hybridization (FISH). Whole cell in situ hybridizations on sections from the polycarbonate filters were performed by Jakob Pernthaler (MPI for Marine Microbiology, Bremen; Pernthaler et al. 2004) using the 16S rRNA targeted oligonucleotide probes EUB I-III (Daims et al. 1999), CF319a (many groups of the *Cytophaga-Flavobacterium* cluster of the *Bacteroidetes*) (Manz et al. 1996), R-FL615 for the freshwater cfIII (*Flectobacillus* sp.) lineage (Šimek et al. 2001), and the newly designed specific probes.

All probes were obtained from ThermoHybaid (Interactiva Division, Ulm, Germany). Hybridizations with directly Cy3 monolabeled probes were performed as described, with 35-40% of formamide in the hybridization buffer (see results for hybridization conditions of newly designed probes) (Pernthaler et al. 2001). After FISH the filter sections were counter-stained with DAPI (final concentration, 1 µg ml^{-1}) and evaluated on a Zeiss Axioplan II epifluorescence microscope equipped with 40 and 63x Plan Neofluar oil objective lenses (Carl Zeiss). The filter sets were Chroma HQ 41007 (Chroma Tech. Corp. Brattleboro, Vt.) for probe fluorescence and Zeiss01 for DAPI. The relative abundances of cells hybridised with the specific probe was determined as a fraction of all DAPI-stained filaments (i.e., cells >5 µm). At least 200 cells per preparation were counted. From lake samples, the length of 40-50 hybridised cells was measured and mean cell volumes estimated as described above. Images of Cy3 and DAPI fluorescence were captured using a SPOT colour CCD camera (Diagnostic Instruments, Sterling Heights, MI) mounted on the Axioplan II microscope.

RESULTS

The lake and the 4 selected mesocosms differed in predation regime and displayed different food web successions. Two control mesocosms without top-down control by crustacean mesozooplankton and a potential dominance of protists where chosen besides two mesocosms seeded with *Daphnia*. The initial conditions in this spring experiment differed markedly from the summer situation and were characterized by lower water temperatures, phosphorus-depletion and low densities of mesozooplankton. Surface water temperatures at start were 11.9 ± 0.1 °C with a maximum on day 12 (18.3 ± 0.1 °C) and a mean over the experimental period of 14.2 ± 2.3 °C.

	PO_4	NO_3	NH_4	TN	TP	TN:TP
Control	0.009	1.60	19.71	34.17	0.40	85.4
dap 5	0.008	1.07	4.92	29.26	0.32	91.4
lake	0.013	1.39	8.61	32.65	0.38	85.9

Table 1:
Initial nutrient data [µM] from selected enclosures and the lake (mean of replicate enclosures).

The initial nutrient data from the lake and the mesocosms indicate P-limited conditions (ortho-phosphate below detection limits) and an initial molar total N: total P-ratio between 85 and 91 (Table 1). Food web dynamics even raised this ratio to values between 106 and 138 in the course of the experiment (see Fig.1).

These nutrient conditions were paralleled by an increase in concentrations of dissolved organic carbon (DOC), as shown for selected mesocosms (Fig.2, data for only 3 treatments available).

Additional DOC-degradation experiments with and without the addition of inorganic nutrients revealed that DOC accumulation was less intense when nutrients were added (data not shown). Chlorophyll a concentrations in the lake ranged around 3 µg L^{-1} and inside the mesocosms they decreased from 5.3(±1.5) to 0.7(±0.6) µg L^{-1}.

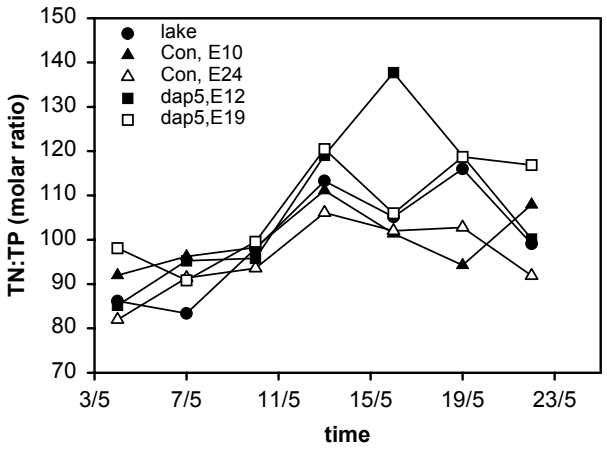


Figure 1 :
Molar ratio of total nitrogen to total phosphorus for selected mesocosms and the lake.

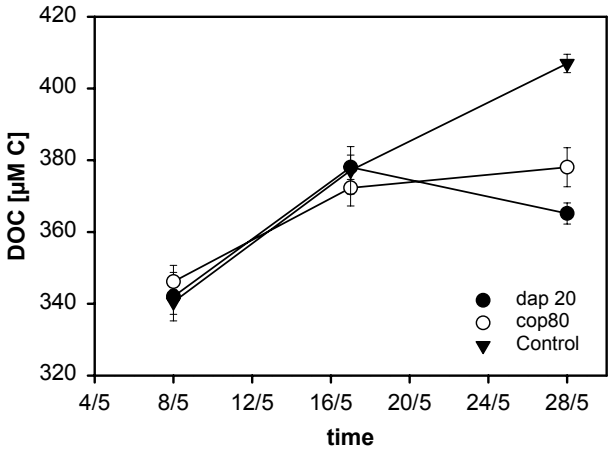


Figure 2:
DOC [µM] in selected enclosures over time.

The phytoplankton community was characterized by very high abundances of *Chrysochromulina* sp (up to 25000 cells ml^{-1}). The weak autofluorescence of these potentially mixotrophic nanoflagellates could be an indication for a shift towards heterotrophic nutrition in order to acquire nutrients for photosynthesis during periods of inorganic nutrient limitation (Nygaard & Tobiesen, 1993). Community grazing experiments with fluorescently labeled bacteria revealed that *Chrysochromulina* cf. *parva* sometimes ingested particulate bacterial prey (data not shown) and therefore should be taken into account as a potential grazer of bacteria. Apart from *Chrysochromulina* also other flagellate taxa (*Cryptomonas*, *Dinobryon*) were noticed in higher abundances and were found to ingest fluorescently labeled bacteria (personal observation), suggesting a high degree of mixotrophy in this P-limited spring situation.

The analysis of zooplankton (B. Santer, personal communication) in the course of the experiment yielded no remarkable mesozooplankton development in the control mesocosms. In the lake, a population increase of *Daphnia* was observed with maximum densities of 18.9 individuals per liter (Table 2). The enclosures seeded with *Daphnia* (dap5), showed a similar incline in the second half of the experiment, which was much stronger, started earlier and peaked at densities of 55.7 (E12) and 43.2 (E19) individuals per liter. The rotifer community has not been extensively investigated, but plankton counts revealed comparably low abundances in most treatments.

Microbial food web successions and filament development

The overall differences between the investigated microbial food webs were astonishingly small with respect to numerical responses of functional groups. Ciliates were most significantly (Repeated-Measures ANOVA, p=0.0141) affected by the experimental treatments “moderate *Daphnia* development, lake”, “no *Daphnia*” (controls) and “strong *Daphnia*” increase (dap5 mesocosms), whereas nanoflagellates, coccal bacteria and thymidine uptake differed not significantly in terms of treatment. Interestingly, total filament biovolume significantly responded regarding time-treatment-interaction (p=0.0010), but not with respect to treatment alone.

Lake

The microbial food web in the lake (Fig. 3) was characterized by low abundance levels of ciliates during the first 2 weeks. Thereafter, mainly nanociliates strongly increased between May 16th and 28th (days 19 and 25) up to a maximum of 12.4 cells per ml. Intermediate-sized ciliates (20-40 µm) showed a peak on day 19 (6.3 cells per ml, not discriminated in figure) and large ciliates (>40 µm) were always below 3 cells per ml.

The nano-sized compartment, mainly consisting of mixotrophic Ch*rysochromulina* and HNF, differentially developed in Schöhsee. HNF contributed with 3 to 59% to the flagellate community and showed 2 smaller peaks on experimental day 4 (May 7th, 2.9 x10³ cells per ml) and day 19 (2.7 x10³ cells per ml) and a maximum on day 10 (5.7x10³ cells per ml). *Chrysochromulina*, instead, had an abundance maximum after 1 week (25.3 x10³ cells per ml). Autotrophic picoplankton (*Synechococcus,* not shown in graph) tended to increase with time and peaked on May 13th with maximal concentrations of 2.8 x10^5 cells per ml, thereafter decreasing slightly.

Throughout the experiment, heterotrophic bacteria (single rods and cocci) mostly ranged from 1.1 to 2.4 x10^6 cells per ml with a peak of 2.5 x10^6 cells per ml on May 19th. Bacterial production, measured as ³H-thymidine incorporation, was very low until May 16th (0.5-1.2 pmol $L^{-1}h^{-1}$) and then increased to 14.4 pmol $L^{-1}h^{-1}$ until May 25th.

In the unmanipulated lake as well as in the four selected mesocosms a strong development of filamentous bacterial morphotypes was observed. However, the extent to which filaments came up, was rather variable. In general, size class heterogeneity decreased towards long filament sizes dominating the community (Fig.3, 4).

In the lake, filamentous bacterial morphotypes increased in abundance and biovolume by more than two orders of magnitude within three weeks, and steeply dropped during the following week (Fig. 3). Filament development started earlier compared to the mesocosms and showed already higher abundances on May 16th. Thereby, the increase reached peak abundances (filaments >5µm: 1.33x10^5 cells ml^{-1}) and biovolumes (1.4x10^{5}µm³ml^{-1}) on May

22nd. The size class 10-20 µm was dominant throughout most of the experiment with a maximal relative contribution of up to 77 % on May 19th, thereafter smaller size fractions (5-10 µm) gained in importance (up to 45%).

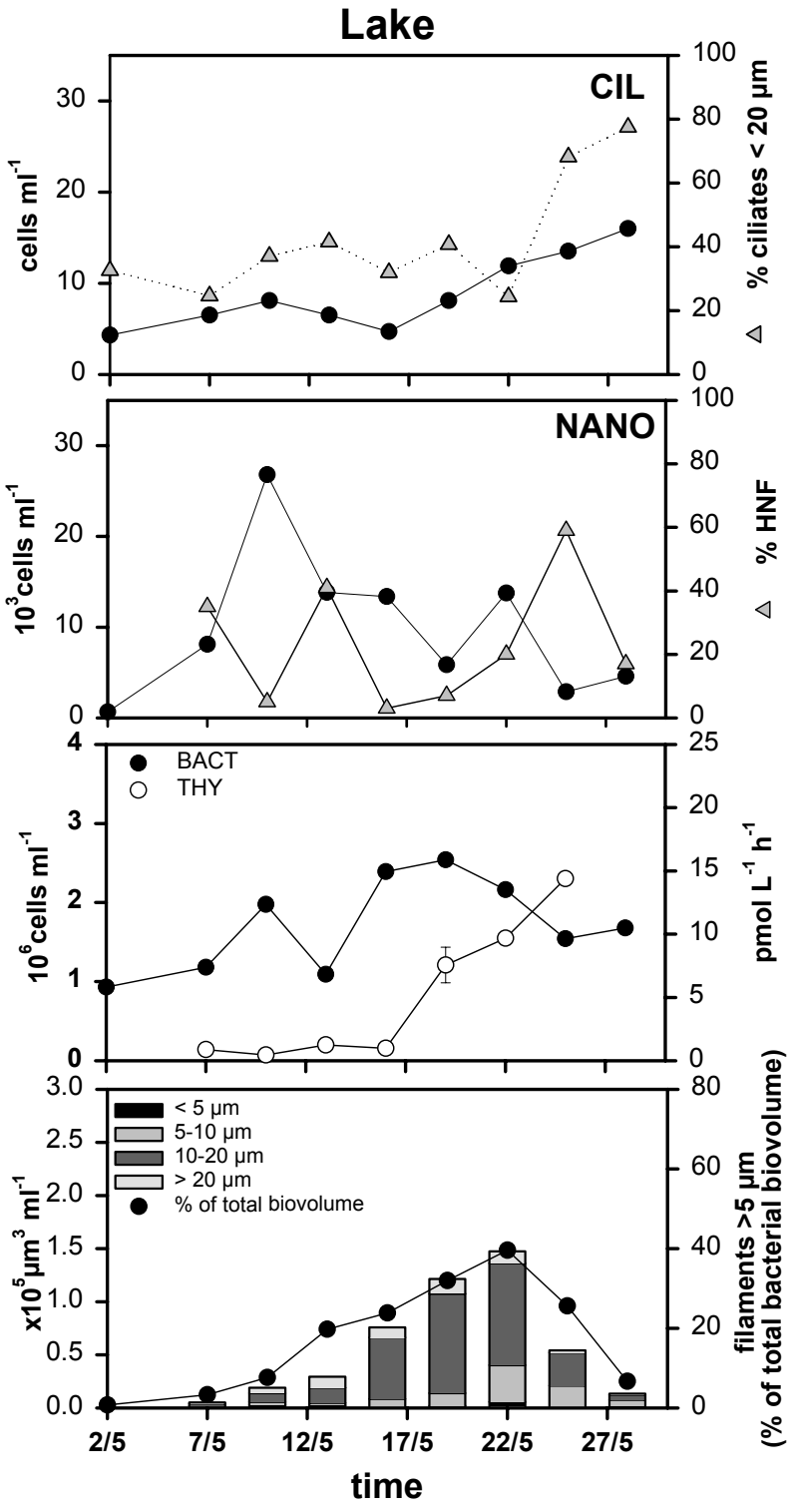


Figure 3:
Succession of microbial food web components in the lake: ciliates (CIL; and % of small ciliates <20µm), nanoflagellates (NANO, consisting of heterotrophic forms (%HNF) and *Chrysochromulina*) and bacteria. Furthermore, bacterial thymidine uptake (THY) and development of bacterial filaments are shown (as biovolume in 4 size classes and relative proportion of filaments larger 5 µm to total bacterial biovolume).

Control mesocosms (no mesozooplankton)

Higher ciliate abundances than in dap5 mesocosms and in the lake were found in control enclosures (Fig. 4) with peak densities of 24.4 (E10) and 28.8 (E24) cells per ml, thereby consisting to 79% and 84% of larger ciliates (>20 µm), respectively (see Fig. 5). The highest proportion of nanociliates (<20 µm) was observed earlier with 7.2 (E10) and 11.9 (E24) cells per ml. Nanoflagellates, in turn, showed strong oscillations and peak densities (33.3x10³ per ml) clearly exceeding those measured in the other treatments. HNF constituted markedly variable relative proportions (4-53%) of the total nanoflagellate community. Although proportions were generally higher in the second half of the experiment, actual density maxima (4.4-5.1x 10³ cells per ml) were achieved until May 13th.

Synechococcus continuously decreased in abundance (from 12.4-14.1x10^4 per ml to a minimum of around 0.3x10^4 per ml, not shown in graph) and also heterotrophic bacteria strongly declined from initial concentrations of 3.1-3.2x10^6 per ml to values between 0.6 and 1.8x10^6 per ml. A second abundance peak of around 3x10^6 cells per ml was registered in only one control mesocosm (E24). Bacterial production was mostly at a low level throughout the experiment (1.2-7.5 pmol L-1H-1). Uptake rates corresponded well with bacterial abundances, and thus showed an incline in E24 to values between 15 (May 19th to 25th).
Maximal total biovolumes for filamentous bacteria of up to 2.0 - 2.2x10^5 µm³ per ml (corresponding to concentrations of filaments >5 µm of 1.5-2.0x10^5 cells ml^{-1}) were registered on May 22nd. During this peak, filamentous bacteria constituted between 42 and 68% of total bacterial biovolume. Long filament sizes (10-20 µm) predominated (43-46%) until May 22nd but were later replaced by smaller forms (around 47% of filament biovolume on May 25th, not shown in graph).

Daphnia - treatments

Ciliates remained at a low abundance level (mostly below 5 cells per ml) in dap5 mesocosms until May 13th and thereafter strongly increased, reaching peak densities of 16.6 (E19) to 19.2 (E12) cells per ml and consisting mostly of oligotrich species larger than 20 µm (Fig. 4, right panel). As in controls, nanociliates dominated the ciliate community during the first experimental week, but at a lower abundance level. Resembling the situation in the lake and controls, nanoflagellates were abundant in the first half of May reaching peak densities of 19.3x10³ cells per ml. Thereafter, the mean proportion of HNF varied between 5 and 50% with maxima of 3.1-4.1x10³ cells per ml. Picoplankton showed a marked initial decrease, *Synechococcus* (not shown in graph) from 12-16x10^4 cells per ml at the start point to values of around 2x10^4 per ml on May 22nd. Heterotrophic bacteria stayed, after a first decrease, at a relatively constant level, with minima on May 22nd (0.94x10^6 per ml in E19, 0.85x10^6 per ml in E12) and a small maximum on May 16th (2.6x10^6 per ml).

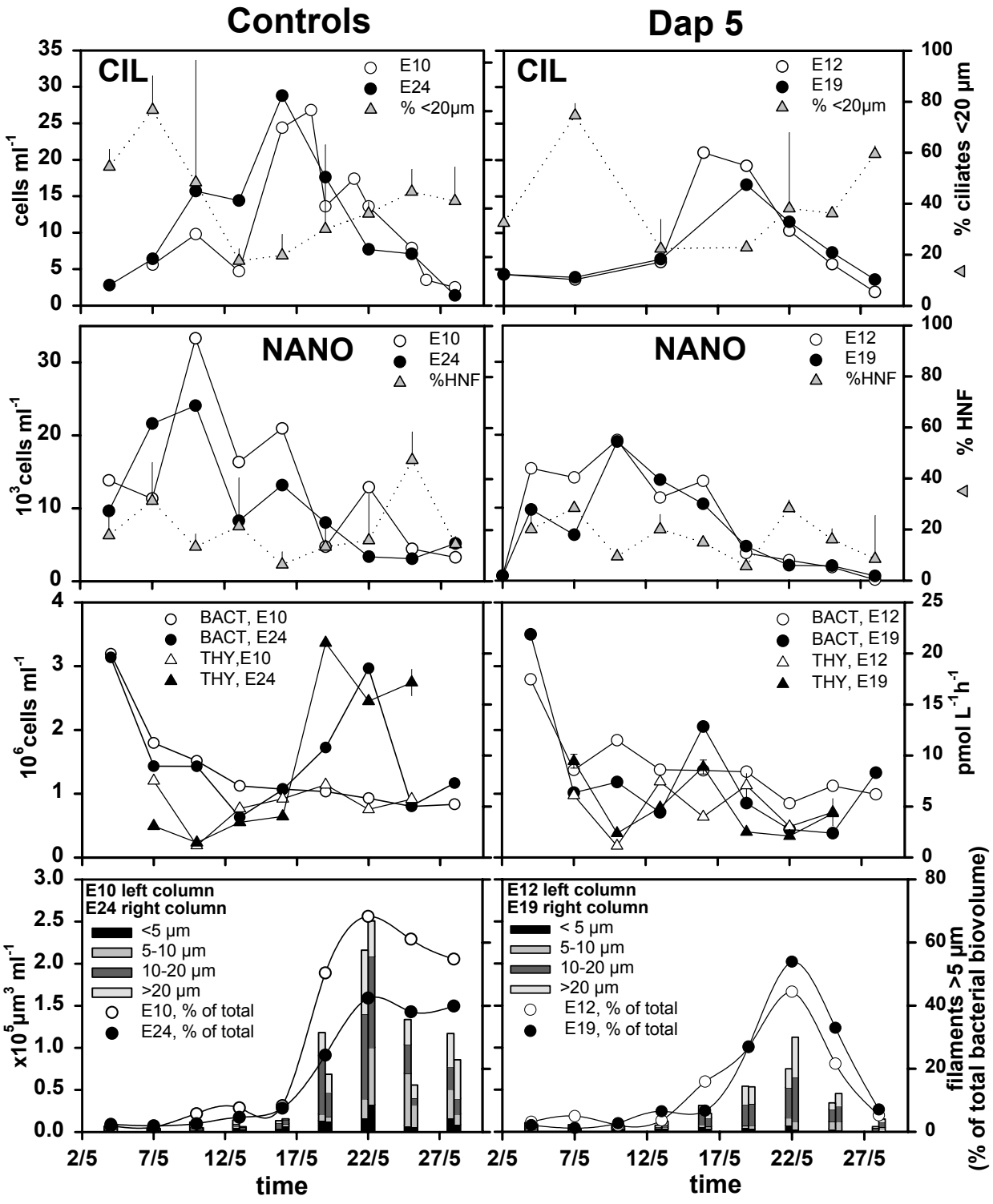


Figure 4:

Development of various microbial food web components in replicate control (left panel, Enclosures E10, E24) and dap5 (Enclosures E12, E19) mesocosms. Ciliates (CIL; and % of small ciliates <20µm), nanoflagellates (NANO, consisting of heterotrophic forms (%HNF) and *Chrysochromulina*) and bacteria are demonstrated. Furthermore, bacterial thymidine uptake (THY) and development of bacterial filaments are shown (as biovolume in 4 size classes and relative proportion of filaments larger 5 µm to total bacterial biovolume).

Accordingly, low ^{3}H-thymidine uptake rates with values persistently below 10 pmol $L^{-1}h^{-1}$ were noted. In the dap5 mesocosms, filament development was far less intense than in controls and in the lake, but showed the same general pattern with a maximum on May 22nd. At that time filamentous bacteria (>5 µm) dominated the community and accounted for 44-54% of total bacterial biovolume. Temporal dynamics of filament formation and filament size class composition proved to be similar to the other treatments, but absolute maximal cells numbers ($0.76x10^5$ cells ml^{-1}) and biovolumes ($1.1x10^5 µm^3 ml^{-1}$) were by far lower than in the control mesocosms and the lake.

Other activity parameters

A selection of other bacterial activity indicators gave support to numerical succession patterns of the investigated microbial food webs and further characterized the different scenarios, under which filamentous bacterial morphotypes could develop. The proportion of HNA bacteria (high nucleic acid, not shown in graphs) slightly increased with time until May 22nd. Time-averaged percentages of selected treatments differed markedly, with the highest value found in the lake and a significantly lower average in dap5 treatments (Fig. 5). Correspondingly, bacterial communities of dap5 mesocosms showed the lowest proportions of actively respiring (CTC-positive) cells (5.6±2.7%), while values for controls (7.0±5.2%) and especially for the lake (25.4%) were considerably higher. Higher CTC+ values were measured earlier in the dap5 and control bags (May 16th, 13.6±1%, 12.3±0.4, respectively).
Interestingly, the determination of ^{3}H-leucine uptake as a measure for protein synthesis (data not shown in graph) yielded earlier maxima and stronger increases than the measurement of thymidine uptake. From May 10th to 16th, when thymidine incorporation rates were still low and the filament bloom was about to start, leucine uptake rates strongly increased (maxima in controls 73-86 pmol $L^{-1}h^{-1}$) and proved to show increasing scatter until May 19th, apparently corresponding to the extent of the subsequent development of filamentous bacteria. Increases in leucine uptake during that period was 3.2-7.8-fold in controls, 3.4-fold in the lake and 2.3-2.7-fold in dap5 mesocosms, respectively.

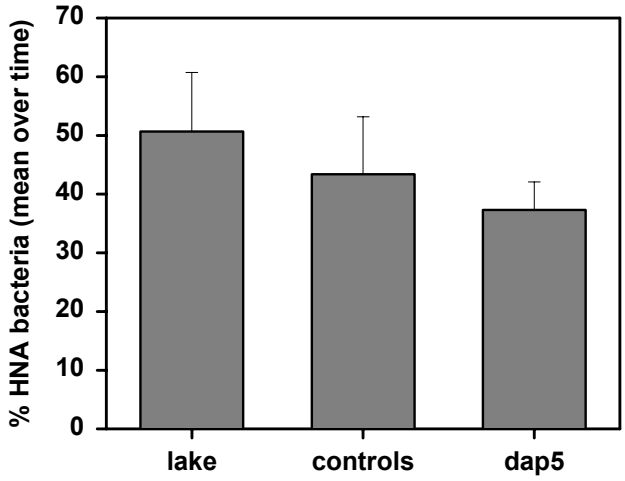


Figure 5:
Time-averaged relative proportions of high nucleic acid bacteria (HNA) of total cells (single, non-filamentous cells), calculated from means of replicate mesocosms). Only differences between lake and dap5 enclosures were significant, ANOVA, p=0.0213)

Furthermore, the increasing proportion of large filamentous morphotypes was reflected by results obtained from a size-fractionated bacterial production measurement (not shown in graph). From May 17th to 23rd, the contribution of bacteria >3µm to total bacterial thymidine uptake was high and further increased from 33.9 to 42.9% in the selected control mesocosm (E10). In *Daphnia*-dominated enclosures, instead, very small cells (<1.2µm) contributed with approximately 77-90% to total bacterial production.

Filamentous bacteria LD2-739

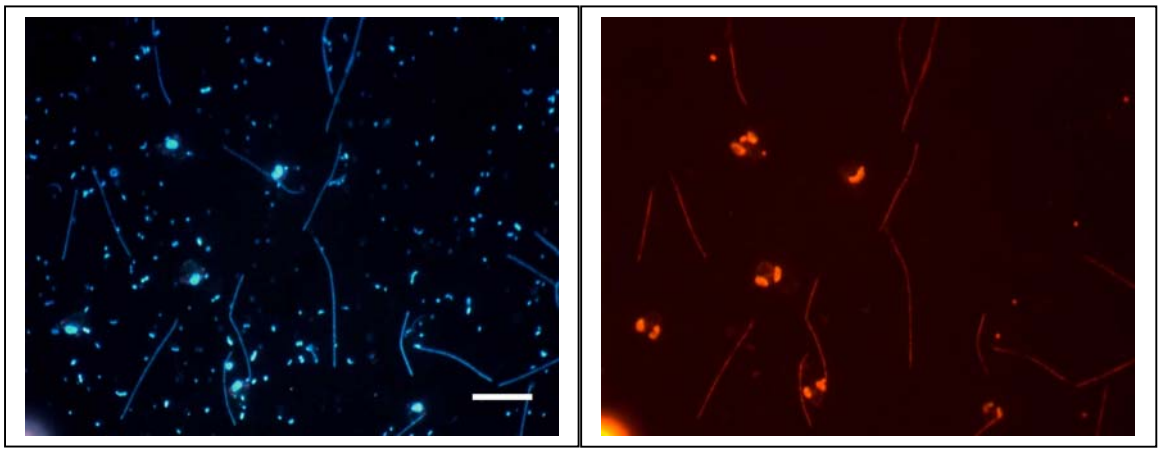


Figure 6:
Photomicrographs of filamentous bacteria stained with DAPI (left) and hybridised with Probe LD2-739 (same microscopic field, scale bar = 10 µm). (Provided by Jakob Pernthaler, MPI Bremen.)

Filamentous morphotypes constituted a substantial component of the bacterial assemblage and obviously belonged to different taxonomic subgroups. Between 33% and 100% of all hybridised filament-forming bacteria in lake water and in the mesocosms could be detected by probe CF319a (*Cytophaga-Flavobacterium* cluster, data not shown, Pernthaler et al. 2004). However, comparably high but variable proportions of bacterial filaments hybridised with the new specific probe LD2-739 (Fig. 6). A detailed phylogenetic affiliation of the LD2 clade and the other clones belonging to the *Cytophaga-Flavobacteria* cluster can be obtained from Figure 7 (Pernthaler et al. 2004).

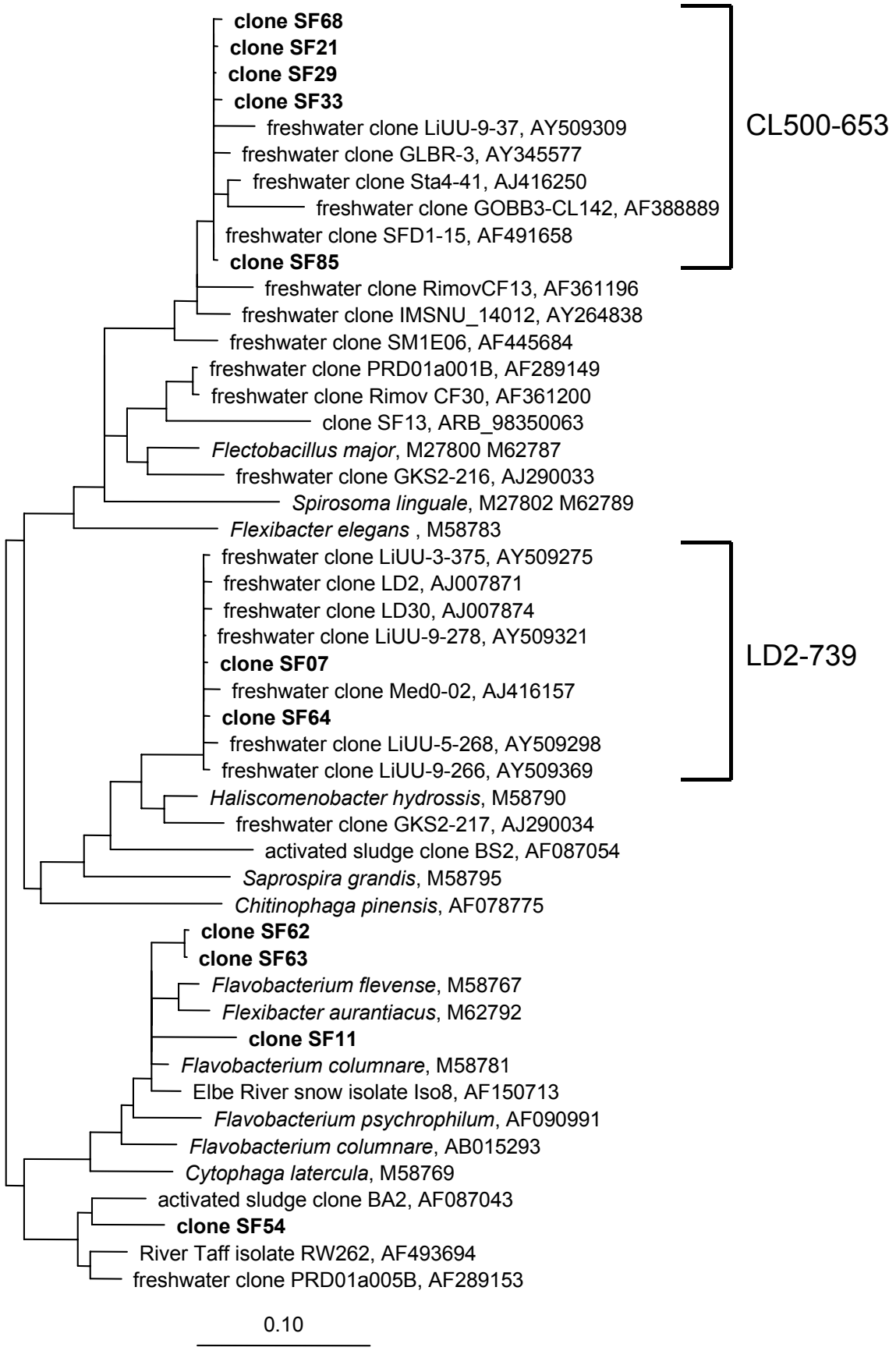


Figure 7:
Phylogenetic tree (provided by J. Pernthaler) showing the taxonomic affiliation of LD2-739 filaments.

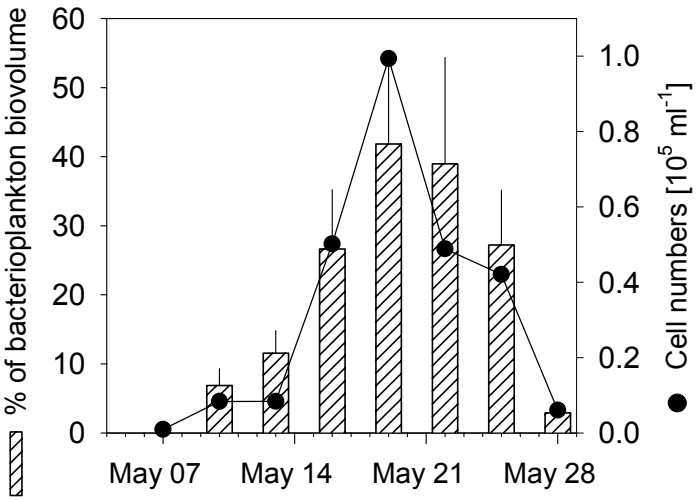


Figure 8:
Development of the filamentous bacteria hybridising with probe LD2-739 in the lake. Absolute abundances and relative contribution to total bacterioplankton biovolume is demonstrated.

While similar proportions were observed in the beginning of the experiment (May 7th), the succession of LD2-739 and their relative importance differed significantly (Repeated-Measures ANOVA, post-hoc comparison of means) between the different treatments. Members of the LD2 clade accounted for most of the filament bloom observed in Schöhsee (up to 97.8% of all filaments), where they achieved peak densities of nearly $1x10^5$ cells per ml on May 19th (Fig. 8). At that time, it was estimated from cell size measurements of all bacteria and of filaments of the LD2 clade that this group transiently formed up to 42% of total microbial biovolume.

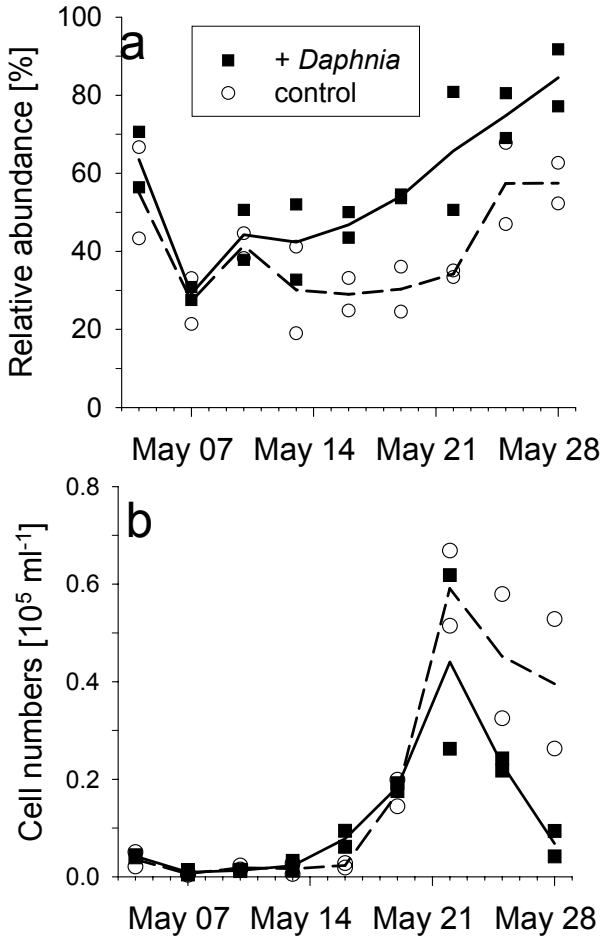


Figure 9:
The relative abundance of LD2-739 given as percent of all filamentous bacteria (a) and absolute cell numbers (b) in controls and mesocosms with *Daphnia* development (dap5).

Bacteria of the LD2 clade formed a significantly higher fraction of total filaments in the mesocosms that had been seeded with 5 ind. L^{-1} of *Daphnia* than in the *Daphnia*-free control ($p<0.05$) (Fig. 9a). Respective time-averaged proportions (as percent of all filaments over the whole experimental period) of LD2-739 cells in the lake (59.3±24.2%) equalled those determined in dap5 mesocosms (E12 53.8±20.7, E19 58.4±17.3, amplitude 27.6-91.8%) and

clearly exceeded mean percentage data obtained for the control treatments (38.9±11.8% E10, 41.6±17.8% E24, amplitude 19-67.8%).

As in lake water, there was a bloom and subsequent decline of LD2 filaments in mesocosms containing *Daphnia*. In contrast, LD2 filaments showed only a slight population decrease in mesocosms without cladocerans (Fig. 9b) at the end of the study period.
Changes were also observed at the level of single-cell characteristics. The mean cell length of LD2-bacteria initially ranged around 20 µm (not shown in graph). It significantly increased to >30 µm when their abundances started to decline, and significantly decreased below the original cell length in parallel with their disappearance in the plankton ($p<0.05$, Kruskal-Wallis ANOVA on ranks, Dunn´s post-hoc comparison).

DISCUSSION

Patterns of food web succession

The general succession of microbial food webs differed remarkably between the lake and the experimentally manipulated mesocosms in terms of abundance and activity and was characterized by considerable dynamics. In all investigated food webs, there was a nanoplankton peak within the first third to half of the experiment, a concomitant decrease in auto- as well as heterotrophic bacteria and the notion of a generally high degree of mixotrophy (abundances mainly of *Chrysochromulina*). Besides a rather low level of thymidine uptake (except for one control mesocosm), all manipulated enclosures showed a maximum in ciliate abundance in the midst of May, following after the peak densities in nanoplankton. Ciliate abundance strongly declined within the last experimental week and was mostly composed of micro-sized forms like *Rimostrombidium* or *Histiobalantium* and species with jumping bristles like *Halteria* (in dap5 mesocosms).

The strong incline in *Daphnia* densities in the lake and even more in dap5 enclosures in the second half of May very likely accounted for most of the observed differences in microbial food web successions. In *Daphnia*-free controls, absolute protist abundances were higher and larger amplitudes in bacterial abundances were observed than in the lake and dap5 mesocosms. Here, the nanoflagellate bloom was less intense and underwent the strongest decline when *Daphnia* came up. This strong top-down impact of *Daphnia* caused substantial reductions of lower food web components like ciliates and nanoplankton as demonstrated in the same system in summer (Zöllner et al. 2003) and by other studies (e.g. Marchessault and Mazumder 1997).

A quite high overall similarity of the succession pattern in filament formation was observed, despite a number of differences in microbial food web structure, which would even increase if accounting for further complexity and heterogeneity within functional

compartments such as HNF. However, by means of a highly specific probe, not only the threadlike morphotype itself could be traced. By following the dynamics of a phylogenetic subgroup a much higher resolution of this filament blooming event could be achieved.

The formation of a filamentous morphotype obviously is a common strategy in various bacterial phylogenetic groups (e.g. Jürgens et al. 1999a). They can constitute very important components in aquatic microbial assemblages and have been documented in a number of studies (Pernthaler et al. 1997, Hahn and Höfle 2001). Few investigations also reported their massive occurrence, for example they accounted for more than 50% of total heterotrophic microbial biomass in the pelagic zone of acidified lakes (Vrba et al. 2003). As the more closely investigated LD2-739 filaments, filamentous bacteria affiliating with the *Cytophaga-Flavobacterium* cluster constituted seasonally even the largest pool of bacterioplankton biomass in Gossenköllesee (Pernthaler et al. 1998). Remarkably, the general occurrence of filaments seems to be restricted to limnic and brackish habitats and almost never observed in marine systems.

In general, bacterial community shifts can be triggered by a number of different factors, of which predation belongs to the most important ones (e.g. Šimek et al. 2002). However, strong and rapid shifts in bacterial community composition mostly occurred when very pronounced alterations in predation regime took place, for example the experimental shift from protist to *Daphnia* grazing (Langenheder and Jürgens 2001, Degans et al. 2002). In other studies on bacterioplankton over different temporal and spatial scales, bacterial community composition proved to be rather stable (Höfle et al. 1999, Riemann and Middelboe 2002, see also chapter 1). Additional factors (e.g. poor nutrient conditions) might set the limits for physiological variability and diversity changes in aquatic bacterial communities. Finally, there is evidence that the observed increases of total filament biovolume (phenotypic diversity shift) to a certain extent can also be enhanced by phenotypic plasticity of individual taxa, as supported by increasing mean cell length in LD2-739 on May 22nd (not shown in graph). The mechanisms of filament formation are extensively discussed in Jürgens and Matz (2002).

Potential causes for filament emergence and elimination and the special case of LD2

Predation

Among the major determinants of filaments occurrence, size-selective predation by protists (mainly HNF) appears to be best investigated (e.g. Pernthaler et al. 1997b, Šimek et al. 1997). In most previous studies, the development of HNF and filamentous bacteria was positively correlated (Jürgens et al. 1994a, Jürgens and Stolpe 1995, Sommaruga and Psenner 1995), indicating that filaments benefit from high grazing pressure. Although the exact protist grazing pressure is hard to predict and the significance of inter- and intraspecific

variation in bacterivorous and herbivorous protists (Weisse 2002) should be underlined, bacterial size matters in terms of grazing susceptibility. Already slightly larger bacteria (2-5 µm) proved to be less vulnerable to HNF grazing (Šimek et al. 1997, Jürgens et al. 1999a). Also in a previous study in Schöhsee, bacterial filaments and not aggregates were considered the main grazing refuge (Jürgens et al. 1994a).

Bacterial beyond a certain length are probably resistant against most types of protist grazers but not all. Recent experiments by Wu et al. (2004) proved that the direct-interception-feeding nanoflagellate *Ochromonas* sp. strain DS successfully preyed on filamentous bacteria and some ciliates are specialised to ingest filamentous bacteria (Shikano et al. 1990). More important are metazoan filter feeders as potential consumers of filaments and other complex morphologies (Güde 1988, Jürgens et al. 1994a).

The mass emergence of bacterial filaments was initiated between May 10th (lake) and May 13th to 16th (mesocosms), subsequent to very high abundances of nanoflagellates. It was paralleled by low concentrations of small bacteria (cocci, rods) and minimal thymidine uptake rates. Although the difference between treatments in terms of total nanoflagellate abundance over time was not significant, peak densities of filamentous bacteria were observed when high proportions of HNF prevailed in the nano-sized community.

Additionally, significant concentrations of *Chrysochromulina* as well as the occurrence of *Dinobryon* spp. indicate a potentially high level of mixotrophy. Grazing by mixotrophic flagellates (*Dinobryon* spp.) mainly induced and maintained seasonal filament formation in acidified lakes (Vrba et al. 2003).

According to other studies, this nutrition strategy is favoured under nutrient poor conditions (Samuelsson et al. 2002, Domaizon et al. 2003, Samuelsson 2003). For a close marine relative (*Chrysochromulina polylepis*) Stibor and Sommer (2003) even found that selectivity in uptake of both dissolved and bacterial P followed the ideas of optimal foraging. Consequently, *Chrysochromulina* might have influenced filament development by enhancing P-depletion on one hand and grazing pressure on bacteria on the other hand (supported by low chloroplast fluorescence and uptake of fluorescently labeled bacteria).

The observed food web successions suggest that strongly increasing densities of *Daphnia* are the most important reason for the termination of the filament mass occurrence. In *Daphnia*-free control mesocosm, in contrast, filament densities and contributions to total bacterial biovolume remained high. Treatments with highest *Daphnia* densities (dap5) not only showed lowest averages in bacterial abundance, production and relative proportions of HNA bacteria, but also lowest absolute concentrations of filamentous bacteria. Filter-feeding daphnids came up in the lake as well and preferentially eliminated larger-sized bacteria. A similar negative correlation between biomasses of cladocerans and filaments were also reported by Vrba et al. (2003).

Interestingly, the LD2-739 type of bacterial filaments reacted less sensitive to *Daphnia*-grazing and achieved larger proportions in the lake and dap5 mesocosms. The few studies examining the impact of unselective *Daphnia* grazing on bacterial community composition found diversity changes in the form of overall diversity reduction (Höfle et al. 1999) or shifts in relative proportions of taxa (Degans et al. 2002). The latter study could provide evidence for differential sensitivity to daphnids by community fingerprinting. However, King et al. (1991) found bacteria to survive *Daphnia* gut passage, suggesting reduced population susceptibility to *Daphnia* grazing and thus a potential selective advantage. To assume a similar strategy of pre-adaptation for LD2-739 cells could help to explain the relative success of these filaments and their ability to develop such amazingly high biomasses, even during periods of *Daphnia* predominance. Sequence types of the apparently widespread LD2 clade have so far been collected from various meso- to eutrophic freshwater systems (Zwart et al. 2002, Pernthaler et al. 2004).

Other factors potentially inducing and indicating filament emergence

Other factors than protist predation might also be of importance in driving filament formation and contribute to explaining succession patterns of heterotrophic bacterial filaments. For example, Holmquist and Kjelleberg (1993) found single bacterial strains to display morphological variability in response to different nutrient conditions, while Tuomi et al. (1995) reported on morphological shifts in bacterial communities due to changes in nutrient availability. Moreover, bacterioplankton community shifts were found to correlate with seasonal changes in dissolved organic matter (Crump et al. 2003).

In all investigated food web scenarios we observed strong filament increases, with differing intensities and, compared to the lake, slightly delayed in experimental treatments. This could suggest on one hand that the absolute intensity of this event is favoured or suppressed by different grazers and grazing intensities. On the other hand this could imply that the underlying mechanisms governing filament emergence are much more complex and dependent also on other factors, although explanations remain speculative to a certain extent.

These findings are consistent with results obtained for eutrophic ponds in a study of Langenheder and Jürgens (2001), who concluded that the dynamics of filamentous bacteria cannot clearly be explained by trophic interactions. The fact that filaments were of minor importance in their study ponds with high input of allochthonous matter, led the authors to the assumption that attached bacteria might be favoured as predation-resistant growth forms. At the same time this could imply that the availability of nutrients and DOC as well as elemental ratios are potential determinants of filament mass occurrences. In the present study, no ortho-phosphate was detectable and total phosphorus concentrations (TP) were minimal

between 13th and 16th of May (6.2-8.5µg L^{-1} in dap5, in controls 8.7-10µg L^{-1} and the lake 8.5-8.7µg L^{-1}), concomitant with the temporal increase in the TN:TP-ratio as well as in DOC concentrations. Bacterial filaments at very P-depleted conditions were also found in the lakes studied by Vrba et al. (2003). Moreover, there is numerous evidence of a tight coupling between the use of DOC by lake bacterial communities and phosphorus availability (Smith and Prairie 2004). An accumulation of degradable dissolved organic carbon is often due to phosphorus limitation (Olsen et al. 2002, Vadstein and al. 2003).

In addition to that, this period was paralleled by very sunny weather and thus clearly increasing water temperatures. According to Chrzanowski and Grover (2001) and the light-nutrient-hypothesis (Sterner et al. 1997), this could have further contributed to driving bacteria towards phosphorus limitation. This coincidence of biotic and abiotic phenomena provides a fairly good indication that the resulting conditions could have induced or at least favoured the mass development of filamentous bacteria.

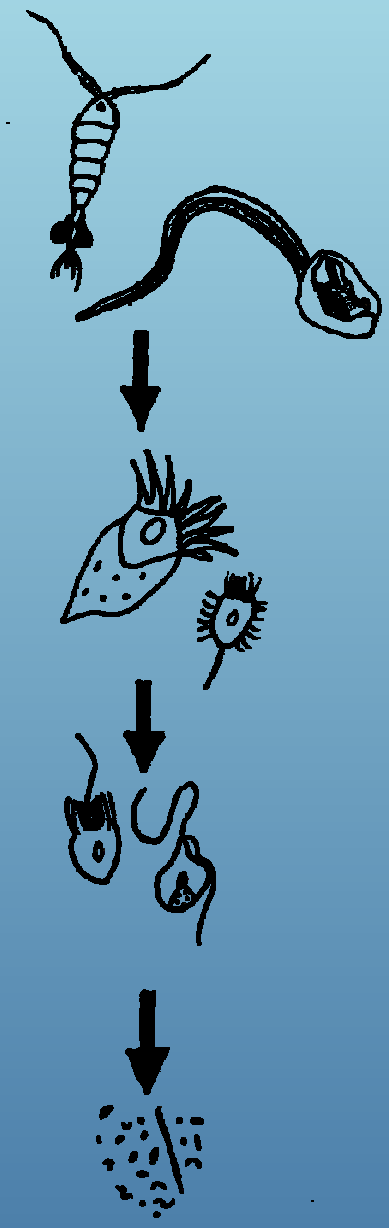
Finally, the observed patterns of bacterial activity could be also interpreted in terms of metabolic responses to the external triggers mentioned above. During the period of beginning filament emergence (May 10th to 13th in the lake, May 13th to 16th in mesocosms), thymidine uptake rates were very low (approximately one seventh of summer values) and filament biovolume increases were not reflected by inclines in relative contribution to total bacterial thymidine uptake. This points at a potentially lower cell-specific activity of threadlike cell, at least with respect to thymidine. These findings were supported by Vrba et al. (2003), who found filaments to be apparently less active in terms of thymidine uptake.

Remarkably, the increase in bacterial leucine uptake during the respective period very likely was due to enhanced protein synthesis, which could be a prerequisite or by-product of filament formation. Recent evidence from chemostat studies by Matz and Jürgens (2003) give some support to this hypothesis as P-limited cells were found to be larger and mainly filamentous with a distinctly higher protein content (two times more).

This present study of natural and experimentally manipulated microbial food web successions revealed resembling and diverging patterns in terms of abundance and activity as a background for a massive development of filamentous bacteria. By means of a specific rRNA-targeted DNA probe, the relative importance of a dominant and apparently widespread group of filaments could be traced. Thus, their functional ecological niche was better defined and the complexity of the underlying succession mechanisms further unveiled. Filament formation varied in intensity and apparently followed a general temporal scheme. In view of the experimental evidence, the development was less tightly linked to protist grazing than previously expected and influenced also by other factors. Finally, *Daphnia* turned out to play a key role in suppressing filamentous bacteria.

III

Chapter 3



Fjord

- microbial food web in spring and summer

-Dynamics of
abundance and activity -

Mesozooplankton (copepods, appendicularians) impact on microbial food web structure in a coastal marine system.

I. Cascading predation effects on protists and bacteria

ABSTRACT

We examined the impact of copepods and appendicularians on the marine microbial food web structure and microbial activity in the Trondheimsfjord (Norway) by manipulating mesozooplankton abundance and composition in large-scale mesocosm experiments performed in spring (2 experiments in April and May) and summer (1 experiment in July). The response of ciliates, nanoflagellates, heterotrophic bacteria and picocyanobacteria was followed to gradients of mesozooplankton density. Despite seasonal differences with deviating initial biotic and abiotic conditions we found a similar general pattern of a copepod-mediated cascading predation impact on microzooplankton and nanoplankton. Ciliates were reduced significantly by increasing copepod density which in turn enabled nanoflagellates to increase in abundance. This cascading predation effect also became obvious under conditions of nutrient enrichment.

Bacterioplankton responded heterogeneously to this clear 3-level trophic cascade and was rather stimulated at low copepod densities and in copepod-free controls. Mainly in the spring experiment, increased HNF-grazing at high copepod densities resulted in a decrease of bacteria and bacterial production after 1 week (4-link trophic cascade) and an increase in potentially grazing-resistant morphotypes. In the summer experiment, increasing copepod and hence nanoflagellate abundances caused decreasing but less clear trends in bacterial concentrations. However, at very high copepod densities (almost 80 ind.L^{-1}) bacterial responses were rather positive.

In contrast, blooming appendicularians (summer experiment) mainly reduced bacterial abundance and production 3- to 5-fold.

Thus, direct and indirect effects of mesozooplankton groups propagated down to heterotrophic and autotrophic bacterioplankton and induced numerical changes as well as shifts in the activity patterns of the lower trophic levels. Remarkably, while protists were similarly affected by copepod predation (ciliates decrease, HNF increase), the more complex and heterogeneous response at the bacterial level could indicate the existence of buffering and compensatory mechanisms.

INTRODUCTION

In marine pelagic ecosystems mesozooplankton link fish and macrozooplankton with primary producers and microbial food web components. All of them can exert direct predation impact on different microbial functional groups but also indirectly stimulate bacterial growth by producing nutrients through sloppy feeding, excretion and leakage from fecal pellets (e.g. Hygum et al. 1997, Møller and Nielsen 2001, Katechakis et al. 2002, Zubkov and López-Urrutia 2003).

The way in which changes in the mesozooplankton community affect lower trophic levels in marine ecosystems is still poorly understood. Most approaches focussed mainly on extrinsic resource regulation of plankton dynamics (Verity and Smetacek 1996) lack the consideration of intrinsic biotic and abiotic interactions to explain the magnitude and temporal dynamics of zooplankton communities (Sipura et al. 2003). The importance of trophic cascades and their ecosystem-level implications have been shown mainly for higher trophic levels (Pace et al. 1999) or with respect to phytoplankton blooms (Carpenter et al. 1985), whereas their impact on microbial food web components remains rather scarcely investigated (Carpenter et al. 2001, Katechakis et al. 2002, Zöllner et al. 2003).

While cladocerans which often dominate in freshwater systems, can directly regulate bacterioplankton as well as protists (e.g. Jürgens 1994), copepods and tunicates are the dominant mesozooplankton guilds in marine environments. Pelagic tunicates (i.e. appendicularians, salps, doliolids and pirosomids) potentially feed on bacteria but also affect their protist predators by direct ingestion (e.g. King et al. 1980, Sommer and Stibor 2002) or clearance by mucous houses (Vargas and Gonzalez 2004a). In contrast, adult copepods can not feed directly on bacteria (e.g. Vargas and Gonzalez 2004b) but can cause an indirect predation impact by grazing on protozoans (e.g. Calbet and Landry 1999, Zöllner et al. 2003).

Studies on short-term impact (1-3 days) in microcosms (Sipura et al. 2003, Zubkov and López-Urrutia 2003) found copepods (single species) to stimulate bacterial growth by excretion. However, the response of protist grazers was not included in that study (Zubkov and López-Urrutia 2003) and limited experimental duration did not take bacterial feedback mechanisms into account as a response to propagating trophic interactions cascading from copepods via ciliates to heterotrophic nanoflagellates and bacteria. So far, results obtained from microcosm studies suggest complexity in the trophic effect of mesozooplankton and the existence of non-linear response patterns and indirect effects.

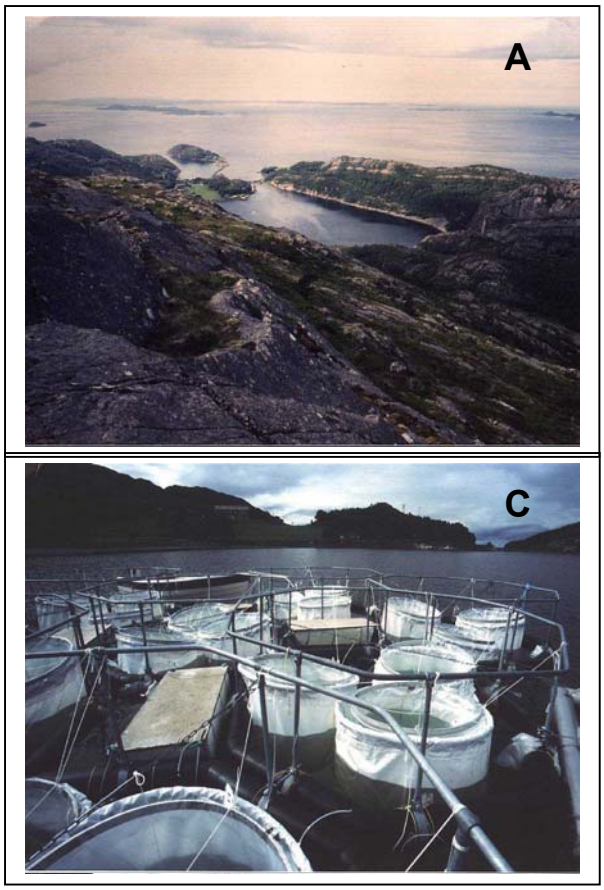
In the present study we investigated by means of large-scale enclosure experiments how copepods and appendicularians as mesozooplankton groups with contrasting feeding modes top-down impact the structure of the microbial food web.

Another objective was to assess the aspect of seasonality (water temperature, nutrient regime) and different initial copepod communities (in terms of size and composition), predominantly with respect to cascading interactions. More information on the interaction of copepods and appendicularians derived from the same summer mesocosm experiment can be obtained from Sommer (200)3 and Sommer et al. (2003, 2004 in press).

This longer-term study not only includes ciliates and heterotrophic nanoflagellates as main predators of bacteria, but furthermore reports how mesozooplankton effects on the metabolic activity of pelagic bacteria in terms of production (including cell-specific uptake rates), DNA content and respiratory activity. The detailed response in composition and diversity of the bacterioplankton assemblage is dealt with in chapter IV.

METHODS

Study site and mesocosm experiments. Mesocosm experiments were performed in a sheltered semi-enclosed marine lagoon situated on the outlet of the Trondheimsfjord (Hopavågen, Norway, 63°34´N; 9°29´O, see image 1A, B) in summer 2001 (15.07.-31.07.) and spring 2002 (20.04.-26.04., 02.05.-08.05.). This fully marine part of the fjord with an area of 27 ha and a maximal depth of 32 m is influenced by a tidal water exchange of 20% per day (www.ntnu.no/trondheim-marine-RI/Hopavaagen.html). Further information characterizing initial water conditions in the different experiments can be obtained from Table 1.



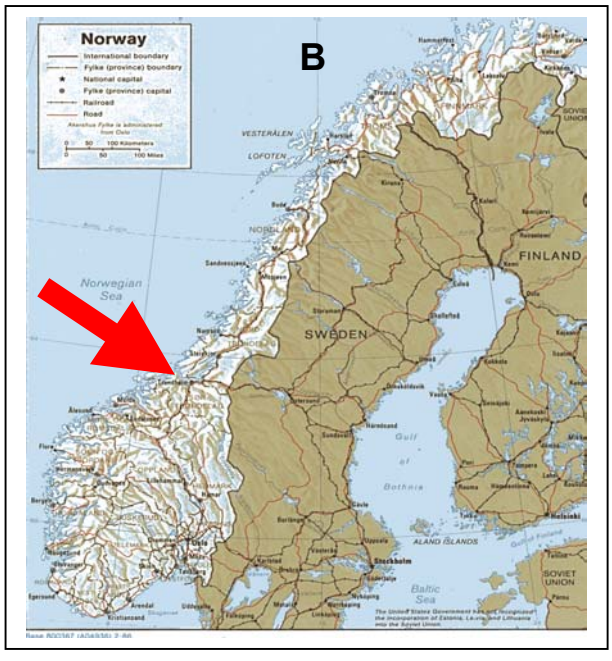


Image 1:

Hopavågen lagoon (A) is located near Trondheim at the outlet of the Trondheimsfjord (B). (C) is showing floats with 24 mesocosms installed in the central part of Hopavågen.

The 24 transparent polyethylene enclosure bags (~1.5 m³ volume, 1.7 m³ in spring, see image 1C) were filled by hauling the submerged bags to the water surface from around 3 m depth. Mesozooplankton were removed by vertical net holes with plankton nets of 250 µm (Ø 80 cm) and 150 µm mesh size.

For the subsequent addition of zooplankton in defined seeding densities to the enclosures, copepods were caught close to the surface by towing a 250 µm plankton net and transferred into a 300 L container. Dead animals were allowed to sink to the bottom and were removed before adding copepods.

Experimental treatments. In the two consecutive spring experiments (April and May 2002), the experimental treatments comprised a copepod-free control and copepod density gradients with replicated treatments (see also Table 1). In addition to a community consisting mainly of small copepod species (*Temora longicornis, Centropages* sp., *Pseudocalanus elongatus*, copepodite stages I-III *of Calanus finmarchicus* and *Oithona* sp., net fraction >250-500 µm, abbreviated with S1 to S4 and referred to as S-gradient) with nominal seeding densities ranging from 0 to 27 individuals per liter, a gradient dominated by *Calanus finmarchicus* (= L-gradient) was established with larger animals being collected with a net of 500 µm mesh size (70-80% *C. finmarchicus* copepodite stages III-V, see also chapter V and Saage 2003). Because of a biomass being approximately three to four times higher, here seeding densities were lower and ranged from 0 to 8.1. Seeding densities in the May experiment were 0-40 (S-gradient) and 0-10 ind L^{-1} (L-gradient), respectively. In general, applied copepod densities included the naturally occurring density amplitude of the investigated systems.

The copepod density gradient in summer consisted of replicated treatments with 5 nominal seeding densities (5, 10, 20, 40 and 80 copepods per liter, named Cop1, Cop2, Cop3, Cop4, Cop5, respectively). Additionally, two copepod-free controls (named Con) were established, in which 10 hauls with a 250 µm net (every 3 days) were carried out to suppress regrowth of zooplankton populations from the residual populations that survived the initial removal. The copepod community consisted mainly of calanoid species *(Temora longicornis* (45±5%), *Centropages hamatus* and *Centropages typicus* (together: 24±4%), as well as *Pseudocalanus elongatus* (24±6%) and few *Acartia longiremis* (5±2%), see Sommer et al. 2003, 2004 in press). In all copepod bags a basic stock of the cyclopoid copepod *Oithona similis* (6.0±0.7 ind L^{-1}) was present whose densities were not increased by the addition of copepods (Sommer et al. 2003).

As appendicularians were not possible to being concentrated by net catches (Sommer et al. 2003), a procedure previously applied to obtain appendicularian treatments was used (Stibor et al., 2003 in press). In order to release appendicularians from predation pressure, copepods (nearly exclusively calanoid copepods, Sommer et al. 2003) were removed with a

250 µm net (10 hauls) from six bags, which then received no further manipulation. In those bags the appendicularian *Oikopleura dioica* showed a mass development after around 9 days, which lasted for almost one week and peaked at densities of 24 and 35 individuals per liter mainly in two mesocosms. Copepods were initially present at ~7 ind. L^{-1} and generally increased in abundance to >20 to 30 ind. L^{-1} during the course of the experiment. Notably, the highest densities of *O. dioica* on day 9 were observed in the bags with the lowest (<10 ind. L^{-1}) copepod abundance (Sommer et al. 2003). Mainly those two mesocosms were selected for investigation of the microbial food web response over time. In the spring experiments no bloom of appendicularians was observed. More details on sampling and population development of *O. dioica* in the investigated enclosures can also be found in Sommer et al. (2003).

In order to assess the impact of nutrients on the food web structure dominated by either copepods or appendicularians, six mesocosms were equally enriched with inorganic nutrients (8 µmol N, 0.5 µmol PO_4^3). Half of them received an addition of copepods (seeding density 40 ind. per liter, EUCOP bags), whereas copepods were removed from the other three bags by net hauls to allow appendicularians to develop (EUAPP bags). In those mesocosms the appendicularian *Oikopleura dioica* showed a mass development after around 12 days (Sommer et al. 2003). The direct comparison with non-enriched mesocosms was maintained over two weeks for appendicularians and over one week for copepods.

Sampling, enumerations and measurements. Samples for zooplankton counts were taken every 3 days with a 50 µm plankton net (contents of 12 l water volume) and preserved with formalin (4% final concentration). Subsamples of at least 200 total individuals were counted to determine zooplankton densities. To account for the time-integrated impact of copepod grazing, copepod densities in the gradient figures (after 1 week, Fig. 2) were shown as geometric means calculated from 3 sampling counts.

Sampling of the mesocosms for microbial components (picoplankton, protozoans) was done every 1 to 3 days using a 10 L-can after gently mixing the water column up to 2 m depth with a Secchi disc. Chlorophyll a measurements were performed with a submersible fluorometer (Fluoroprobe, BBE Moldaenke, Kiel, Germany) that is able to register in vivo fluorescence of phytoplankton (e.g. Beutler et al. 2002). Pre-screened (<100 µm) subsamples for the analysis of inorganic dissolved nutrients as well as total nitrogen (TN) and total phosphorus (TP) were measured immediately after sampling by an autoanalyzer (Skalar SANplus, Skalar, Breda, the Netherlands) according to standard procedures (Grasshoff et al. 1999). Sampling, fixation and enumeration of ciliates and heterotrophic nanoflagellates as well as bacterial biovolume determinations and production measurements of heterotrophic bacterioplankton were performed as described in chapter I. Ciliate identification followed the

"user-friendly guide to coastal planktonic ciliates" by Strüder-Kypke et al., which is available in the internet at www.liv.ac.uk/ciliate/index.htm.

Flow cytometric measurements of abundances of heterotrophic bacteria, autotrophic pico- (*Synechococcus,* data for autotrophic picoeukaryotes not shown) and nanoplankton were based on the method published by Del Giorgio et al. (1996) and Gasol et al. (1999) as discussed in Gasol and Del Giorgio (2000). Samples were fixed with 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration), then after 15 min. deep frozen in liquid nitrogen and stored frozen at –80°C. For analysis, the samples of bacteria were unfrozen and stained with SYTO 13 (Molecular Probes, final concentration 5 µM, diluted in DMS) for 15 min. in the dark and run through a FACScalibur flow cytometer (Becton & Dickinson, San José, CA, USA) with a laser emitting at 488 nm until around 10000 events have been acquired (log mode).

According to their cellular DNA content, 3 different subgroups (LNA, MNA, HNA) of bacteria were discriminated by their signature in a plot of Side Scatter (SSC) vs. FL1 parameters. Picocyanobacteria (*Synechococcus*) were detected by their signature in a plot of orange fluorescence (FL2) vs. red fluorescence (FL3). Yellow green 0.92 µm latex beads (Polysciences) calibrated with TRUCOUNT™ controls (Becton & Dickinson) were used as internal standard in all measurements.

The analysis of actively respiring bacteria is based on the use of the redox dye 5-Cyano-2,3-ditolyl tetrazolium chloride (CTC, *Polysciences*) which was applied according to Del Giorgio et al. (1997) and Sieracki et al. (1999). After preparation of a 50 mM CTC stock solution (filtered by 0.2 µm, stored at 4°C), for experimental measurement at two time points, 0.9 ml of sample (+ 1 fixed control) and 0.1 ml of this stock solution (5 mM final) were incubated in a 2 ml cryovial for 45 minutes at in situ temperature. Incubation was stopped by addition of 1% paraformaldehyde + 0.05% glutaraldehyde (final concentration) followed by freezing in liquid nitrogen (after 15 minutes) and storage in a freezer (-80°C). Later the samples were measured with a FACScalibur (Becton & Dickinson) flow cytometer with the following settings: FSC: E01, SSC: 400, FL1: 500, FL2: 675, FL3: 629, threshold in FL3: 90. Acquisition and analysis of all flow cytometric data was done using Cell-Quest software (Becton Dickinson).

Determination of bacterial grazing losses. For estimation of protist community grazing impact on bacterioplankton, a microcosm grazing experiment using fluorescently labeled bacteria (FLB, Sherr et al. 1987) was performed with water of selected enclosures (S1, S3, S4, L1, L3, L4) on day 5 of the second spring experiment. 500 ml Duran bottles (3 replicates + GF/F-filtered control) were filled with enclosure water, which was prefiltered by 100 µm mesh size to remove larger zooplankton. DTAF-stained bacteria (cultured bacteria with a mean size of 1.15 µm, biovolume of 0.20 µm³, similar to measured mean cell volume

of 0.18 µm³ obtained from bacterial communities in copepod mesocosms after 1 week) were added at a final concentration of 4 x 10^5 ml^{-1} to the bottles, which were then incubated in cages in situ at around 1 m depth. Subsamples of 10 ml, taken immediately after the addition of FLB (T0) and after 24 h, were fixed with formalin (2 % final concentration), filtered onto 0.2 µm polycarbonate filters, stained with DAPI and stored at –20°C until enumeration. At least 500 FLB were counted per sample under blue light excitation.

Grazing losses (after 24 h, t_0-t_{24}) for each selected community, shown as cell loss of initial FLB concentration N_{t0} (means), were calculated according to :

$$\% \text{ of } \bar{N}_{t0} = \frac{(\bar{N}_{t0}-\bar{N}_{t24}) - (\bar{F}_{t0}-\bar{F}_{t24})}{\bar{N}_{t0}} \text{ x } 100$$

Here values of GF/F-filtered controls ($Ft_{0,}t_{24}$) without protists were subtracted from means of treatment bottles to correct for non-grazing losses e.g. due to adsorption of FLB.

Statistical analyses. In order to analyse the effects of mesozooplankton on the abundances and incorporation rates of pico- and nanoplankton regression analyses were performed. Thereby, each dependent parameter (Y) was tested against zooplankton biomass as an independent parameter (X) and regression models using first and second order polynomial equations or power regressions ($y = ax^b$) were calculated. For analysis of changes and treatment effects over time, 1- and 2-way Repeated-Measures ANOVAs and post-hoc comparisons were performed (means and Tukey HSD in Statistica 6.0).

RESULTS

Mesocosm experimental conditions

The general initial situations differed markedly among the five independent copepod gradients regarding abiotic and biotic parameters (Tab.1). Averaged water temperatures were 4-5°C higher in summer than in spring, but varied only slightly during the experiments. Initial salinity values were comparable during the first spring experiment (around 31 PSU) and in summer, and slightly higher in the second spring experiment (33 PSU). More differences were due to nutrient conditions. While the initial summer TN:TP ratio of 28.5:1 exceeded those in spring, the starting concentrations of dissolved nutrients (P, N) were much lower (sometimes at the detection limit) than those measured in spring, especially when compared with the May experiment. In terms of chlorophyll a, initial values were found to be in a similar range in all experiments with spring concentrations achieving a slightly higher level (Tab. 1).

While diatoms prevailed in spring with *Sceletonema* sp. dominating the phytoplankton assemblage (ca. 60% of total phytoplankton cells; Feuchtmayr, unpubl. data) and dinoflagellate and nanoflagellate (< 5 µm) densities being low, the initial summer plankton community was characterized by low abundances of large (> 10 µm) marine phytoplankton (*Rhizosolenia* and *Rhodomonas*) and high densities of ciliates and flagellates (< 5 µm) (Sommer et al. 2003).

Table1: Parameters characterizing treatments and the water conditions during the experiments.

treatments	L1-L4	S1-S4	L1-L4	S1-S4	COP 1-5
month	April	April	May	May	July
seeding densities [ind L^{-1}]	0-8.1	0-27	0-10	0-40	0-80
geometric mean densities [ind L^{-1}]	0-10.1	0-30.8	0.9-4	0-27.6	2.3-73.4
water temperature °C	7.8-8.4	7.8-8.4	7.8-9.1	7.8-9.1	12.6-13.5
initial salinity	31.1±0.1	31.1±0.1	33.1±0.1	33.1±0.1	30.9±0.1
mean initial molar TN:TP	25:1	25:1	22:1	22:1	28.5:1
initial PO_4^{3-} [µg L^{-1}]	3.0 ± 0.6	2.9 ± 0.4	8.3 ± 0.3	8.4 ± 0.3	< 2.5
initial NO_3^- [µg L^{-1}]	3.0 ± 1.9	3.0 ± 2.0	41.8 ± 1.9	42.2 ± 2.1	< 1.8
initial chlorophyll a [µg L^{-1}]	2.1 ± 0.4	2.6 ± 0.4	2.1 ± 0.2	2.0 ± 0.2	1.6 ± 0.2

Copepod gradients were always well established and maintained throughout the experiments with a wider density range and maximal values of 73.4 ind. L^{-1} in summer (Tab. 1) corresponding to higher naturally occurring copepod abundances during that time (Tokle, unpubl. data). Mainly in the highest density enclosures some copepod mortality was observed (approx. 20-40% Feuchtmayr, personal communication; Sommer 2003), but did not affect the persistence of a clear density gradient. Calculations for the summer experiment yielded estimations that maximal copepod mortality corresponded to ~14% and ~15% of the total N and P pools, respectively (Sommer 2003). As it was not possible to establish a strong density gradient of *Calanus finmarchicus* in the May experiment due to high mortality (geometric means 0.9 to 4 ind. L^{-1}), microbial food web components showed rather weak responses and data were thus excluded from the figures. More details on the development of the zooplankton communities can be obtained from Sommer (2003).

The addition of copepods generated no significant trends in molar TN:TP ratios, ranging from 23.3-25.5:1 (S-gradient) and from 23.4-26.6:1 (L-gradient) in April and from 19-22.8:1 in May (S-gradient). Measurements of nutrient conditions in summer yielded a slight decrease in mean TN:TP from 28.5:1 (before addition) to 27.5:1 (after addition, day 3) and almost no changes in dissolved inorganic nutrients.

Copepod effects on microbial food webs in spring and summer

Temporal development of microbial components

In terms of the development of microbial food webs in selected mesocosms (controls, lowest and highest copepod densities) over time, similar patterns were observed at the protist level during both seasons. In the first spring experiment, total ciliate numbers showed a significant treatment-dependent abundance alteration (Fig. 1A) and substantially increased in mesocosms without copepods and with lowest copepod densities (S1, L1) and remained on a high level (25-40 cells ml^{-1}).

The initial ciliate community consisted mainly of cells smaller than 40 µm and a high proportion of ciliates <20 µm (50%). Absolute densities showed a wide range from 0.02 to 43 cells ml^{-1} (means of enclosure replicates) in the course of the experiment. Apart from mostly oligotrich species like *Strombidium* spp., *Lohmanniella* sp., *Leegaardiella* sp. and *Laboea* sp., *Myrionecta rubra* was the predominant non-oligotrich ciliate in spring. In mesocosms with high copepod abundances (S4, L4), ciliate numbers clearly declined and showed an order-of-magnitude reduction after 1 week.

Similarly, in the summer situation with higher initial ciliate numbers (25.4 cells ml^{-1}), ciliates were significantly affected over time and significantly responded to the selected copepod treatments (see Fig 1 E, Table 2A,B). The increase in ciliate abundance observed in the low density copepod treatments (Cop1) was even stronger than in the control mesocosms (Fig. 1E), while ciliate densities in the Cop5 bags strongly declined ($p<0.0001$). The summer ciliate community consisted mainly of small oligotrich species (>80%) like *Strombidium dalum*.

Conversely, heterotrophic as well as auto-/mixotrophic (data not shown) nanoflagellates (Fig. 1B, F) mostly consisted of forms <5 µm (>88%) and increased over experimental time in high density copepod treatments (S4, L4, cop5) in spring and summer, thereby reaching maximal spring abundances of $19.4x10^3$ cells ml^{-1}. In contrast, they decreased in the control and lowest cop-density mesocosms to numbers below $1x10^3$ cells ml^{-1} (Fig. 1B) in spring or remained at low level in summer (Fig. 1F). Thus, besides significant time-dependent changes, HNF abundance significantly responded to copepod treatments in spring ($F_{4,5}=27.24$; $p<.0014$) as well as in summer ($F_{2,3}=9.82$, $p<0.0483$, post-hoc comparison of means, Table 2B).

For bacterial abundances significant time-dependent and time-treatment interaction effects became obvious in spring, but treatment effect alone was not significant ($p=0.0972$; Table 2B). Abundances decreased (Fig. 1C, Table 2B) and remained low in S1 and L1 until day 4 ($0.52\text{-}0.87x10^6$ ml^{-1}). In the S4 mesocosms, they decreased only slightly and remained at a comparably low level until day 3, whereas they increased in the L4 bags during that time.

While bacterial concentrations in S4 and L4 (with increasing HNF cell numbers) stayed low or decreased from days 3 to 6, they increased in the low density copepod treatments (S1, L1).

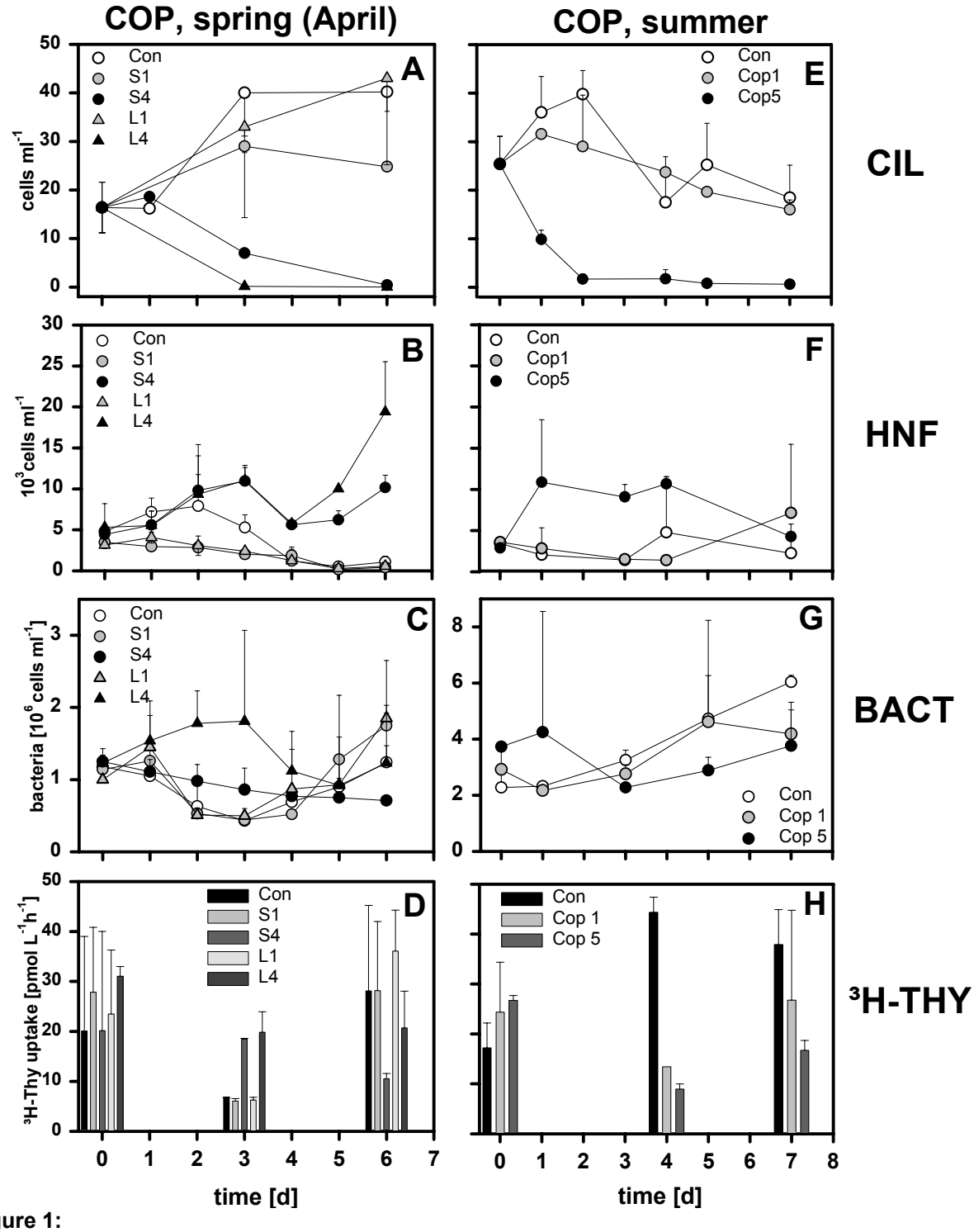


Figure 1:
Time course [in spring (April, A-D) and summer (July, E-H)] of the response of ciliates (CIL), heterotrophic nanoflagellates (HNF) and autotrophic/mixotrophic nanoflagellates (AMNF) as well as heterotrophic bacteria with respect to abundances (BACT, HNA bacteria in summer) the proportion of actively respiring cells (CTC-positive cells, in summer) and bacterial production (^{3}H-thymidine incorporation, ^{3}H-THY) to copepod grazing. Selected mesocosms are controls (Con, without mesozooplankton), lowest (S1, L1, COP1) and highest (S4, L4, COP5) density copepod treatments. Error bars show the range between replicate mesocosms.

Table 2:
Analysis of parameter time courses by Repeated-Measures ANOVA for selected copepod treatments (controls, lowest and highest densities) in spring and summer. Results for time effects of each treatment are displayed in the upper part (A), whereas those for differences between treatments are shown in the lower part (B).

A

Spring		single time effect			Summer		single time effect		
treatment		***df***	***F***	***p***	**treatment**		***df***	***F***	***p***
CON	CIL	2	9.69	*0.0935*	**CON**	CIL	5	7.40	**0.0233**
	HNF	6	20.62	**0.0009**		HNF	4	0.61	0.6802
	BACT	6	5.20	**0.0325**		BACT	4	4.31	0.0932
	THY	2	1.43	0.4118		THY	2	21.70	**0.0441**
S1	CIL	2	1.94	0.3402	**COP1**	CIL	5	8.71	**0.0165**
	HNF	6	12.47	**0.0036**		HNF	4	1.74	0.3015
	BACT	6	3.63	*0.0711*		BACT	4	3.57	0.1228
	THY	2	3.55	0.2197		THY	2	2.35	0.2984
S4	CIL	2	7.86	0.1129	**COP5**	CIL	5	406.59	**<0.0001**
	HNF	6	6.09	**0.0225**		HNF	4	4.40	0.0902
	BACT	6	5.35	**0.0304**		BACT	4	0.56	0.7050
	THY	2	0.85	0.5403		THY	2	824.94	**0.0012**
L1	CIL	2	4.00	0.2001					
	HNF	6	26.91	**0.0004**					
	BACT	6	7.60	**0.0131**					
	THY	2	7.91	0.1122					
L4	CIL	2	60.98	**0.0161**					
	HNF	6	13.77	**0.0028**					
	BACT	6	1.57	0.2988					
	THY	2	4.76	0.1735					

B

	all effects (RM-ANOVA)				*all effects (RM-ANOVA)*			
		df	***F***	***p***		***df***	***F***	***p***
CIL	***treat***	4	29.99	**0.0011**	***treat***	2	138.4	**0.0011**
	time	2	2.07	0.1770	***time***	5	23.24	**<0.0001**
	time x treat	8	7.262	**0.0026**	***time x treat***	10	9.285	**0.0001**
HNF	***treat***	4	27.24	**0.0014**	***treat***	2	9.816	**0.0483**
	time	6	14.45	**<0.0001**	***time***	4	0.846	0.5224
	time x treat	24	11.87	**<0.0001**	***time x treat***	8	3.018	**0.0416**
BACT	***treat***	4	3.58	0.0972	***treat***	2	0.481	0.6587
	time	6	6.626	**0.0002**	***time***	4	3.172	0.0538
	time x treat	24	2.956	**0.0027**	***time x treat***	8	1.638	0.2126
THY	***treat***	4	3.186	0.1178	***treat***	2	8.731	0.0561
	time	2	7.417	**0.0106**	***time***	2	2.09	0.2047
	time x treat	8	1.793	0.1908	***time x treat***	4	15.04	**0.0028**

	treatment effect *post-hoc comparison of means*		***treatment effect*** *post-hoc comparison of means*
CIL	F(4,5)=29.99; **p<.0011**	**CIL**	F(2,3)=138.39; **p<.0011**
HNF	F(4,5)=27.24; **p<.0014**	**HNF**	F(2,3)=9.82; **p<.0483**
BACT	F(4,5)=3.58; p<.0972	**BACT**	F(2,3)=0.48; p<.6587
THY	F(4,5)=3.19; p<.1178	**THY**	F(2,3)=8.73; p<.0561

In summer, on the other hand, neither time nor treatment effects turned out to be significant (Fig. 1G, Table 2A) . In Cop1 (from 2.92 to 4.62 x10^6 cells ml^{-1}) and in controls (from 2.28 to 6.04 x10^6 cells ml^{-1}) bacteria increased until day 5 and day 7, respectively, while after a first

substantial increase they remained at lower concentrations in the Cop5 treatments (2.89 to 3.77 x10^6 cells ml^{-1}). In the experimental time course, significant changes were noted for bacterial ^{3}H-thymidine uptake (Table 2A) in spring and summer (Fig.1H), ranging from 6.1 to 36.1 pmol $L^{-1}h^{-1}$. Values for this production parameter were roughly in accordance with bacterial abundances, showing first a decrease (day 3) then an increase (to day 6) in the controls and low copepod density treatments. In high density treatments (S4, L4), instead, thymidine uptake rates decreased in the course of the experiment (Fig. 1D). In spring as well as in summer, amplitudes for thymidine uptake were greater in magnitude than for abundances. Summer maxima on day 4 in the control mesocosms reached 44.4 pmol $L^{-1}h^{-1}$, thereby clearly exceeding those from copepod treatments. Here, the interaction effect of time and treatment yielded a significant response (Table 2A).

Abundances of autotrophic picoplankton were clearly lower than 10^4 cells per ml in spring and were thus considered as rather negligible, whereas higher densities were found in summer (data not shown in graph). Here, *Synechococcus* densities decreased to 41% of their initial values in controls (from 7.95 - 3.28x10^4 ml^{-1}, whereas they were reduced less in the cop1 and cop5 mesocosms (77 and 89% of initial densities, respectively).

Response to gradients of copepod abundance

Microbial food webs displayed distinct responses after being exposed to copepod density gradients (shown as geometric means) for 1 week (Fig. 2, Table 3). Among the investigated components, ciliates and nanoflagellates revealed very similar patterns in the two spring and one summer experiments (Fig. 2, A-L, Table 3), while more complex response characteristics were noted at the picoplankton level. Compared to the spring experiments, the response of the microbial community in summer (Fig. 2I-L) took place over a wider range of copepod densities with maximal values of 73.4 copepods per liter (geometric mean).

After 1 week of copepod grazing, ciliates of all size classes showed a significant decrease with increasing copepod abundances in spring and summer. Along the *Calanus finmarchicus* (L) gradient, this reduction was even more pronounced than in small copepod (S) gradients (Fig. 2A, E) and showed a density range of more than one order of magnitude (Fig.2A). An opposite response pattern with a highly significant and nearly exponential increase was found for heterotrophic ($p<0.0001$, $p<0.01$ in May) and even more pronounced for auto-/mixotrophic (AMNF) nanoflagellates (Fig. 2 B, F, J). HNF spring densities ranged from 0.19 to 10.9x10^3 cells ml^{-1}along the small copepod density gradient and from 0.47 to 22.5x10^3 cells ml^{-1} along the *Calanus* gradient (Fig. 2B). Thus, the HNF incline was even more distinct in the latter gradient. In summer, autotrophic and heterotrophic nanoflagellates responded differentially with increasing copepod density (Fig. 2J). While AMNF showed a

significant linear increase with peak numbers of 16.5x10³ cells ml^{-1}, the abundance curve of HNF levelled off and rather decreased towards highest copepod densities.

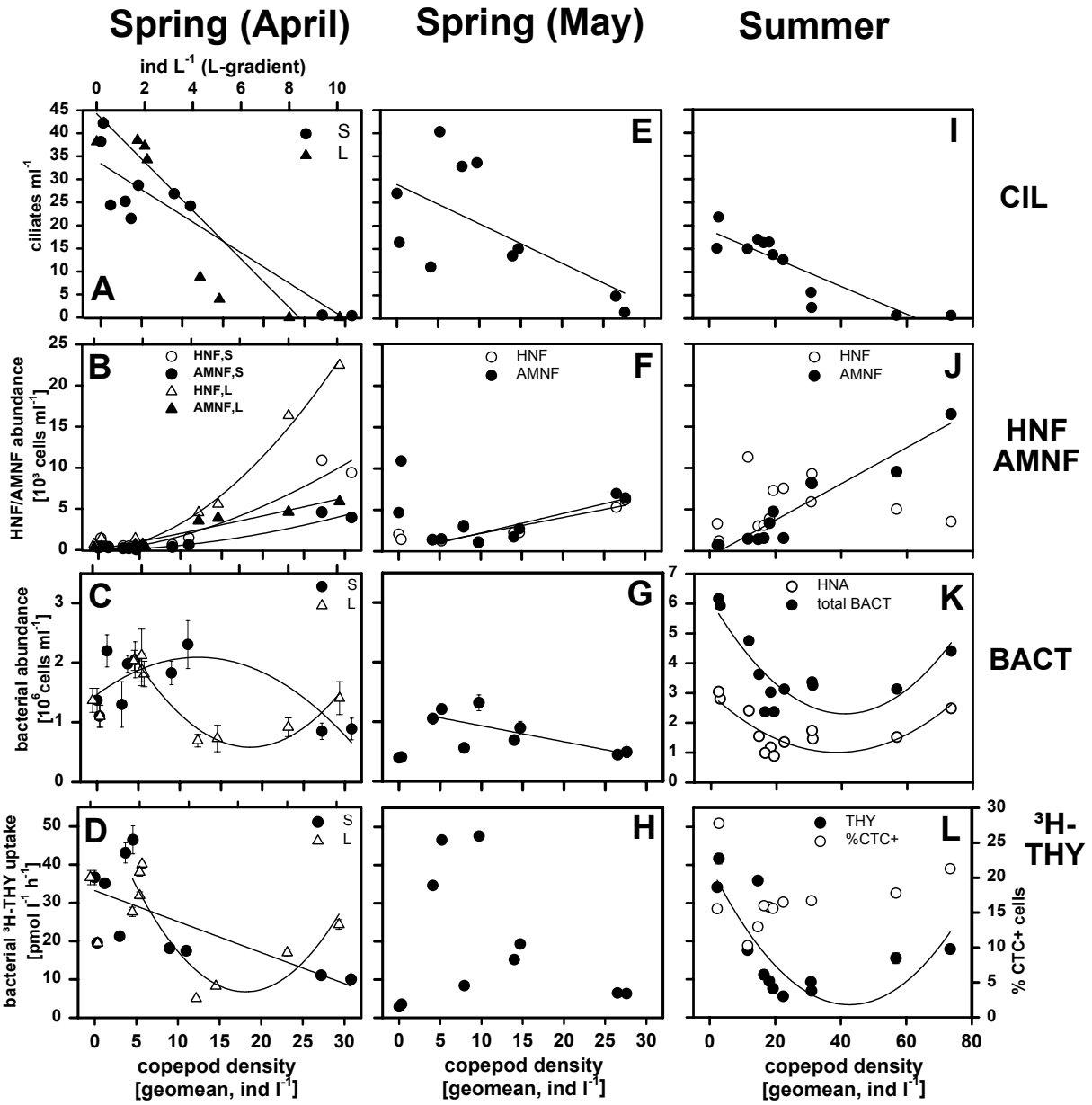


Figure 2:
The response of ciliates (CIL), heterotrophic nanoflagellates (HNF), autotrophic/mixotrophic nanoflagellates (AMNF) as well as heterotrophic bacterial abundance (BACT; and HNA in summer) and production (³H-thymidine incorporation, ³H-THY) as well as respiratory active bacteria (L) along the respective copepod density gradients (geometric means, S = small copepods, 250-500 µm, L= *Calanus* gradient, copepods >500µm) after 1 week of experimental duration. Gradients are shown for experiments in spring (April, A-D; May, E-H) and summer (July, I-L). For significant regression functions see table 2.

The response curves of bacterioplankton (Fig. 2C, G, K) showed highest spring values of 2.12 (L) to 2.30 (S) $x10^6$ cells ml^{-1} at low copepod densities in April and then levelled off and strongly decreased towards high densities with abundances of around 10^6 cells per ml. In comparison, the response amplitude in bacterial abundance was smaller and ranged from 0.44 to $1.32x10^6$ cells ml^{-1} in May. Bacterial abundances in summer were maximal on both ends of the copepod gradient (Fig. 2 K). Moreover, the strongest response along the copepod gradient arose from high nucleic acid bacteria , whereas the low and medium nucleic acid bacterial subgroups remained less affected (data not shown in graph). From intermediate (approx. 15-20 copepods L^{-1}) to very high copepod abundances, we observed rising concentrations of HNA bacteria, paralleled by an increase in the proportion of actively respiring (CTC-positive) bacterial cells (Fig.2 K,L).

Table 3:

Regression statistics for analysis of parameter responses along density gradients of copepods.

	Spring (April)		**S-gradient**	**Spring (April)**		**L-gradient**
Dependent variable (Y)	***R²***	***p***	**regression equation**	***R²***	***p***	**regression equation**
CIL	0.83	0.0002	$y=33.396-1.121x$	0.77	0.0009	$y=44.262-5.261x$
HNF	0.93	<0.0001	$y=36.32x^{1.664}$	0.99	<0.0001	$y=287.57x^{1.898}$
ANP	0.93	<0.0001	$y=7.965x^{1.843}$	0.91	<0.0001	$y=504.79x^{1.085}$
BACT	0.61	0.037	$y=1466629+101779x-4156x^2$	0.95*	0.0006	$y=3377338-868032x+67407x^2$
THY	0.46	0.031	$y=33.236-0.81x$	0.76*	0.027	$y=62.46-17.68x+1.403x^2$
	Spring (May)		**S-gradient**	**Summer (July)**		
Dependent variable (Y)	***R²***	***p***	**regression equation**	***R²***	***p***	**regression equation**
CIL	0.40	0.0483	$y=28.93-0.850x$	0.77	0.0002	$y=18.943-0.301x$
HNF	0.83*	0.0017	$y=217.064+193.08x$		n.s.	
NANO		n.d.		0.70	0.0007	$y=1996.69x^{0.533}$
ANP	0.83*	0.0015	$y=-146.582+235.153x$	0.89	<0.0001	$y=-674.09+219.474x$
BACT	0.52*	0.0434	$y=1198896-26851x$	0.72	0.0032	$y=6076738-184627x+2257x^2$
HNA		n.d.		0.67	0.0067	$y=2953374-101100x+1314x^2$
THY		n.s.		0.65	0.0088	$y=39.83-1.714x+0.0201x^2$
				0.55	0.0056	$y=56.36x^{-0.451}$
% CTC+**		n.d.		0.73	0.003	$y=0.509x^{0.071}$

*** control mesocosms not included in regression**
****arcsin-transformed**
n.s. not significant
n.d. not determined

Patterns well corresponding to abundances but with a larger amplitude (mainly in May and in low density bags) were observed for bacterial ^{3}H-thymidine uptake rates. Absolute values ranged from 10.0 to 46.5 pmol $L^{-1}h^{-1}$ along the S-gradient (Fig. 2D) and from 5.0 to 40.2 pmol $L^{-1}h^{-1}$ along the L-gradient (Fig. 2D) . Estimated bacterial doubling times were short (13-26h, μ=0.04-0.05 h^{-1}) at very low copepod densities) and longest at intermediate to high cop-densities (up to 48h, μ=0.015 h^{-1}). In spite of lower bacterial numbers compared to the April experiment, the absolute values of bacterial production were similar (6.3-47.6 pmol $L^{-1}h^{-1}$) in

May and peaked at low copepod densities (corresponding to μ= 0.08 h^{-1}). In the controls, values for bacterial numbers and production were very low and with a mean volume of 0.13 μm³ cells were even smaller than in the April experiment (0.18 μm³ MCV). The response curve of bacterial production in summer resembled the U-shaped one of bacterial abundance and HNA cell densities (Fig. 2 L). In general, lower bacterial growth rates were measured than in spring, with corresponding maximal doubling times of 162-197h at copepod densities of 20-30 ind L^{-1} and minimal doubling times of 35-62h at low copepod densities and in control mesocosms.

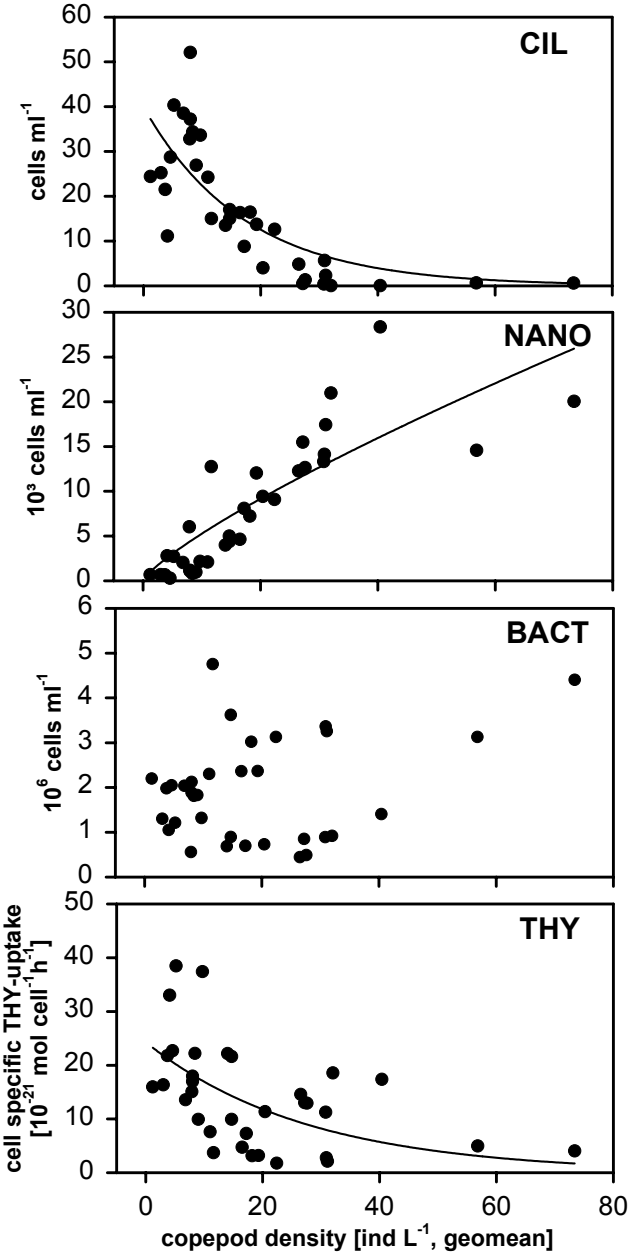


Figure 3:
Impact of increasing density of copepod (after 1 week geometric means, without control mesocosms) on pooled data from 4 independent gradients (copepod densities for L-gradient taken times four. Regressions:
For ciliates $y=39.96e^{(-0.058x)}$, <0.0001,R^2=0.61, for nanoplankton $y=831.8848x^{0.8007}$; p<0.0001, R^2=0.70), for bacterial abundance (n.s.) and cell-specific bacterial ³H-thymidine uptake $y=24.29e^{(-0.036x)}$; p=0.0013, R^2=0.28.

In order to summarise the copepod density-dependent and time-integrated response of main functional groups of the investigated microbial food webs, data sets obtained from 4 independent copepod gradients (S and L from April, S from May and summer gradient) were pooled (Fig. 3). Copepods exerted a strong top-down impact on ciliates and thus enabled auto- and heterotrophic nanoplankton to increase via a clear trophic cascade.

Complex and differing responses at the bacterial level in spring and summer did not yield a comparable relationship between copepods and bacterial abundances. Nevertheless, such a trend was observed for cell-specific bacterial thymidine uptake rates. Highest thymidine uptake rates at moderate bacterial concentrations (Fig. 2 G, H) in low density cop-treatments resulted in very high cell-specific bacterial activity (Fig. 3).

Bacterial grazing losses

Altered protist grazing pressure due to changes in copepod grazing activity affected bacterial grazing losses as assessed by the disappearance of fluorescently labeled bacteria (FLB) after 24 hours (Fig.4). At the end of the second spring experiment, water from the selected enclosures showed high abundances of HNF in treatments with highest copepod densities (S4, L4 not shown in graph) varying between 6.2 and 8.3x10³ cells ml^{-1} and very low ciliate abundances (Fig. 2 E,F). In bottles with water from these enclosures, highest values of FLB grazing loss were registered in L4 (54.4%) and S4 (40.7%) treatments. Furthermore, FLB grazing loss was higher in L1 (23.2%) compared to S1 (8.7%) bottles.

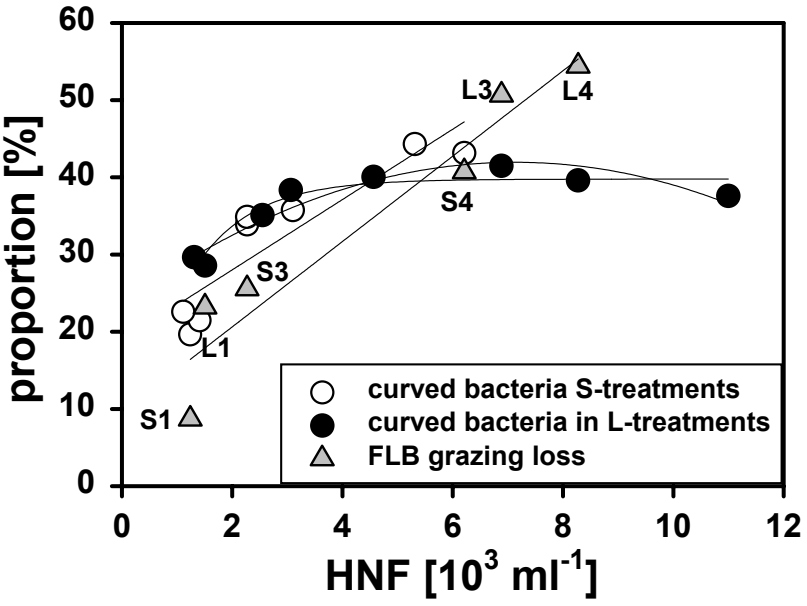


Figure 4:
Impact of increasing numbers of HNF with increasing copepod densities is shown as proportions of curved *Vibrio*-like bacteria (presumably motile and potentially grazing resistant/ tolerant) as well as FLB grazing loss (as % of initial FLB concentration). Coefficient of variation of FLB loss between 3 replicate bottles: T_O: S1=0.099, S3=0.109, S4=0.057, L1=0.028, L3=0.084, L4=0.016; T_{24}: S1=0.019, S3=0.075, S4=0.124, L1=0.144, L3=0.236, L4=0.314. Regressions significant are at $p<0.05$.

Bacterial size and morphologies

The bacterial community was predominantly composed of single-cell morphotypes (large rods, cocci), whereas particle-associated, aggregates and filamentous bacteria were almost absent. Highest mean cell volumes (MCV, 0.18-0.28 µm³) were calculated for intermediate copepod density treatments (cop20, cop40). In spring copepod gradients, no trends in MCV became obvious with averages (over all treatments) of 0.18 µm³. Only in controls (May experiment) the assemblage consisted mainly of cells < 0.125 µm³ (MCV). Most interestingly, with increasing HNF abundance we observed an incline in the proportion of small curved *Vibrio*-shaped bacteria from 19.6 to 43.2% in May (Fig. 4). The increase was significant for a HNF density range of 0 - 7x10³ cells per ml. Thereafter, the curve leveled off and showed no further increase with increasing HNF grazing pressure. Cells of this morphotype also occurred in the summer experiment mainly at high copepod densities. TEM images (Transmission Electron Microscope) of these bacteria revealed a long flagellum, presumably allowing for high motility.

Appendicularians and nutrient enrichment

Out of six appendicularian bags (copepod reduction treatments, Sommer et al. 2003), the two mesocosms with maximal appendicularian population densities developing after 9 days (mean 30 ind. L^{-1}, see Fig. 5A) were selected in order to show the response in protists (Fig. 5B, C) and bacteria (Fig. 5D, E).

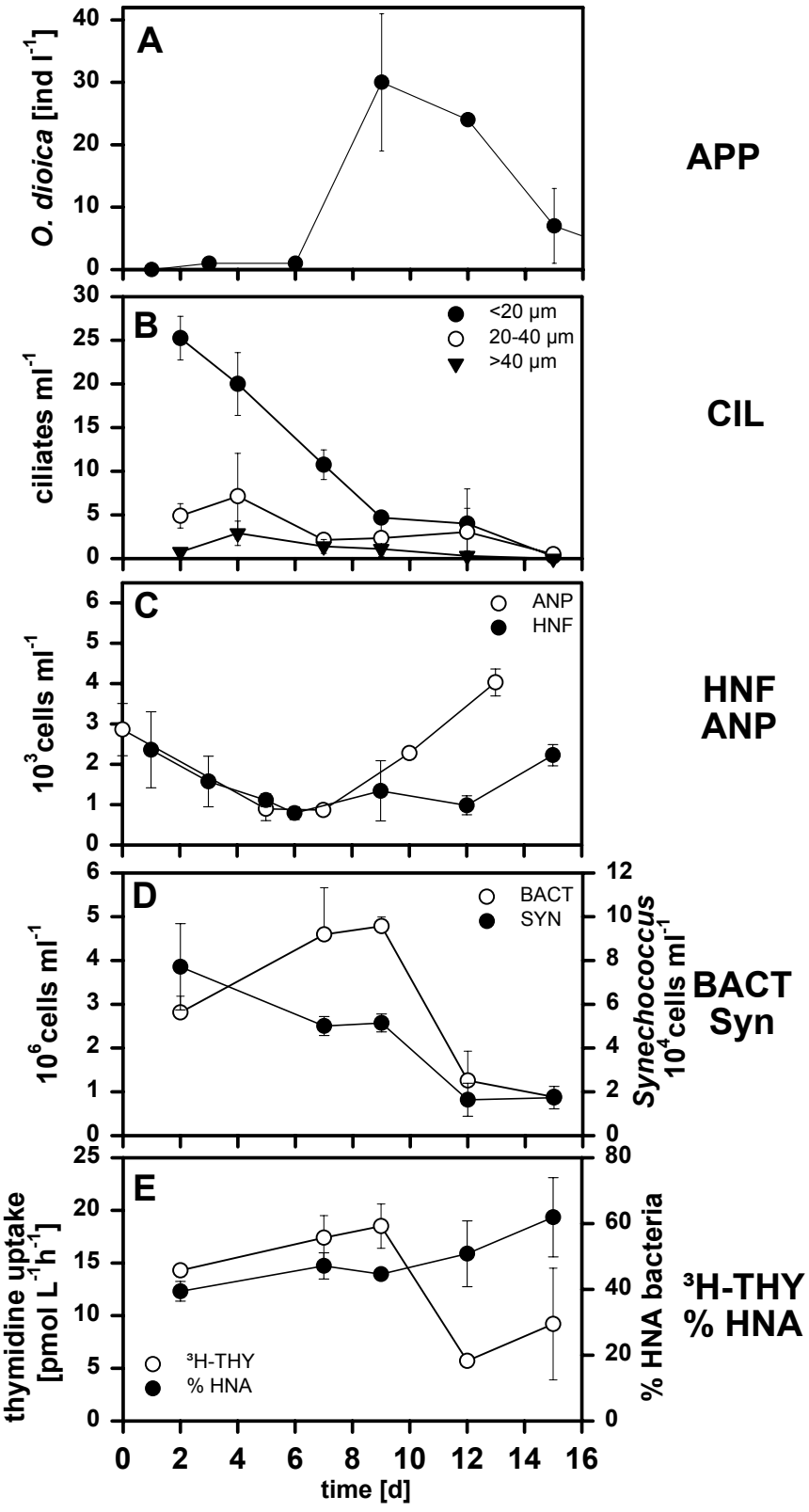


Figure 5:
Population development of *Oikopleura dioica* in the APP mesocosms (A, copepod reduction treatments) and the time-dependent responses of ciliates (CIL), heterotrophic nanoflagellates (HNF), autotrophic nanoplankton (ANP), heterotrophic and high nucleic acid bacteria (BACT, HNA), *Synechococcus* (SYN, only in summer) and bacterial production (^{3}H-thymidine incorporation, ^{3}H-THY). Error bars indicate the range of replicate mesocosms.

time effect				
APP		***df***	***F***	***p***
	CIL	5	40.65	**0.0005**
	HNF	6	4.408	**0.0470**
	ANP	4	105.3	**0.0003**
	BACT	4	47.28	**0.0013**
	SYN	4	21.54	**0.0057**
	THY	4	13.06	**0.0145**
	%HNA	4	9.445	**0.0257**

Table 4:

Analysis of parameter time courses by Repeated-Measures ANOVA for treatments (APP) with highest appendicularian densities (abbreviations as in figures).

Unfortunately, a contamination with copepods occurred within the second half of the experiment, which affected the phytoplankton community (Sommer 2003) and likely impacted also on the protist assemblage. For all investigated microbial food web components as well as for bacterial activity significant time-dependent changes were observed (Table 4). While the abundances of larger-sized ciliates showed only few changes until day 12, nanociliates were more intensely reduced over time (Fig. 5B). Simultaneously, auto-/mixotrophic nanoflagellates first decreased until day 7 and thereafter increased up to $4x10^3$ cells ml^{-1} (see Fig. 5C). A very similar population development was noted for HNF, but with slightly lower abundances and a delayed and less intense increase after day 12. In addition to that, HNF numbers were much lower than in high density copepod enclosures.

In response to the appendicularian bloom registered on day 9, the picoplankton fraction showed the most pronounced numerical reduction, whereby *Synechococcus* cells declined continuously from 7.7 to $1.7x10^4$ cells ml^{-1}. Heterotrophic bacteria, after a first increase, were reduced 1.6 to 3.8-fold from day 9 to day 12 (Fig. 5D). Simultaneously, bacterial production values followed the same pattern and decreased from 18.5 to 5.7 pmol $L^{-1}h^{-1}$ after the appendicularian bloom (Fig. 5E). Corresponding bacterial doubling times increased from 68h (day 2) to 92h on day 7 and thereafter decreased from 90h (day 9) to 33h (day 15). The numerical decline in bacteria was paralleled by a constant increase in the mean proportion of the HNA bacterial subgroup from 38.8±1.8 % (day 2) to 56.6±10.9 % (day 15) and an incline in the mean proportion of CTC-positive cells from 12.6±3 to 19.5±3.4% between days 7 and 15.

Nutrient enriched mesocosms should demonstrate whether eutrophication effects on the microbial functional groups would be modified under the given mesozooplankton dominance (Fig. 6, Table 4). Therefore, all six appendicularian treatments were pooled and time series (over two weeks) of lower trophic level parameters were tested against pooled data obtained from the respective enriched bags (EUAPP). Functional groups in both mesocosm sets were subjected to comparable *Oikopleura* blooming events. Samplings

during that time yielded very similar appendicularian densities (means over time) of 10.3 ind. per liter (non-enriched, APP) and 10.8 ind. per liter (EUAPP treatments) and an equal mean background density of copepods (~ 16 per liter). All investigated components of the pico- and nano-sized fraction were markedly stimulated by nutrient addition and showed significant time and treatment dependent effects in terms of abundance and production level (Fig. 6, Table 4), although in both mesocosm sets substantial picoplankton reductions were observed after blooming of *Oikopleura*.

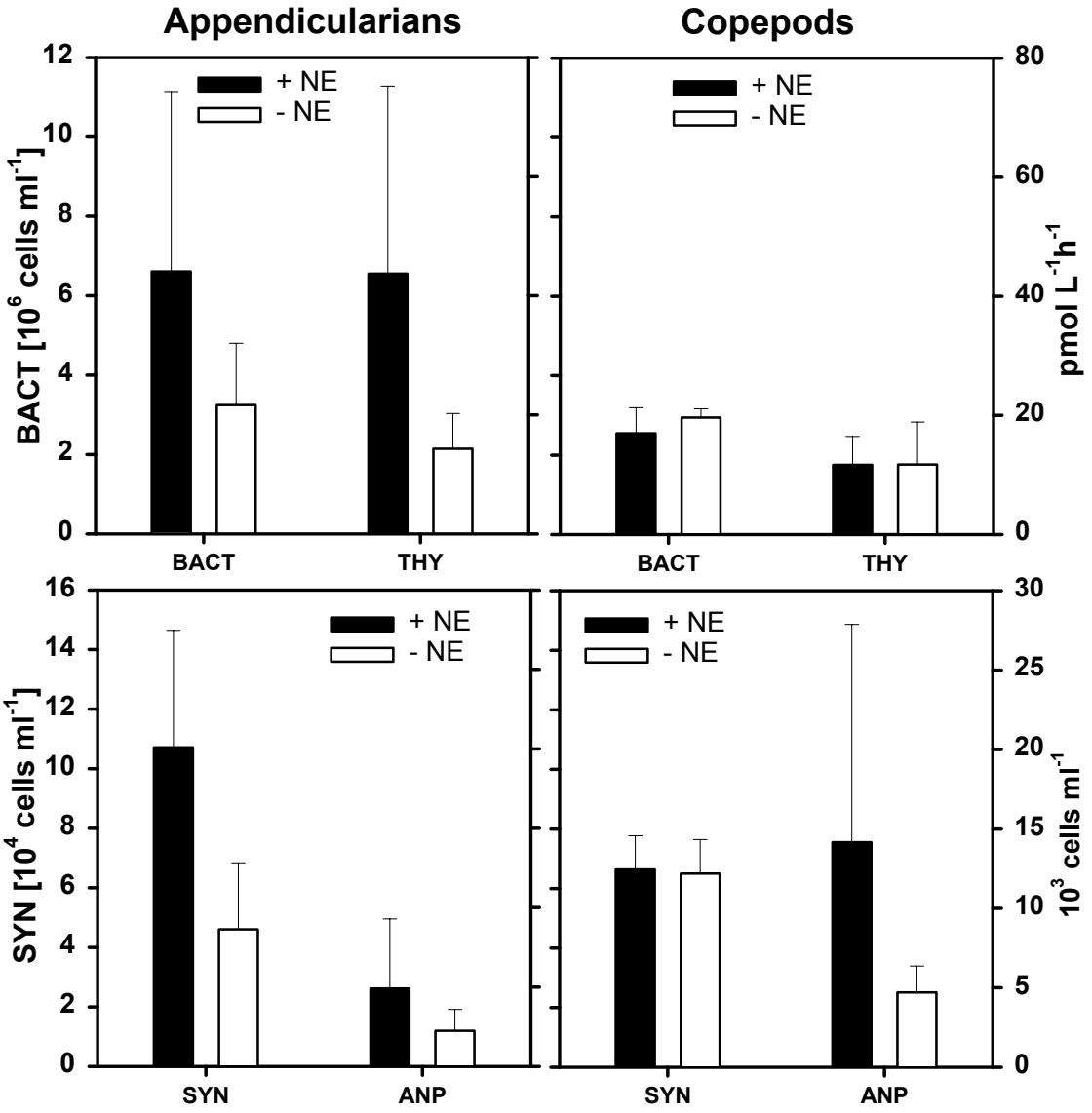


Figure 6:
Effect of nutrient enrichment (+NE) on lower trophic level components in mesocosms with an appendicularian bloom (left panel) and with copepod dominance. Upper graphs demonstrate bacterial abundance (BACT) and thymidine uptake (THY), while lower graphs show *Synechococcus* (SYN) and autotrophic nanoplankton (ANP). Mean values and standard deviations are time-integrated averages (3 to 6 enclosure replicates).

Nutrient enrichment effects

		APP versus EUAPP					*COP(3+4) versus EUCOP*		
		df	*F*	*p*			*df*	*F*	*p*
ANP	***treat***	1	190.04	**<0.0001**	**ANP**	***treat***	1	74.43	**0.0003**
	time	4	65.13	**<0.0001**		***time***	2	52.39	**<0.0001**
	time x treat	4	25.80	**<0.0001**		***time x treat***	2	41.34	**<0.0001**
SYN	***treat***	1	71.53	**<0.0001**	**SYN**	***treat***	1	0.04	0.8447
	time	4	5.16	**0.0031**		***time***	2	8.41	**0.0072**
	time x treat	4	41.00	**<0.0001**		***time x treat***	2	2.79	0.1091
BACT	***treat***	1	83.22	**<0.0001**	**BACT**	***treat***	1	1.59	0.2636
	time	4	28.69	**<0.0001**		***time***	2	1.86	0.2056
	time x treat	4	19.21	**<0.0001**		***time x treat***	2	2.90	0.1015
THY	***treat***	1	191.71	**<0.0001**	**THY**	***treat***	1	0.00	0.9575
	time	4	213.15	**<0.0001**		***time***	2	32.41	**<0.0001**
	time x treat	4	183.68	**<0.0001**		***time x treat***	2	42.07	**<0.0001**

Table 5:
Time and treatment-dependent nutrient enrichment effects in appendicularian (left) and copepod dominated mesocosms using Repeated-Measures ANOVA with post-hoc comparison of means.

For a comparison of enriched and non-enriched copepod-dominated communities, treatments with intermediate copepod densities and similar temporal successions (cop3+4) were pooled and tested against eutrophicated mesocosms (EUCOP). Again, both series contained comparable time-averaged copepod densities of 25.9±6 and 26.4±3 individuals per liter, respectively. Interestingly, treatment-dependent differences were only found for autotrophic nanoplankton (Fig. 6, Table 5), with very high peak densities of 29.5 x 10^3 cells per ml (not shown in graph) on day 7. Neither heterotrophic bacterial abundance and production nor *Synechococcus* densities significantly differed between enriched and non-enriched copepod mesocosms.

DISCUSSION

This study of mesozooplankton grazing effects illustrated the impact of copepods and appendicularians on a coastal marine microbial food web. Differing from microcosm experiments on mesozooplankton-microbe interactions (Katechakis et al. 2000, Zubkov and Lopez-Urrutia 2003, Sipura et al. 2003), this investigation is going one step further by using large in-situ enclosures allowing for conclusions on food web structuring patterns on a larger scale. Thereby, the chosen initial zooplankton densities were within the range naturally occurring in the respective system. Differing from treatments with presence or absence of zooplankton only, the gradient design of copepod density made it possible to reveal the mode and amplitude of the microbial food web response.

These experiments could demonstrate mesozooplankton-triggered changes within lower trophic levels and detected alterations along a mesozooplankton density gradient as well as temporal responses of microbial food web components. Moreover, experimental duration of 1 to 2 weeks (appendicularians) enabled cascading trophodynamic effects and feedback mechanisms.

The investigated systems proved to be highly dynamic revealing a large response amplitude. However, despite different initial conditions and spring as well as summer plankton scenarios, the experiments displayed general structuring effects of copepods on the protist assemblage and illustrated the importance of trophic cascades for structuring pelagic microbial food webs. As initial nutrient conditions differed and copepod addition led only to minor changes in the overall nutrient regime and did not create significant trends after 1 week of copepod grazing (via recycling etc.), we assume the occurrence of these general structuring patterns and the observed order-of-magnitude responses mainly to be a result of direct and indirect predation effects.

Trophic impact of copepods on microbial food webs

Effects on functional protist groups

Although some copepod mortality occurred (mainly in the second spring experiment), the remaining abundances were still high and the gradient design was maintained throughout the experiments. Independent of the initial plankton situation and composition of the copepod communities, copepods exerted highly significant trophic impact on the protist level and contrastingly affected both ciliates and HNF (e.g. Table 2). The “small copepod communities” as well as *Calanus finmarchicus* preferentially preyed upon larger-sized prey and significantly reduced ciliates. Likely due to ciliate grazing pressure relief, nanoflagellates were enabled to increase significantly. The more pronounced increase in autotrophic compared to heterotrophic forms might be explained by more nutrients being available, as copepods potentially removed the larger-sized competing algae. Calculated HNF net growth rates in spring (not shown in graph, estimated from abundance data) revealed mostly positive values in S4 and L4 treatments ($\mu = 0.01 d^{-1}$, time averaged), whereas ciliate-rich mesocosms (controls, S1, L1) caused mostly negative rates with maximal values of $\mu = -0.1 d^{-1}$. The size-shift towards nanoplankton proved more rapid at high copepod densities and comprised orders-of-magnitude changes in protist abundances.

The evolving inverse patterns of ciliate and nanoflagellate abundance can be interpreted as a clear 3-link trophic cascade. Changes of that type were revealed also in some other studies performed in freshwater (Adrian and Schneider-Olt 1999, Zöllner et al. 2003) and marine systems, whereby the marine studies were mostly based on microcosm experiments (Katechakis et al. 2002, Sipura et al. 2003). In general, such community level trophic cascades (Paine 1980, Pace et al. 1999) which we found during both seasons are supposed to occur rather rarely and point at a uniform size-class preference and a rather low level of heterogeneity (Strong 1992, Polis 1999, Sipura et al. 2003). Ciliate escape behaviour can be efficient in reducing copepod grazing susceptibility (Jonsson and Tiselius 1990,

Broglio et al. 2001), but apparently was not successful at high copepod densities used in the present study.

The resulting differences in HNF concentrations likely accounted for marked differences in the grazing pressure on bacteria as shown by clearly differing proportions of FLB disappearance. Thereby, treatments with high HNF abundances caused much stronger FLB grazing loss than those with high ciliate densities.

Effects on bacterioplankton

Predation by protists is known to be a structuring and shaping force for the heterogeneous community consisting of autotrophic and heterotrophic prokaryotes (Jürgens and Matz 2002). In this bacterial compartment we found complex time- and treatment-dependent responses to changes in the mesozooplankton and thus protist grazing regime with considerable and partly significant fluctuations in bacterial densities over experimental time.

Interestingly, bacterial abundances and production did not show significant differences in terms of treatment effects (post-hoc comparison of means over experimental time, Table 2B). This strikingly contrasts to results obtained for higher trophic level protists, demonstrating highly significant treatment-dependent changes. While combined effects of time and treatment yielded significant differences between spring mesocosms, bacterial communities in summer displayed enhanced stability in terms of both aspects.

This could suggest that bacteria were more buffered against food web shifts and relatively more impacted by bottom-up forces. These could at least partly account for the observed stimulating effects of copepod addition during the first 1-3 days (L4, cop5), resulting in an increase in bacterial numbers and production. A stimulatory impact of copepods with positively responding bacteria is consistent with short-term effects found in different microcosm experiments (Sipura et al. 2003, Zubkov and Lopez-Urrutia 2003). This fuelling of bacterial activity is mostly due to DOC release by copepod sloppy feeding and faecal pellet leakage (e.g. Møller et al. 2003). In this context, a relatively wide amplitude in bacterial abundance and activity at low copepod densities could indicate the existence of alternative stable states and different successions or scenarios being possible.

A further increasing and shaping predation stress due to cascading trophodynamic effects, might force bacterial populations into a certain direction. This conspicuous negative impact on bacterial abundance and activity at very high HNF densities in spring (S4, L4 mesocosms after 1 week) even suggests a community-wide 4-link trophic cascade. This cascading HNF predation effect thus contributed to explain the more dynamic response of spring bacteria mentioned above.

Remarkably, HNF also could have influenced patterns of bacterial activity. While low HNF numbers accounted for high bacterial abundances as well as a high cell-specific activity

S1, L1 after 1 week), high ambient protist abundances (at approx. 15-40 copepods L^{-1}) caused low bacterial numbers and growth rate estimates. Here, the significant decrease in cell-specific activity towards higher copepod and thus HNF densities could result from the fact that large active cells were preferentially preyed upon in the course of the experiments.

In summer, the bacterial community response was apparently less clear. At least after 1 week, rather a 3-link trophic cascade can be assumed, since bacterial abundances are higher at highest copepod densities. Results from the summer experiment further suggest that mesozooplankton directly and indirectly (trophic cascade) triggered shifts in bacterial production and respiration, thereby very likely affecting mineralization rates and carbon dynamics. The experiments revealed a considerable variability in actively respiring bacteria with proportions ranging from 9.7 to 27.8%. Moreover, the proportion of CTC+ cells and of HNA bacteria as the most active part of the bacterial assemblage (Lebaron et al. 2001a) as well as bacterial production clearly corresponded.

Various studies proved CTC to be an ecologically meaningful measure of active bacterial abundance in aquatic systems, and that CTC-active bacteria are likely responsible for the bulk of community metabolic activity (Smith 1998, Sherr et al. 1999). Furthermore, quite huge variations in CTC-positive cells were found in other aquatic systems, ranging from 3.5-47.4% of the total bacterial population activity (Smith 1998). As bacteria are responsible for 50 to >90% of the total community respiration (Jahnke and Craven 1995, Sherr and Sherr 1996), conspicuous shifts in actively respiring bacteria due to altered mesozooplankton grazing regime could significantly impact carbon-flux and the ratio of net heterotrophy to net autotrophy of the respective system.

In cop5 mesocosms, bacterial abundances slightly increased, whereas HNF numbers declined, although ciliate predation pressure was obviously low. This converse development led to comparably high numbers of HNA bacteria in these two mesocosms, coinciding with high thymidine uptake and elevated proportions of CTC-positive cells. The fact that these apparently very active cells were not effectively controlled by HNF, could be interpreted as an indication of bacterial feed-back mechanisms and reduced grazing susceptibility.

High proportions of a small and curved bacterial morphotype were observed under intense HNF grazing pressure in spring (S4, L4) as well as in summer. Here, TEM images (Transmission Electron Microscope) revealed a long flagellum, presumably allowing for high motility. Other studies on marine microbial food webs reported only a modest development of grazing-resistant bacteria (Massana and Jürgens 2003). As no obvious and typical morphological responses were noted such as the development of long filamentous bacteria commonly observed in freshwater microbial communities (see chapter II), high motility, also useful for resource exploration, could be a potential mechanism to reduce grazing susceptibility (Grossart et al. 2001, Jürgens and Matz 2002).

Effects of the appendicularian Oikopleura dioica

Appendicularians as most abundant marine microphage metazoans (Fernandez and Acuña 2003) are capable of capturing particles in the colloidal and bacterial size fractions (King et al. 1980, Deibel and Lee 1992, Fernandez et al. 2004) and can significantly contribute to carbon fluxes (Vargas et al. 2002). Due to their high fecundity they are able to reach high densities within short time periods (Acuña and Kiefer 2000). The highest densities of *Oikopleura dioica* (ca. 35 ind l^{-1}) developing after 9 days in our mesocosms in the summer experiment are comparable to peak values found in other studies (Fernandez and Acuña 2003). Nevertheless, in most periods throughout the year appendicularian biomass in Hopavågen is an order of magnitude lower than copepod biomass (COMWEB 1999).

In our experiment, the strong increase of appendicularians was followed by a significant reduction of bacterial abundance and production. All DNA-subgroups of bacteria were reduced by the increase in appendicularians, but the relative proportion of HNA bacteria stayed constant or even increased. Moreover, a temporal decrease of ciliates and also nanoflagellates was noted in *Oikopleura*-mesocosms. After day 10, densities of autotrophic nanoplankton increased again. Contrasting to the copepod-mediated cascade, heterotrophic forms stayed at a very low abundance level, presumably due to low bacterial numbers.

A reduction of bacterial abundances and growth by appendicularians was also reported by Zubkov and Lopez-Urrutia (2003) from the English Channel. Moreover, measured transfer efficiencies (contribution ratio of bacterial to photosynthetic production as mesozooplankton food resources) were reported to be highly variable (from 6 to 41%), thereby being lower in the community dominated by copepods and higher when pelagic tunicates are more abundant (Koshikawa et al. 1996, 1999). The low numbers of bacteria competing with phytoplankton for nutrients after bloom events of appendicularians as well as low numbers of copepods could contribute to explain the observation that appendicularians can rather enhance phytoplankton blooms (Fernandez and Acuña 2003).

The temporal decrease of ciliates and also nanoflagellates in *Oikopleura*-mesocosms could be at least partly due to increasing appendicularians, as according to (Vargas and Gonzalez 2004a) ciliates were not directly ingested but trapped in houses of *Oikopleura* and thus cleared from the water column. Nanoplanktonic organisms, in turn, could have been ingested directly (King et al. 1980, Urban et al. 1992). However, the observed specimen of *Oikopleura* from the enclosures were rather small (Sommer et al. 2003). So, at least for ciliates more likely, the remaining copepods in these bags accounted for their decline and thereby released nanoflagellates from predation pressure.

Although appendicularian bloom events in general are apparently rather transient scenarios (roughly 1 week in our case), during these periods of mass development they can exert significant trophic impact mainly on the pico- and nanoplankton size fraction

(Koshikawa et al. 1996, Koshikawa et al. 1999). The decrease of appendicularian density in the mesocosms after day 12 very likely was due to suppression by the copepods still remaining in the APP-treatments (Sommer et al. 2003). This phenomenon finally could provide field evidence for recent results by Lopez-Urrutia et al. (2004) proving calanoid copepods to directly prey on *Oikopleura dioica* in bottle experiments.

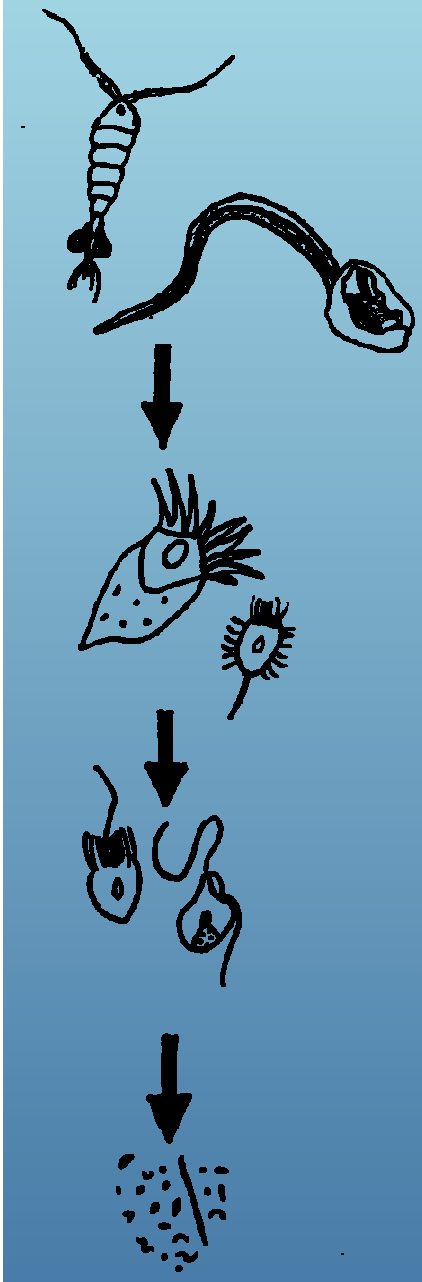
Eutrophication and food web structure

Enrichment of mesocosms with inorganic nutrients differentially affected microbial food web structure. Besides differences between appendicularian and copepod-dominated mesocosms, also the nutrient addition itself caused significant effects when mesozooplankton of the same type was present. Mostly, effects turned out to be related to the absolute level of abundance and production, which was higher in enriched bags. However, according to mesozooplankton-mediated food web structure, nutrients were channelled into different compartments. While in mesocosms with *Oikopleura* blooms picoplankton was favoured, fertilized systems permanently dominated by copepods revealed strong increases in nanoplankton organisms, significantly exceeding those of non-fertilized bags (e.g. up to 29.5×10^3 ml^{-1} ANP and 13.8×10^3 ml^{-1} HNF on day 7). Here, contrasting to EUAPP-enclosures, micro-sized ciliates strongly decreased and allowed nanoplankton to increase (not shown in graph). Apparently, this cascading predatory effect seemed to be a rather stable phenomenon under enriched and non-enriched conditions. Heterotrophic bacteria, in turn, stayed at a constantly low level and were preyed upon by increasing numbers of HNF. At the same time, they also could have been outcompeted by ANP taking advantage out of the food web constellation during that week. According to Jacquet et al. (2002), the favouring of larger autotrophic plankton by the addition of mineral nutrients could also point at a potential carbon limitation of bacteria in EUCOP-mesocosms.

Altogether there is still comparably little known about the biogeochemical consequences of bacterial grazing (Strom 2000). However, cascading direct and indirect effects on bacteria and order-of-magnitude responses in bacterial activity as well as differential responses of bacterial community subgroups indicate the importance of biotic interactions at higher trophic levels for bacterial function and turnover rates (C-demand, C-flux, nutrient and DOC-uptake). As bacteria can contribute ~50->90% to total community respiration (in Rivkin and Legendre 2001), strong alterations in bacterial activity due to shifts in food web structure can have major implications for various matter fluxes. A challenge for future research will be to find out how close the linkage between bacterial community structure and function is (e.g. by STARFISH, Fuhrman 2002) and how the trophic and biogeochemical contribution of individual bacterial taxa changes due to shifts in the composition of higher trophic levels.

IV

Chapter 4



Fjord

- microbial food web in spring and summer

- Bacterial diversity and community composition -

Mesozooplankton (copepods, appendicularians) impact on microbial food web structure in a coastal marine system

II. Patterns of bacterial community composition and diversity

ABSTRACT

The impact of copepods and appendicularians on bacterial diversity, community composition and dynamics was studied based on the same large-scale mesocosm experiments that were analysed in chapter III for microbial food web responses. I investigated the composition, qualitative and partly quantitative responses of bacteria to changes in mesozooplankton regime by Denaturing Gradient Gel Electrophoresis (DGGE) combined with sequencing of excised bands. From DGGE-derived data, I calculated Shannon´s diversity index H´, Evenness and richness of operational taxonomic units (OTUs) and analysed patterns of community similarity by non-metric multidimensional scaling (NMDS).

41 DGGE bands were sequenced, of which 25 were of bacterial origin. The communities were dominated by members of the alpha- (mainly *Roseobacter*) and gamma-Proteobacteria as well as taxa belonging to the *Cytophaga-Flavobacterium* cluster of the *Bacteroidetes*.

Bacterial community composition (BCC) proved to be remarkably stable in time and across copepod density gradients with most OTUs found in all experimental treatments. Thereby, eukaryotic bands contributed to a large extent to deviations in community profiles. Nevertheless, I noted increases in bacterial OTU richness and Shannon's diversity index H´ over time and across gradients in spring and in summer, whereby the spring communities, according to numerical and activity changes of microbial food webs (chapter III), showed pronounced dynamics and amplitudes (H´ ranging from 1.8 to 2.4 in spring and 2.1-2.4 in summer, after 1 week). BCC proved to be more dynamic in mesocosms without copepods or lowest copepod densities, whereas treatments with highest copepod abundances deviated with a generally higher and more persistent bacterial diversity. Furthermore, relative frequencies of selected OTUs significantly correlated with environmental variables and food web parameters. During growth of *Oikopleura dioica*, bacterial communities showed only modest changes, while a shift towards copepod-dominated mesozooplankton triggered pronounced alterations in bacterial assemblages (range of H´ 1.3 - 2.3).

Equal enrichment with inorganic nutrients of both appendicularian- and copepod-dominated mesocosms yielded different temporal successions of bacterial communities. Again, a transient dominance shift in mesozooplankton caused conspicuous deviations in overall similarity of bacterial communities.

INTRODUCTION

Recent studies in microbial ecology are about to characterise bacterial diversity in various pelagic aquatic ecosystems and put considerable effort in approaching one of the big aims of linking function and diversity in marine bacterial communities (Fuhrman 2002). Although bacteria are of an apparently ubiquitous nature only a limited number of bacterial species seems to be abundant at a given time and space (e.g. Chapter II). As there is a general assumption of functional diversity correlating with taxonomical composition in bacterioplankton communities, it appears to be crucial to elucidate patterns of bacterial diversity change or shifts in relative contributions of most abundant bacterial species in order to assess their biogeochemical implications. Under certain situations like algal blooms (α-proteobacteria, DMSP uptake) single species can dominate bacterioplankton and can contribute 30-50% to total bacterial biomass (Zubkov et al. 2001). There are still impediments to the understanding of bacterial biogeochemical impact, based mainly on a lack of knowledge of the temporal and spatial scales at which bacterial populations vary (Riemann and Middelboe 2002). These authors stated that large variations between the community changes had been observed in various recent studies.

Most investigations of marine bacterial community dynamics have attended to large temporal (Murray et al. 1998, Riemann et al. 1999, Pinhassi and Hagström 2000) and spatial (Ferrari and Hollibaugh 1999, Schauer et al. 2000) scales or both (Acinas et al. 1997, Riemann and Middelboe 2002), or the bacterial communities have been studied in response to environmental disturbance events like strong dinoflagellate blooms (Fandino et al. 2001). Experimental studies by means of mesocosms have focused mostly on diversity changes due to nutrient enrichment (Schäfer et al. 2001, Øvreås et al. 2003) or DOC (protein, starch) manipulation (Pinhassi et al. 1999). Comparably few experimental studies in marine systems have addressed trophically induced bacterial community dynamics (e.g. Zubkov and Lopez-Urrutia 2003).

Copepods, tunicates and to a smaller extent cladocerans are the main mesozooplankton grazers in marine pelagic ecosystems and impact directly (predation) and indirectly (nutrient release, DOC from sloppy feeding and leakage from faecal pellets) (Møller et al. 2003) lower trophic levels. Drastic shifts in BCC due to changes in the predation regime were mostly documented from freshwater systems and were most significant when a dominant grazers from *Daphnia* to small protists (or vice versa) (Jürgens et al. 1994, Langenheder and Jürgens 2001, Degans et al. 2002, Posch et al. 1999). However, there is much less known how interactions at higher trophic levels and bacterial diversity correspond in marine systems. Here, experimental evidence is rather scarce since food web manipulation studies with marine microbial communities are comparably rare.

Massana and Jürgens (2003) investigated the impact of HNF grazers on bacterial community composition and population dynamics by means of chemostats. Only very few studies focused on the trophic impact of mesozooplankton like copepods (Sipura et al. 2003) or both copepods and appendicularians (Zubkov and Lopez-Urrutia 2003) on lower trophic levels. These experiments were performed in short-term microcosm experiments, were restricted to bacterial abundance and activity (Sipura 2003) or excluded intermediate consumers and trophic feedback effects (Zubkov and Lopez-Urrutia 2003).

As in the present marine food web study significant order-of-magnitude responses in abundance and physiological activity as well as shifts in protist grazing pressure on bacteria were noted (as described in chapter III), this chapter goes even one step further in gaining insight in the bacterial response and investigates how mesozooplankton affects bacterial community composition and diversity dynamics. Propagating trophodynamic impact of copepods and appendicularians was followed at the level of the whole bacterial assemblage (community similarity, Shannon diversity) and with respect to individual bacterial taxa by means of DNA-fingerprinting and sequencing.

METHODS

Study site, experimental set-up and measurements. This study is based upon the same marine mesocosm experiments described in chapter III, performed in the Trondheimsfjord (Norway) in spring and summer. Mesocosm treatments (small and large copepods, appendicularians, nutrient enrichment) and sampling intervals resemble those explained in chapter III and results obtained for numerical and activity responses of the microbial food web constitute the basis for the following investigations on diversity and composition of the bacterial community.

PCR-DGGE. For investigation of bacterial diversity, 250 ml of samples (prefiltered by 3 µm in spring and 5 µm in summer) were filtered onto 0.2 µm Durapore filters (25 mm, Millipore), covered with 1 ml lysis buffer (40 mM EDTA, 50 mM Tris-HCl, 0.75 M Sucrose) and stored at –80°C until DNA extraction. Phenol-chloroform extraction of total DNA followed the protocol reported by Schauer et al. (2000). It started by incubating the filters with lysozyme (1 mg ml^{-1} final concentration) at 37°C for 45 min. Then sodium dodecyl sulfate (1% final concentration) and proteinase K (0.2 mg ml^{-1} final concentration) were added before the filters were incubated at 55°C for 60 min. The lysate was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1), and the residual phenol was removed by extraction of an equal volume of chloroform-isoamyl alcohol (24:1). DNA was further purified, desalted and concentrated with a Centricon-100 concentrator (Millipore). The integrity of the DNA extract was checked on a 0.8% agarose gel stained with ethidium bromide. After quantifying spectrofluorometrically the extracted microbial DNA with a Hoechst fluorescence

assay (Paul and Myers 1982), equal amounts of template DNA were used for each PCR (polymerase chain reaction) amplification of bacterial 16S rDNA. I used the bacterial specific primer 358f (5'-CCT ACG GGA GGC AGC AG-3'), with a 40 bp GC-clamp and primer 907rM (5'-CCG TCA ATT CMT TTG AGT TT-3'), which amplifies a 550 bp DNA fragment of bacterial 16S rDNA (Muyzer et al. 1998). Deviating from previous DGGE analyses of freshwater samples from enclosure experiments of the same project (see chapter I), primer 907rM was used instead of primer 907rC (5'-CCG TCA ATT CCT TTR AGT TT-3'), since the latter one may not result in efficient amplification of 16S rRNA gene fragments of some marine representatives of the γ–Proteobacteria (e.g. the genera *Alteromonas*, *Pseudoalteromonas*, *Aeromonas*, and *Shewanella*) due to a mismatch in the target site (Schäfer et al. 2001).

PCR was performed with a Biorad thermal cycler using the following programme: initial denaturation at 94°C for 5 min; 10 touchdown cycles of denaturation (at 94 °C for 1 min), annealing (at 65-55 °C for 1 min, decreasing 1 °C each cycle) and extension (at 72 °C for 3 min); 20 standard cycles of denaturation (at 94 °C for 1 min), annealing (at 55°C for 1 min) and extension (at 72 °C for 3 min) and a final extension at 72 °C for 7 min. PCR products were verified by agarose gel electrophoresis (1.2% agarose) with a standard in the gel and quantified again spectrofluorometrically (see above).

DGGE was carried out with a D-Code Universal Mutation Detection System (Biorad) as described in Muyzer and Smalla (1998) and Schauer et al. (2000, 2003). A 6% polyacrylamide gel with a gradient of a DNA-denaturing agent was cast by mixing solutions of 40% and 80% denaturant agent (100% denaturant agent is 7 M urea and 40% deionised formamide) with a gradient former (Biorad). Equal amounts of PCR product (600 ng) were loaded and the gel was run at 100 V for 16 h at 60°C in 1x TAE buffer (40 mM Tris, pH 7.4, 20 mM sodium acetate, 1 mM EDTA). The gel was stained with the nucleic acid stain SYBR Gold (Molecular Probes) for 45 min, rinsed with Milli-Q water, removed from the glass plate to a UV-transparent gel scoop and visualised with UV. By using the software GeneSnap 4.00. (SynGene, Cambridge, England) high-resolution images were saved as computer files.

To obtain quantitative information from DGGE fingerprints, digitized DGGE images were analyzed with the software GeneTools 3.00.22 (SynGene, Cambridge, England). The software performs a density profile through each lane, detects the bands (defined as operational taxonomic units, OTUs) and calculates the relative contribution of each band to the total band signal in the lane after applying a rolling disk as background subtraction. Bands with a relative intensity of less than 1% of the total intensity of the lane were disregarded. The bands occupying the same position in the different lanes of the gel were identified by visual inspection. To allow for a direct comparison of different gels (in one case only), a multi-band-standard was run on every denaturing gel.

Sequencing of bands and phylogenetic analysis. For sequencing, individual DNA bands were excised from DGGE gels with a sterile scalpel and stored in 50 µl of sterilised HPLC water overnight at 4°C. Subsequently, 1µl of eluted DNA was taken as template in a PCR using the same temperature programme and primers 358f-GC and 907rM (see above). The PCR product was checked in another DGGE to reconfirm the relative position of the respective band and primarily to ensure that only one single band has been excised. If this was the case, duplicate 50 µl PCRs were performed with the same primers, except that the primer 358f did not contain a GC-clamp (MWG Biotech-AG). Afterwards, the size and quality of the PCR product was verified on 1.2% agarose gels and the pooled PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and quantified with a Hoechst fluorescence assay (Paul and Myers 1982). Sequencing reactions were performed by MWG Biotech-AG (Ebersberg, Germany). The obtained partial (up to 550bp) 16S rDNA sequences were aligned, and a consensus sequence was assembled using the Seqman II software (Lasergene, DNAStar) These consensus sequences were compared to available 16S rRNA sequences in GenBank database using the NCBI BLAST programme.

Statistical analysis of PCR-DGGE. The gel was inspected visually on a computer screen and presence and absence of individual bands in each lane was recorded. Based upon this information, a binary matrix (1/0 matrix) was compiled. Alternatively, the relative contribution of each band to the total band signal was used to obtain quantitative information on the banding patterns. From the binary matrix I calculated a distance matrix using the single linkage method with Euclidean distances (root-mean-squared differences). To assess changes of the genetic diversity (fingerprint profile) of the bacterial assemblages in selected mesocosms over time, the complex DGGE banding patterns were analyzed by a powerful data reduction method, the so-called non-metric multidimensional scaling (NMDS) as described in van Hannen et al. (1998).

A complex DGGE pattern is reduced to one point in a two dimensional space. Thus, relative changes in the community structure can be visualised by connecting the consecutive points (e.g. Schäfer et al. 2001). The closer the points are to each other, the more similar are the DGGE banding patterns. Various distance matrices obtained from 0/1- data and in one case also from relative intensities (expression used tantamount to relative frequency) constituted the basis for NMDS analysis, which was computed in STATISTICA 6.0 using two dimensions and a minimum number of 200 iterations. In a second step, these NMDS analyses were repeated after excluding OTUs derived from eukaryotic plastid DNA (see sequencing results). Therefore, presence/absence matrices were adjusted and relative band frequencies newly calculated. To reveal relationship to food web parameters, Pearson´s product-moment correlations and regression analyses were performed (relative OTU frequencies were arcsin-square-root transformed before regression analysis).

On the basis of position and relative intensities of DGGE bands, the Shannon diversity index H´ and Evenness E of the bacterial communities were calculated (Shannon 1948, see also Schauer et al. 2000).

RESULTS

PCR-DGGE analysis

Analysis of BCC by means of PCR-DGGE displayed both temporal and density dependent effects, thereby corresponding manifold to numerical and activity responses of the microbial food web (see chapter III). Banding patterns obtained from several DGGEs were highly reproducible (see Fig. 1 A-D, exemplary selected DGGE images), as seen from samples repeatedly amplified and run on different gels. DGGE bands were referred to as operational taxonomic unit (OTU) when contributing more than 1% to total gel lane intensity, which was the case for 9 to 22 bands per lane. Most bands occurring in various gels and dominating the bacterial community were sequenced in order to exclude non-bacterial OTUs from community analysis (see Table 1). Bacterial community shifts observed in the assemblage were compared with DGGEs regarding all OTUs. All sequencing results are listed in table 1. From the 45 sequenced DGGE bands 4 bands gave only poor sequence quality and were not used at all. 25 bands were of bacterial origin, whereof 3 sequence affiliations were based upon a single strand sequence and not a consensus. 15 bands turned out to derive from eukaryotic plastid DNA or from autotrophic picoplankton (1 band, *Synechococcus*). Bacterial sequences showed a similarity between 77 and 100% with already known sequences obtained from GenBank, thereby roughly two third of the affiliations yielded a similarity of 97% or higher.

Bacterial OTUs primarily belonged to alpha- and gamma-proteobacteria with *Roseobacter* as a predominant taxon. The position and identity of *Roseobacter* bands 4 and 5 were reconfirmed on independent gels showing gradients and time series. In the course of the experiment from day 0 to day 3 and 6, one single band seemed to split into two closely related bands (NAC11-3, NAC 11-7). Sequencing results obtained from two independent gels led to identical results. As these two bands were not always discernible, bands 4 and 5 were regarded as one *Roseobacter* band in further analyses. Moreover, three DGGE-bands with identical sequence affiliation were found in the spring and summer experiments, whereby band 5 equalled band 16, 2 equalled 14 and 13 was equivalent to 17. Although sequences of two OTUs (no. 34 and 28) yielded the same matching similarity with bacteria (90, 97% respectively), but with 1% instead of 0% gaps, they were conservatively considered as chloroplasts.

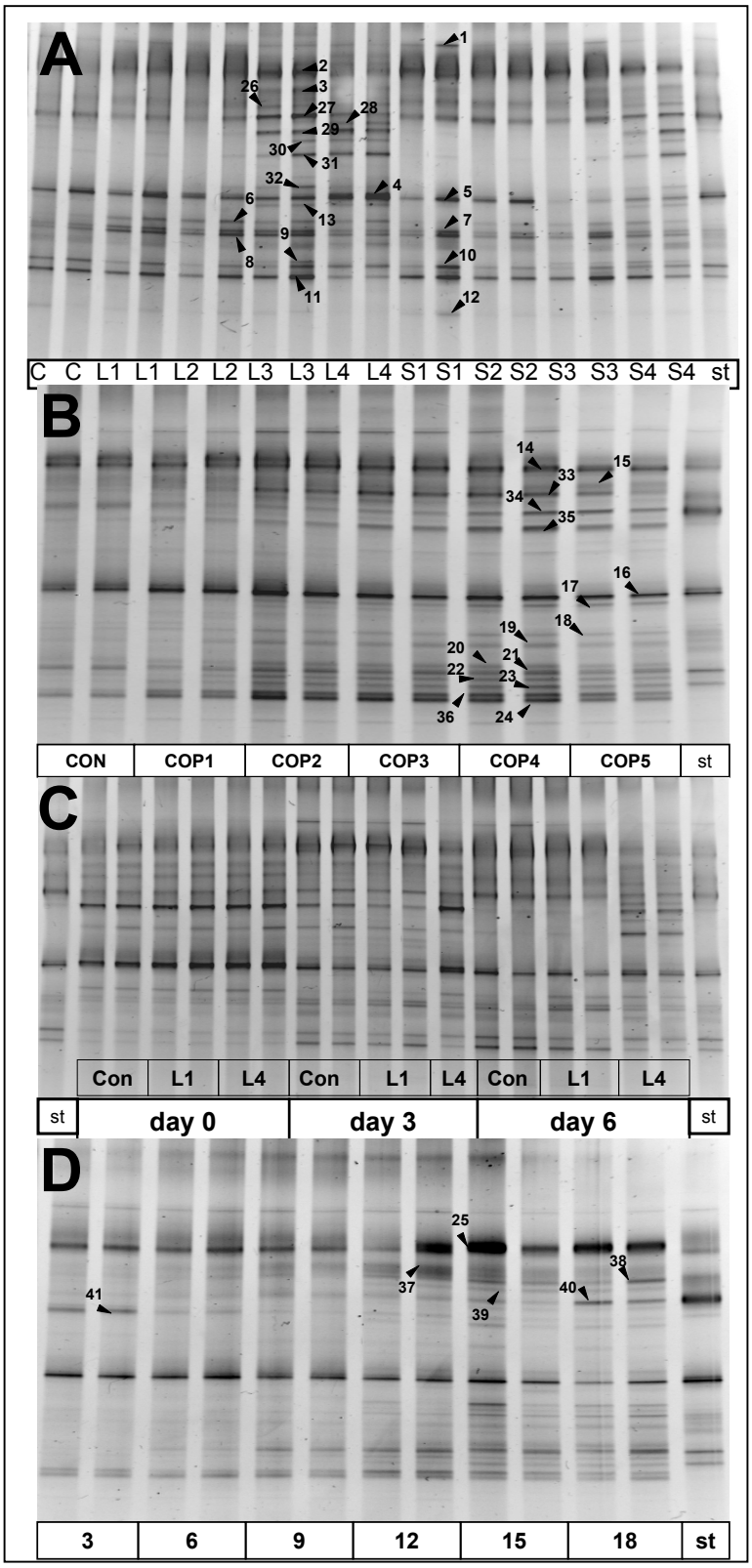


Figure 1:

PCR-DGGE gel images obtained from enclosures with different mesozooplankton treatments (abbreviations see text, st = standard). Little arrows and numbers mark the bands that were excised and sequenced (see Table 1). Banding patterns of copepod gradients after 1 week of experimental duration are demonstrated for spring (A) and for summer (B). In (C) selected spring mesocosms (C, L1, L4) were followed over time and in (D) bacterial community profiles are shown as a time series in mesocosms with highest appendicularian densities (day 3 to day 18).

Table 1:
Sequencing results obtained for bands 1-41 (see Fig. 1) with best matches (BLAST). Sequences derived from eukaryotic DNA and *Synechococcus* are shown in lower part.

Band no.	% similarity	NCBI number of best match
1**	77	AF353229: Uncultured alpha proteobacterium clone Arctic96B-22
2	99	AJ298376: Uncultured marine bacterium BY-65
3**	85	AY033312: Uncultured alpha proteobacterium MB12A07
4	99	AF245635: Uncultured *Roseobacter* NAC11-7
5	99	AF245632: Uncultured *Roseobacter* NAC11-3
6	97	AF100168: Uncultured alpha proteobacterium Shippagan
7	93	AY135674: Uncultured bacterium clone ANT18/2_48
8	98	AF354595: Uncultured gamma proteobacterium Arctic96B
9	87	AF382112: Uncultured bacterium clone ZA3235c
10	97	AY386344: Marine gamma proteobacterium HTCC2188
11	97	AY317112: Beta proteobacterium BAL58
12	98	AJ575519: Uncultured actinobacterium, clone SV1-10
13	97	AY033324: Uncultured alpha proteobacterium MB11C09
14	97	AJ298376: Uncultured marine bacterium BY-65
15	95	AY354737: Uncultured Bacteroidetes bacterium clone PLY-P2-28
16	100	AF245632: Uncultured *Roseobacter* NAC11-3
17	99	AY033324: Uncultured alpha proteobacterium MB11C09
18	94	AF268234: Uncultured proteobacterium EBAC36F02
19	97	AF354611: Uncultured gamma proteobacterium Arctic96A
20**	84	AF468280: Uncultured bacterium clone ARKIA-12
21	95	AY386344: Marine gamma proteobacterium HTCC2188
22	96	AF235120: Uncultured gamma proteobacterium KTc1119
23	96	AY317112: Beta proteobacterium BAL58
24	98	AY033315: Uncultured alpha proteobacterium MB12D10
25	99	AF207850: Uncultured marine eubacterium OTU_A; close of Cytoph-Flavob.
Eukaryotic OTUs		
26	97	U32671: Unidentified eukaryote clone OM21. Cape Hatteras picopl.
27	91	AJ319823*: Coscinodiscus* sp. chloroplast
28	97	AJ319828: *Asterionella glacialis* chloroplast
29	96	AF406549: Uncultured prasinophycean clone AEGEAN_115
30	98	AB073114: *Dinophysis acuminata* plastid gene for 16S rRNA
31	98	AB073116: *Dinophysis norvegica* plastid gene for 16S rRNA
32	90	AF268235: Uncultured prasinophycean EBAC36H07
33	97	AF172719: *Chrysochromulina polylepis,* 16S ssu ribosomal RNA gene
34	90	AJ319823: *Coscinodiscus* sp. chloroplast partial
35	89	AF406549: Uncultured prasinophycean clone AEGEAN_115
36	99	AY172800: *Synechococcus* sp. Almo3
37**	78	AF172719: *Chrysochromulina polylepis*
38**	89	AY135677: Uncultured phototrophic eukaryote clone ANT18/2_
39**	82	U32671: Unidentified eukaryote clone OM21. Cape Hatteras picopl.
40	89	AF418972: Uncultured diatom clone HTB2
41	96	AF406549: Uncultured prasinophycean clone AEGEAN_115

** single strand DNA, no consensus

Copepod impact on bacterial community composition

Temporal development – richness, diversity and community similarity

In the investigated systems, the total number of OTUs per gel varied between 21 and 25 (9 to 22 OTUs per lane) and revealed temporal and treatment-dependent shifts in spring and summer. When following OTU richness (not shown in graph) of selected enclosures (Con, L1, L4) in spring over time, 12-19 OTUs (of 23 total) were registered per lane (Con 14-19, L1 12-17, L4 14-17). Taxa richness increased in all treatments from 14 in the beginning to 15-19 on day 3. While band number further increased towards day 6 in the L4 enclosures, the number of bands decreased in the control and L1 enclosures.

Time-dependent analysis of DGGE patterns in summer revealed as well 23 OTUs, with 14-17 found in the control treatments, 16-19 in the cop1 and 20-22 in the cop5 enclosures.

As a summarising indicator for microbial diversity **Shannon's diversity index H´** varied most strongly when all OTUs were included compared to bacterial OTUs being the only basis for calculation. Clear patterns of temporal diversity dynamics became visible in spring and summer (Table 2). In all selected mesocosms (e.g. controls, lowest and highest density treatments) different degrees of diversity increase (all OTUs) were found from the start to the midst (day 3, day 4 in summer) of the experiments. In spring this rise was apparently more pronounced (28% increase), whereas summer diversity remained rather constant (5.4-10% increase).

While diversity decreased again in controls and low density treatments from the midst towards the end of the experiments, it remained high or further increased in highest density treatments (also in cop1 in summer, all OTUs). Unfortunately, the first sampling for bacterial diversity took place one day after copepod addition in summer, so the cop 5 treatments already showed some initial deviation from the other treatments with a higher number of bands at start, coinciding with strong bacterial abundance alterations in the beginning). Nevertheless, the relative increase in H´ derived from all OTUs was comparable within the first 3-4 days.

These initial differences were further buffered and temporal diversity shifts further clarified when only sequenced bacterial OTUs constituted the basis for H´-calculation (Table 2, lower part). Average diversity values at start were 1.54-1.56 in spring and 1.82-2.23 in summer. Again, diversity increases were enhanced in spring and relative inclines proved stronger for controls and low density treatments compared to high density mesocosms.

Table 2:
Mean (± range of replicate enclosures) values for Shannon diversity index H´ and evenness E for selected copepod treatments in spring and summer. Temporal shifts of H´ are demonstrated (for both all OTUs and sequenced bacterial OTUs only) and relative increases in bacterial diversity indicated.

Time effect: Shannon diversity H´ and Evenness

Spring

All OTUs

	Con		L1		L4	
days	**H´**	**E**	**H´**	**E**	**H´**	**E**
0	**2.07 ± 0.01**	**0.78 ± 0.002**	**2.08 ± 0.01**	**0.79 ± 0.004**	**2.10 ± 0.04**	**0.80 ± 0.02**
3	**2.62 ± 0.05**	**0.90 ± 0.03**	**2.55 ± 0.08**	**0.90 ± 0.03**	**2.39**	**0.88**
6	**2.43 ± 0.01**	**0.92 ± 0.01**	**2.37 ± 0.18**	**0.92 ± 0.02**	**2.56 ± 0.04**	**0.91 ± 0.02**
BACT OTUs						
0	**1.50 ± 0.03**	**0.65 ± 0.01**	**1.55 ± 0.01**	**0.67 ± 0.01**	**1.54 ± 0.02**	**0.67 ± 0.01**
3	**1.96 ± 0.14**	**0.76 ± 0.07**	**1.99 ± 0.14**	**0.78 ± 0.05**	**1.97**	**0.86**
6	**2.02 ± 0.02**	**0.89 ± 0.03**	**2.04 ± 0.06**	**0.93 ± 0.03**	**2.05 ± 0.03**	**0.89 ± 0.01**

Summer

All OTUs

	Con		COP1		COP5	
days	**H´**	**E**	**H´**	**E**	**H´**	**E**
1	**2.26 ± 0.04**	**0.83 ± 0.02**	**2.43 ± 0.15**	**0.84 ± 0.02**	**2.78 ± 0.01**	**0.90 ± 0.002**
4	**2.45 ± 0.17**	**0.86 ± 0.06**	**2.48 ± 0.02**	**0.88 ± 0.01**	**2.88 ± 0.06**	**0.93 ± 0.02**
7	**2.26 ± 0.09**	**0.86 ± 0.03**	**2.49 ± 0.01**	**0.89 ± 0.02**	**2.80 ± 0.05**	**0.94 ± 0.02**
BACT OTUs						
1	**1.82 ± 0.09**	**0.79 ± 0.04**	**1.95 ± 0.15**	**0.80 ± 0.03**	**2.23 ± 0.05**	**0.87 ± 0.02**
4	**2.13 ± 0.17**	**0.83 ± 0.07**	**2.14 ± 0.02**	**0.86 ± 0.01**	**2.35 ± 0.06**	**0.91 ± 0.02**
7	**1.87 ± 0.09**	**0.81 ± 0.04**	**2.09 ± 0.01**	**0.85 ± 0.03**	**2.35 ± 0.09**	**0.81 ± 0.03**

A decrease in bacterial diversity took place in the former and an increase or level maintenance in the latter mesocosms in the second half of the experiment. However, the level of bacterial diversity in general was elevated at the end compared to assemblages in the beginning and the increase more pronounced in spring than in summer. Interestingly, the increase in H´ and E was similar in controls, L1 and L4 mesocosm, indicating that treatment had nearly no effect on the development of overall bacterial diversity over time (Tab. 2, upper part, BACT OTUs). In summer, instead, some more differences became obvious.

In terms of **community similarity,** bacterial assemblages turned out to be comparably stable with most community members being present in all investigated food web constellations. Over time and across gradients, the maximal distances between communities based on all OTUs (with potential plastid DNA included) were generally larger than those based exclusively on bacterial OTUs (e.g. Fig. 2). Although changes were less intense and apparently more buffered when OTUs derived from eukaryotic plastid DNA were excluded, shifts in bacterial community dynamics and similarity were noted in response to

mesozooplankton impact, within as well as among bacterial assemblages. In community analysis by NMDS (mainly based upon the presence and absence of bands), treatment replicates stayed mostly close together and showed very similar successions, thereby reflecting dynamics at higher trophic levels.

As in temporal patterns of Shannon diversity in spring, BCC in low density treatments and controls showed strongest shifts from day 0 to day 3 (Fig. 2A). After 6 days, assemblages in these treatments were more similar to the start community, whereas changes in highest density treatments were less intense but more persistent, leading to communities with highest dissimilarity. When looking at selected small copepod treatments (C, S1, S4; with all OTUs), a similar pattern became obvious with S4 treatments deviating the most from the rest and the initial community (not shown in graph). Communities of replicate highest copepod density mesocosms always showed a very high similarity to each other and a further decreasing similarity to the initial assemblages within the period from day 3 to day 6. In summer, time-dependent community changes were observed within one week (Fig. 2B), whereby changes became more apparent in controls and cop1 than in cop5 mesocosms.

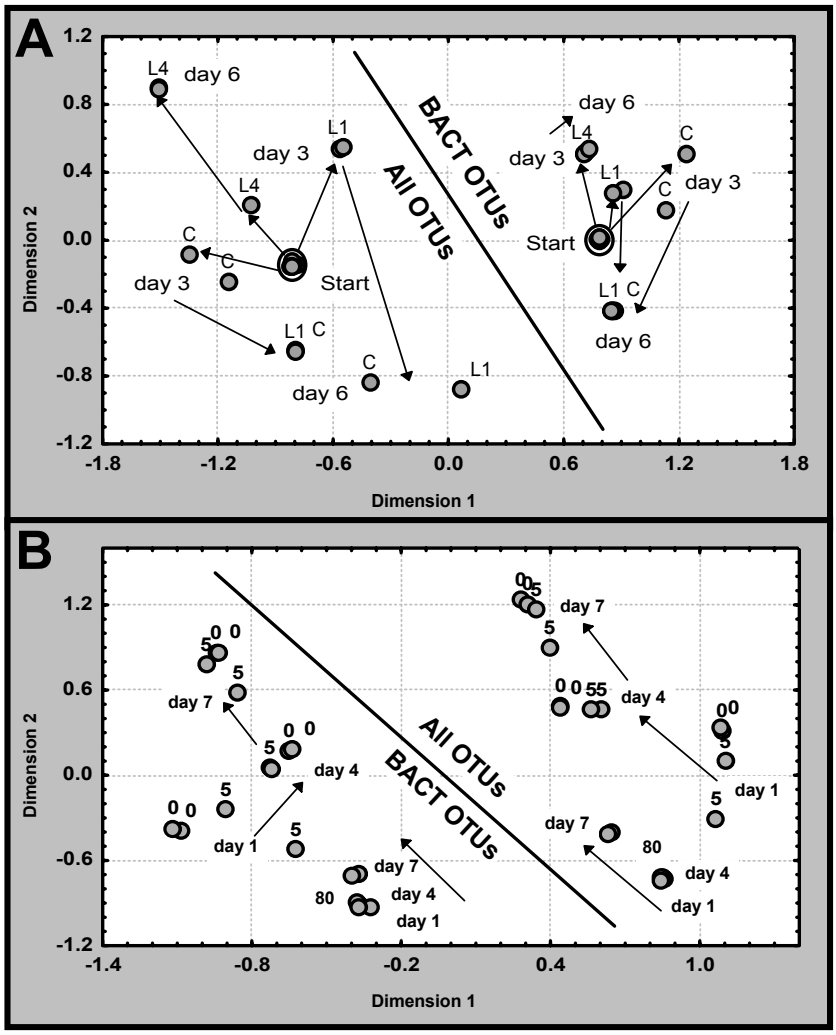


Figure 2:
NMDS plots (based on presence/absence matrices) demonstrating temporal patterns of bacterial community dynamics and similarity in the spring (April, A) and the summer experiment (B). Selected treatments are shown over time (C, L1, L4 on days 0, 3, 6 in (A), Con=0, cop1=5, cop5=80 on days 1, 4, 7 in (B)) with successions derived from all OTUs and sequenced bacterial OTUs only (BACT). Stress ("goodness of fit") values are: 0.1216 (A), 0.1648 (B).

Copepod gradient effects – richness, diversity and community similarity

Along the copepod gradients in spring (Fig. 1A) 21 different OTUs were found in total after 1 week of experimental incubation, of which 12-20 occurred in the *Calanus* bags (L-gradient) and 12-19 in the S-gradient (small copepods, >250-500 µm) treatments.

Various OTUs were found in all treatments and remained during the whole experimental period and along the complete copepod gradients, although their relative contribution to the community was variable. Out of the 14 sequenced bacterial bands obtained from the L-gradient, 5 bands (corresponding ~ 36%) changed regarding presence or absence across the gradient, i.e. they disappeared or became detectable. Similar values were measured for the S-gradient (4 out of 13, ~ 31%). In the summer community, 23 OTUs were observed in total, of which 17-21 occurred per lane. Out of the 13 sequenced bacterial OTUs, 3 disappear or become detectable (~23%), whereas the rest was present along the whole gradient. Four weak bands were not successfully sequenced.

Gradients in spring as well as in summer showed an increase in taxa richness with increasing copepod abundance, which was clearly less intense, when eukaryotic OTUs were excluded from regression (shown for the S-gradient in spring, Fig.3).

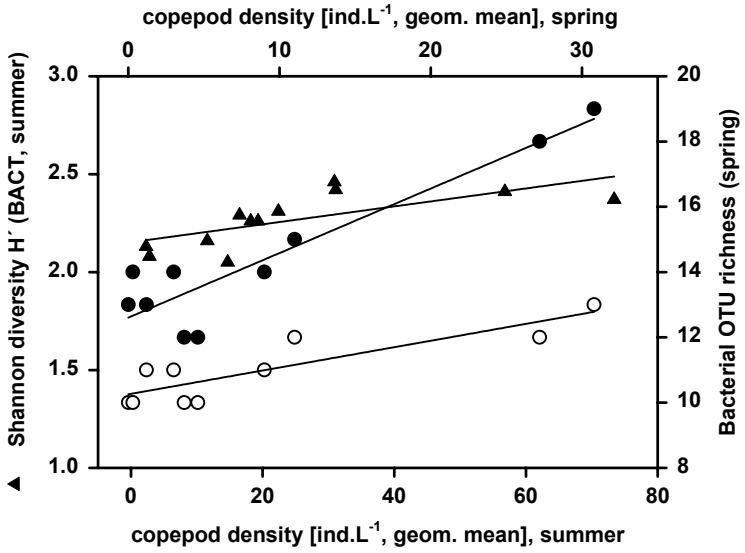


Figure 3:
OTU richness (bacterial OTUs only = white circles, all OTUs = black circles (S-gradient, in spring) and Shannon´s index H´ (summer) across copepod gradients after 1 week (geometric means).
Linear regressions are significant at $p<0.05$

Copepod density-dependent effects on Shannon's diversity index H´ after one week of experiment resulted in a larger range (from 2.10 to 2.80, minimal and maximal values found in single mesocosms) in spring than in summer (from 2.45 to 2.87) along the copepod density gradients (Table 3), when all OTUs were considered. In spring and summer, increases were significant or nearly significant.

The range between minimal and maximal diversity was reduced (1.93-2.42 in S and 1.83-2.28 in L-gradient; more strongly in spring than in summer 2.05-2.46) when eukaryotic DGGE-bands were excluded, leading to a significant linear increase in bacterial diversity along the summer gradient (Fig.3).

Table 3:
Mean (± range of replicate enclosures) as well as minimal and maximal values for Shannon diversity index H´ and Evenness for selected copepod treatments in spring and summer. Copepod density-dependent differences after 1 week of experiment (all OTUs, BACT). Values in brackets are minima and maxima measured in single enclosures. Regression were calculated with geometric mean copepod densities.

All OTUs

			H´	Evenness		regression	p	R²
	Controls		2.46 ± 0.01	0.94 ± 0.02				
	L-Gradient	min	2.26 ± 0.32 (2.10)	0.87 ± 0.05 (0.85)	H´	y=2.3612+0.0095x	***0.057***	0.38
spring		max	2.75 ± 0.10 (2.80)	0.92 ± 0.02 (0.93)	E	y=0.9144-0.0000x	0.968	0.00
	S-Gradient	min	2.16 ± 0.05 (2.10)	0.85 ± 0.11 (0.80)	H´	y=2.2668+0.0125x	**0.040**	0.43
		max	2.68 ± 0.05 (2.70)	0.92 ± 0.03 (0.93)	E	y=0.8930+0.0002x	0.885	0.00
	Controls		2.48 ± 0.03	0.87 ± 0.01	H´	y=2.5669+0.0038x	***0.065***	0.30
summer	Gradient	min	2.47 ± 0.04 (2.45)	0.85 ± 0.02 (0.85)	E	y=0.8725+0.0010x	**0.014**	0.47
		max	2.84 ± 0.04 (2.87)	0.93 ± 0.01 (0.94)	H´	y=2.3902+0.0197x-0.0002x²	0.003	0.72
					E	y=0.8448+0.0035x-0.0000x²	0.004	0.70

BACT OTUs

			H´	Evenness		regression	p	R²
	Controls		2.18 ± 0.02	0.95 ± 0.01				
	L-Gradient	min	2.05 ± 0.25 (1.93)	0.86 ± 0.02 (0.84)	H´	y=2.1621-0.0004x	0.909	0.00
spring		max	2.36 ± 0.12 (2.42)	0.92 ± 0.02 (0.93)	E	y=0.9273-0.0017x	***0.067***	0.36
	S-Gradient	min	1.92 ± 0.10 (1.83)	0.83 ± 0.13 (0.76)	H´	y=2.0465+0.0060x	0.233	0.17
		max	2.26 ± 0.05 (2.28)	0.90 ± 0.05 (0.92)	E	y=0.8787-0.0002x	0.923	0.00
	Controls		2.10 ± 0.04	0.88 ± 0.02	H´	y=2.1523+0.0046x	**0.011**	0.49
summer	Gradient	min	2.11 ± 0.11 (2.05)	0.86 ± 0.01 (0.85)	E	y=0.8873+0.0009x	***0.060***	0.31
		max	2.44 ± 0.04 (2.46)	0.95 ± 0.01 (0.96)	H´	y=2.0221+0.0163x-0.0002x²	0.002	0.76
					E	y=0.8549+0.0038x-0.0000x²	0.019	0.59

In terms of community similarity, bacterial assemblages showed some copepod density-dependent trends after one week (Fig. 4A), but the overall variation in community composition was far less striking when chloroplast-derived OTUs were excluded from NMDS analysis. Bacterial assemblages from high copepod density mesocosms (L3, L4, S4) were very similar to each other (mainly L4) and most separated from the rest.

In summer (Fig. 4B), comparably small shifts along the gradient were registered and most bands were present in all treatments, but with differing relative intensities. Nevertheless, a gradual response according to copepod densities was noted and replicate treatments were always highly similar to each other. Again, community similarity along the gradient was much higher when non-bacterial OTUs were excluded, indicating that eukaryotic bands thus contributed more to the separation of the communities.

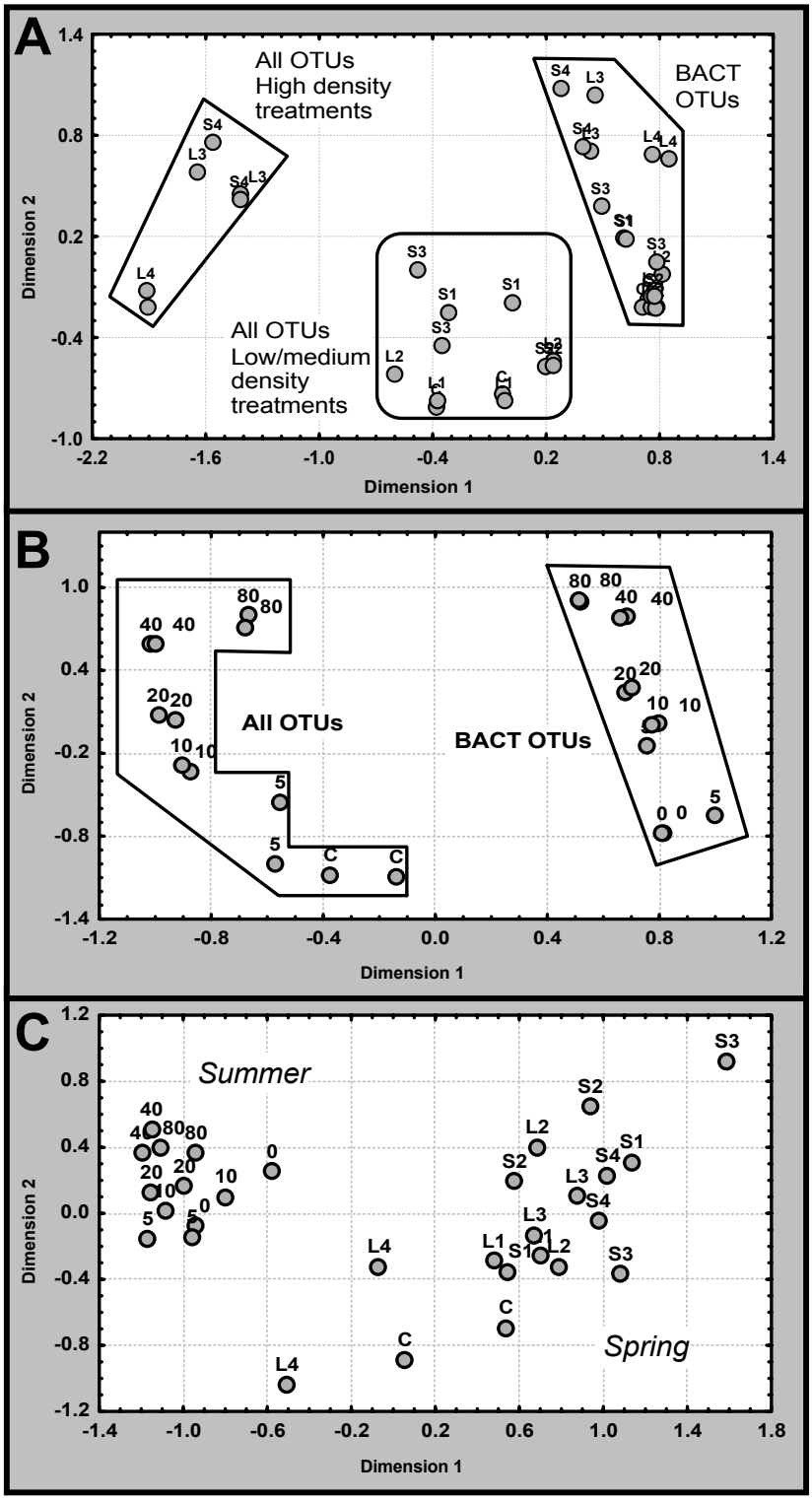


Figure 4:
NMDS plots demonstrating patterns of bacterial community dynamics and similarity dependent on copepod density in experiments in spring (S- and L-gradient, A) and summer (B).
Patterns are shown derived from all OTUs and bacterial OTUs only. A comparison of spring and summer communities (along gradients) is summarised in plot (C), whereby relative intensities of DGGE bands were taken into account. Stress (`goodness of fit´) values: 0.1239 (A), 0.1030 (B) and 0.0992 (C).

With respect to a spring and summer comparison of cascading effects of the food web manipulation at higher trophic levels, bacterial assemblages proved to be more dynamic in spring, thereby reflecting the larger range found for Shannon's diversity index (see above). Density-dependent patterns of community similarity showed a wider amplitude after 1 week in spring, especially when relative band intensities were taken into account (Fig. 4C).

Relative frequencies of OTUs and relationships to food web parameters

Spring bacterial communities appeared to be more dynamic over time than assemblages in summer mesocosms, as already shown by NMDS analysis (Fig. 4). These dynamics were underlined by shifts in relative frequencies of dominant OTUs (Fig. 5). Thereby most OTUs could be taxonomically assigned and only a small proportion (average 6%) remained undetermined. Communities in selected spring enclosures (Con, L1) and especially in L4 were dominated by *Roseobacter* (OTU 4/5).

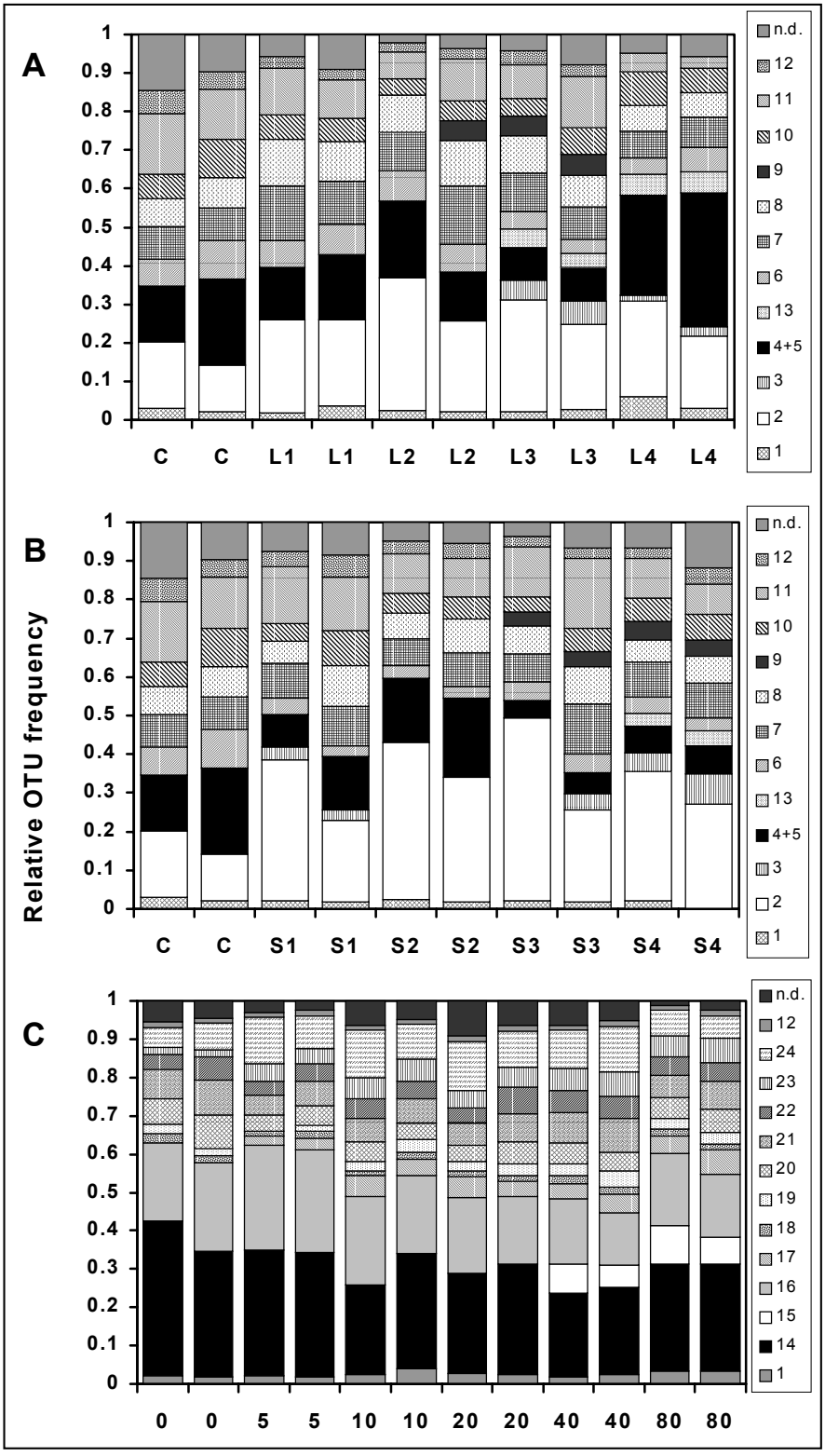


Figure 5:
Relative frequencies of sequenced OTUs along 3 copepod density gradients in spring (L-gradient (A) and S-gradient (B)) and summer (C). Taxonomic affiliations of numbered OTUs can be obtained from table 1.

As can be inferred from temporal community changes, also patterns of relative OTU frequencies changed over time (not shown in graph) and were very similar in Con and L1. While relative contribution of *Roseobacter* decreased in these treatments, other OTUs gained in relative importance. In summer (not shown in graph), the vast majority of OTUs remained comparably constant in terms of relative frequencies in cop5 and also cop1 over the 1 week of investigation period.

Interestingly, trends in relative frequencies of dominant OTUs also became obvious along gradients of copepod density after 1 week of experiment (Fig. 5). The genus *Roseobacter* (OTU 4/5) was a very important component in spring, in L-treatments more and with increasing density (A), in S-treatments less and rather with decreasing copepod density (B). OTU 9, instead, was observed only in intermediate density L- and high density S-mesocosms. Furthermore, the occurrence of OTUs 3 and even more of 13 was restricted to high copepod density treatments, whereas OTU 2 was most important at intermediate copepod densities. The latter one (equivalent to OTU 14) occurred also in summer and rather decreased with increasing copepods. Contrastingly, OTU 15 was only found under high copepod abundances. Only small variations in relative OTU frequencies were noted along the summer gradient (Fig. 5C)

Another objective of bacterial community analysis was to detect significant relationships between response patterns of single OTUs or community subgroups (in terms of relative intensity) and relevant food web parameters/constellations (Fig. 6).

In spring, 9 OTUs significantly correlated with one or more of the dominant food web parameters copepod, ciliate or HNF abundance, whereas in total 12 correlations were positive and 12 negative (Tab.4). Instead, only 5 OTUs exhibited significant correlations with the selected parameters in summer, of which 5 were positive and 3 negative. When substituting HNF density by total nanoplankton abundance (thus including mixotrophic forms like *Chrysochromulina*), 4 additional significant relationships became apparent, one was negative (OTU 14) and 3 positive (OTUs 15, 17, 23).

	L-gradient			S-gradient				Summer gradient			
OTU	**COP**	**CIL**	**HNF**	**COP**	**CIL**	**HNF**	**OTU**	**COP**	**CIL**	**HNF**	**NANO**
1	**0.67**		**0.76**			-0.63	**14**		**0.62**		-0.64
3		-0.77		**0.77**	-0.82	**0.80**	**15**	**0.88**	**-0.94**		**0.84**
4+5			**0.65**				**16**		**0.65**		
6	-0.71	**0.83**					**17**	**0.64**	**-0.60**		**0.63**
7							**19**				
8			-0.66				**20**				
9				**0.88**	-0.71	**0.72**	**23**	**0.65**	**-0.67**		**0.62**
11	-0.75		-0.73				**24**				
12	-0.84		-0.78								
13	**0.90**	-0.95	**0.84**	**0.93**	-0.87	**0.99**					

Table 4:
Positive and negative correlations (Pearson Product Moment Correlation coefficients, significant at p>0.05) of OTUs with selected food web parameters in spring and summer.

To exemplary demonstrate the relevance of heterogeneity with bacterial communities in regarding environmental changes and to illustrate the existence of gradient responses and dependencies at the level of community composition, the relative band intensities of similarly responding OTUs were pooled, together constituting a considerable proportion of total community lane intensity (on average 14-56%). While some OTU groups increased with increasing *Calanus* densities in spring, others declined in relative importance with increasing HNF abundances (Fig. 6A). In summer, positive correlations were also unveiled in the summer experiment (Fig. 6B, C) for OTU subgroups and HNF numbers or the proportion of actively respiring cells (CTC-positive, chapter III). One subgroup of OTUs, for example, correlated significantly positive with bacterial thymidine uptake and at the same time responded negatively to increasing HNF abundances (Fig. 6C).

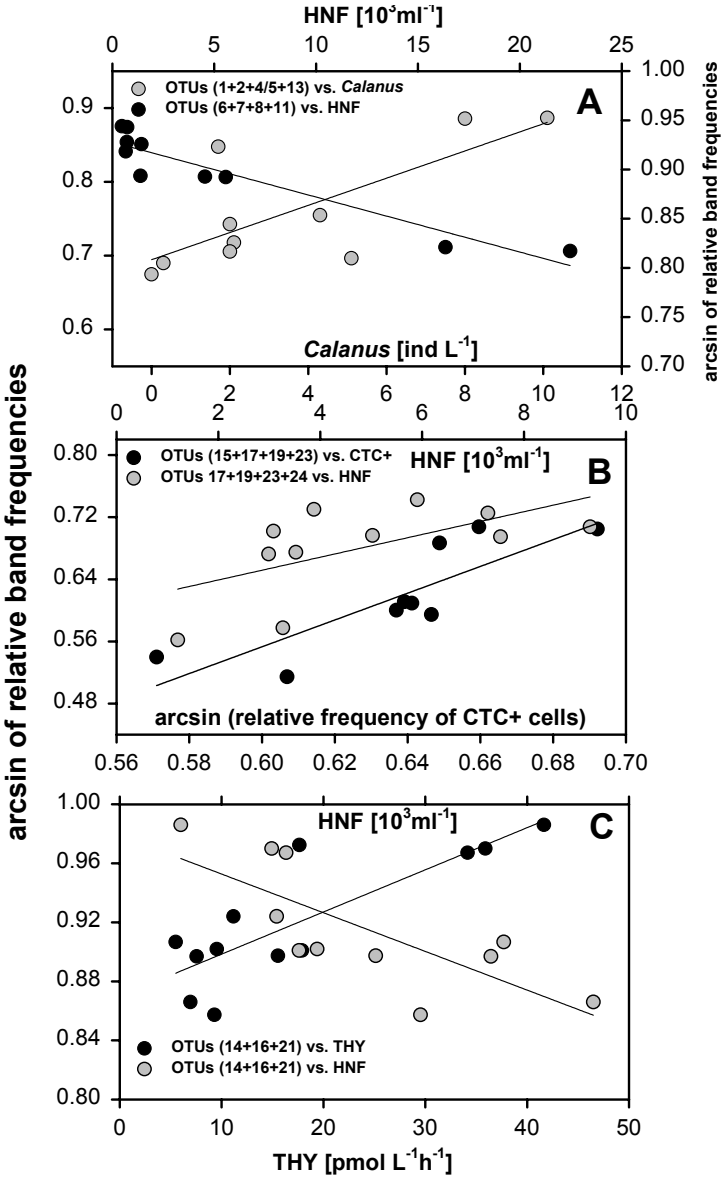


Figure 6:
Relationships of selected bands to other explanatory community variables along spring (A) and summer (B,C) copepod gradients. In (A) significant regressions were y=0.8539-0.0000007x, R^2=0.89 p<0.0001, HNF) and y=0.8083+0.0138x (R^2=0.55, p=0.0137, *Calanus* geomean). In (B) significant regressions were y=-0.4814+1.7243x, R^2=0.71, p=0.0045; CTC without controls) and y=0.6098+0.00001*x, R^2=0.37, p=0.048; HNF plots without 1 outlier) and in (C): y=0.8699+0.0029x (R^2=0.68, p=0.0009, ³H-thymidine) and y=0.9790-0.00001x, (R^2=0.58, p=0.0067, HNF).

Appendicularian impact on bacterial communities

Oikopleura dioica proved to significantly affect bacterial abundances and activity (see chapter III) in the course of the experiment. In order to illustrate if the quantitative impact of these appendicularians was reflected at the level of bacterial diversity and community composition, two mesocosms showing the strongest appendicularian bloom event were chosen for analysis.

In terms of mesozooplankton grazing regime, two periods either dominated by appendicularians or copepods could be discriminated. Until day 9, *Oikopleura* increased strongly (peak densities of 24 and 35 ind. L^{-1}, respectively, Sommer et al. 2003, chapter III), thereafter decreasing again while copepods, surviving the initial removal, became more abundant. Following the time course of the appendicularian enclosures, 25 different OTUs were registered in total with a broad range of 9-22 bands per lane (Fig. 1D). During the first three sampling time points taxa richness was rather low (9-14 OTUs, 5-9 bacterial OTUs), but increased after day 9 (19-22 OTUs). Shannon's diversity index H´ initially was low and increased from day 3 to day 12 (Fig. 7), when calculated based on all OTUs (from 1.90 to 2.63, means) as well as on a community without identified eukaryotic bands (from 1.33 to 2.27, means)

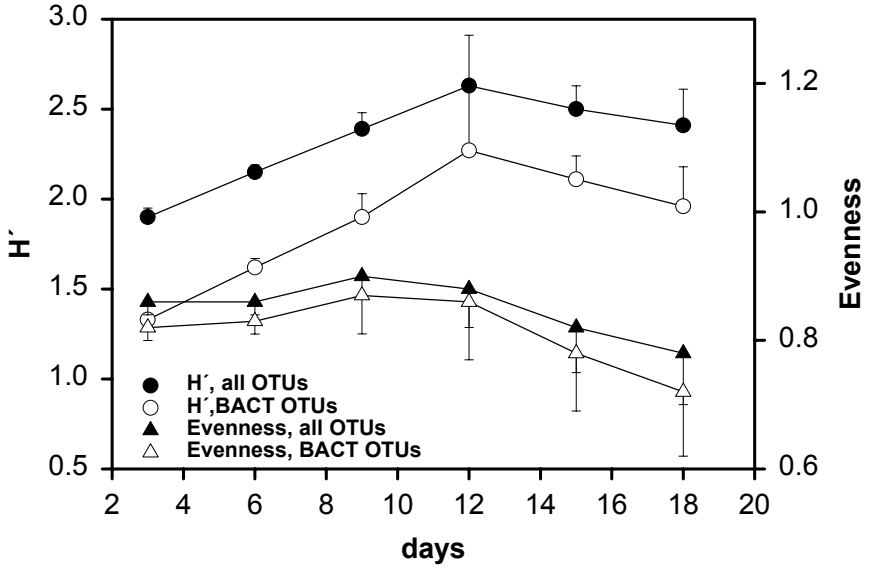


Figure 7: Shannon diversity H´ and Evenness calculated for all OTUs and bacterial OTUs (BACT) as time course in appendicularian mesocosms with maximal densities of *Oikopleura dioica.*

After that shift from appendicularian to copepod dominance, diversity slightly decreased again. During the phase of appendicularian growth (first three data points), the mean evenness of the bacterial community rather increased from 0.82 to 0.87 and decreased with a declining *O. dioica* population to a minimum of 0.72 on day 18.

A shift in mesozooplankton dominance was furthermore mirrored in a distinct pattern of community similarity as analyzed by NMDS (Fig.8), regardless of the exclusion of non-

bacterial OTUs. Bacterial communities showed a high similarity and few changes during the first 9 days (growth period of appendicularians), whereas a strong shift became apparent between days 9 and 12. Subsequent changes (until day 18) were comparably small again (Fig.8).

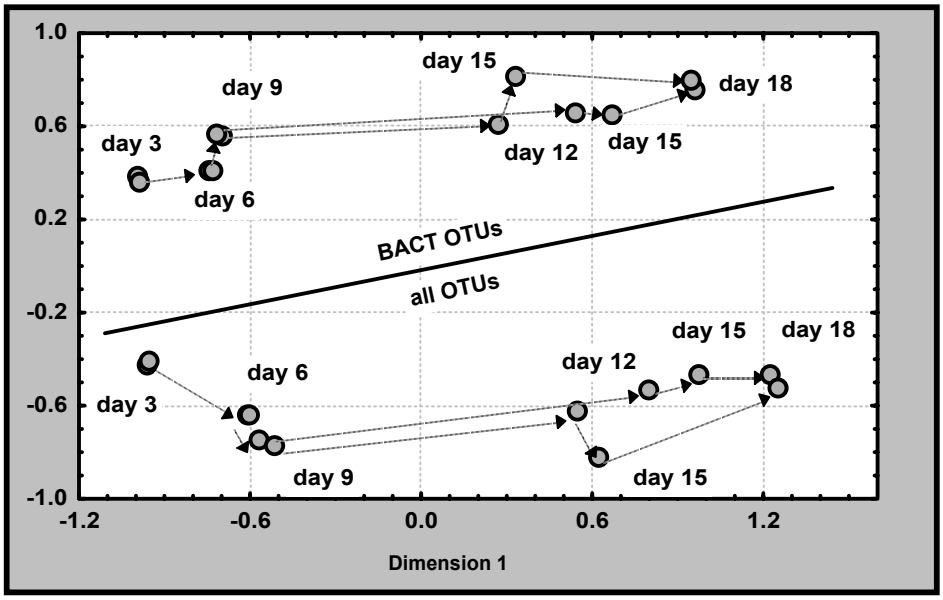


Figure 8:
NMDS analysis for temporal development of communities (comprising all OTUs and bacterial OTUs only, BACT) in mesocosms with maximal densities of *Oikopleura dioica* (stress 0.08808).

The two most dominant bacterial OTUs 16 and 25 first decreased in relative frequency until day 12 (Fig. 9). Thereafter, the contribution of OTU 16 to the total bacterial community was further reduced, whereas OTU 25 strongly gained in importance. In addition to that, a number of OTUs mostly belonging to γ-proteobacteria appeared within the period from day 9 to 12 and remained with a minor relative frequency until the end of the experiment.

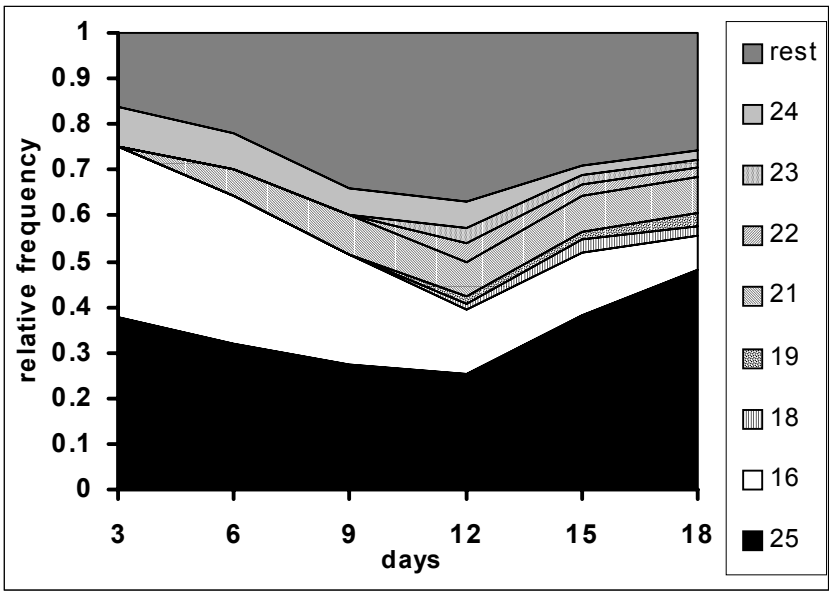


Figure 9:
Relative frequencies of selected bacterial OTUs in appendicularian mesocosms with highest densities of *Oikopleura dioica* (means of replicate enclosures) in the course of the experiment.

Mesozooplankton dominance and nutrient enrichment

Microbial communities in eutrophicated enclosures displayed 28 OTUs in total, with 14-20 bands found in the EUCOP mesocosm and 13-22 being noted in the EUAPP enclosure (not shown). Both treatments showed an OTU overlap of 68%, while 14% and 18% of all OTU were observed only in EUAPP and EUCOP, respectively. Moreover, OTU richness tended to increase over time.

As no bands from the nutrient enrichment study were sequenced so far and samples from enriched mesocosms were applied on a separate gel, the investigation of the microbial diversity response rather focuses on the temporal comparison of copepod- and *Oikopleura*-dominated assemblages under conditions of identical enrichment (referred to as EUAPP and EUCOP) and the aspect of a dominance shift (in EUAPP, as in non-enriched appendicularian bags) than on comparing eutrophicated and non-eutrophicated treatments (as done in chapter III). As described in chapter III, microbial food web assemblages subjected to nutrient enrichment differentially responded in terms of abundance and activity. When *Oikopleura* predominated, nutrient addition significantly affected picoplankton (heterotrophic bacterial abundance and production and *Synechococcus*), whereas copepod dominance led to significantly increased numbers of autotrophic nanoplankton.

However, combined nutrient and grazer impact also affected microbial community composition and caused differences in temporal successions of communities dominated either by *Oikopleura* or copepods (Fig. 10).

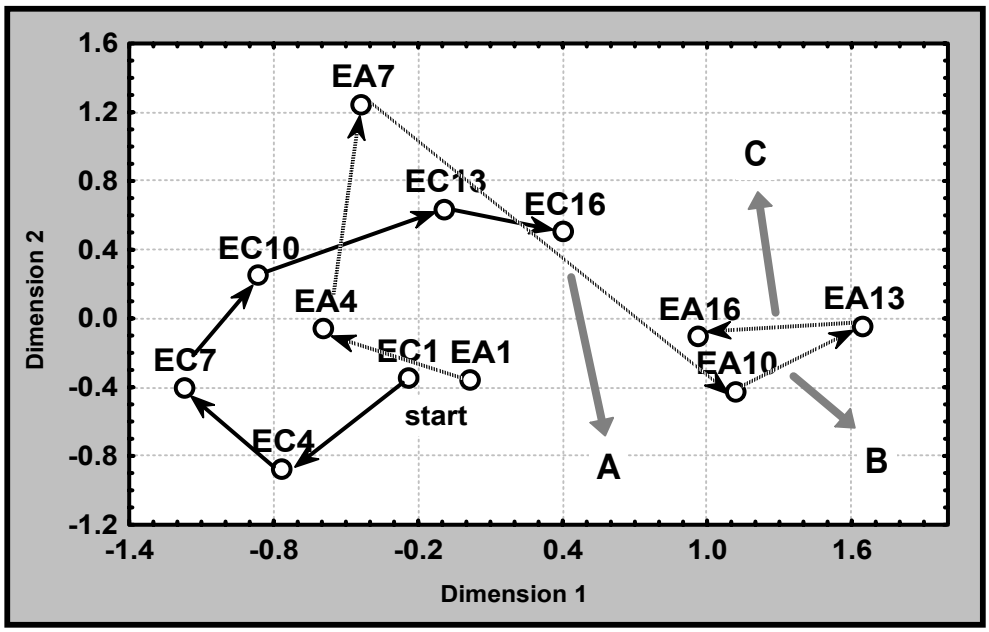


Figure 10:
NMDS plot (from presence/absence distance matrix) showing the temporal succession of bacterial communities in eutrophicated mesocosms with either Appendicularian (EA) or copepod (EC) dominance on experimental day 1 to 16. Stress (`goodness of fit´) =0.1307. Grey arrows mark distinct transition periods with pronounced shifts mesozooplankton composition. A= copepod increase from 8 to 25 ind.L^{-1}, B= increase of *Oikopleura* from 5 to 26 ind.L^{-1}; C= decrease of *Oikopleura* from 26 to 1ind.L^{-1}.

As the initial budget of added inorganic nutrients was identical, the observed alterations should be largely due to grazer-mediated effects. Thereby, most community shifts appeared to be roughly proportional to the length of the respective time intervals. Remarkably, the most pronounced changes in overall community similarity occurred concomitant with a strong increase in density of copepods surviving the initial removal (period A, Fig.10). Thereafter, smaller distances between microbial communities pointed to comparably minor composition alterations between subsequent assemblages, although marked variations in *Oikopleura* density were observed (periods B and C, Fig. 10). Microbial communities at the termination of the experiment were again rather similar and subjected to comparable densities of copepods (23 in EUAPP, 30 in EUCOP).

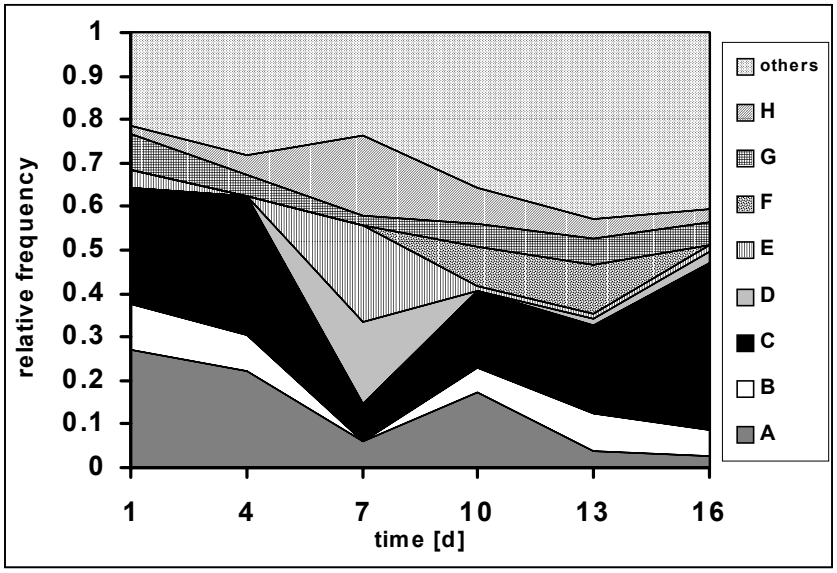


Figure 11:
Relative frequencies of dominant OTUs (not sequenced) in EUAPP mesocosm. The positions of some bands in the DGGE gel (according to multi-band standard) were identical with those of sequenced bands from other gels. Accordingly, OTU A was assigned to OTU 5 and 16 (related to *Roseobacter*), whereas bands B and C equaled bands belonging to OTU 36 (*Synechococcus*) and OTU 25, respectively.

Nevertheless, although copepod appearance triggered pronounced changes in overall qualitative BCC, relative frequencies of dominant OTUs indicated marked shifts of EUAPP microbial assemblages in the first half of the experiment (Fig. 11), where general mesozooplankton densities were still low (6-11 copepods per L, up to 5 *Oikopleura* per L). Compared to the non-enriched treatments with *Oikopleura* (see Fig. 9), stronger changes were noted mainly in the period between days 4 and 10 (see also Fig. 10). This observation coincided with pronounced increases in bacterial production (peak on day 7) and abundance (peak on day 10), while autotrophic as well as heterotrophic nanoplankton remained at a constantly low level. Dominant bands in EUAPP steeply dropped in relative importance (A, B, C in Fig.10). According to their position in the gel (multi-band standard); These bands were found to equal already sequenced bands from other gels (OTUs 5/16, 36, 25, respectively). When comparing relative intensities of these important OTUs under enriched (Fig.11, OTUs A and C) and non-enriched (Fig.9, OTUs 16 and 25) conditions, these respective taxa strongly declined under the former and remained rather constant under the latter conditions.

DISCUSSION

General aspects and methodology

Marine bacterioplankton communities are regulated by nutrients (Ducklow 2000), protist predation (e.g. Del Giorgio et al. 1996, Zubkov et al. 2000) and viral infections (Fuhrman 1999). The presence of copepods can increase bacterial abundance and growth (e.g. Møller and Nielsen 2001, Maar et al. 2002) or alternatively trigger a 4-link trophic cascade negatively affecting bacterioplankton (chapter III). Appendicularians, in contrast, can reduce bacterial concentrations and activity by direct predation (King et al. 1980, Zubkov and Lopez-Urrutia 2003, chapter III). Consequently, I expected both mesozooplankton groups to cause shifts in bacterioplankton community composition. The study objectives were to investigate trophodynamic effects of different mesozooplankton on microbial food web components, thereby including key zooplankton organisms (*Calanus finmarchicus, Oikopleura dioica*) and allowing for longer-term (more than 1-3 days) cascading temporal and density–dependent effects. This detailed and comprehensive analysis was composed of independent large-scale experiments in different seasons and food web scenarios. While many studies end at the level of protists (Broglio et al. 2001) or of bacterial biomass and activity (Hansen et al. 2000), my primary goal was to link patterns of numerical and/or activity responses of functional microbial food web components to patterns of BCC using 16S-DNA fingerprinting and sequencing.

Constraints of the semi-quantitative PCR-DGGE are well known (Muyzer et al. 1993, Muyzer et al. 1998, Riemann et al. 1999), amongst those the fact that only more abundant species are detected. Cloning approaches should be involved to resolve total bacterial diversity (Fuhrman and Ouverney 1998, Kerkhof et al. 1999, Fuhrman 2002). Another potential obstacle could be that ecologically different bacterial groups might not show differences in 16S-DNA.

Nevertheless, PCR-DGGE, especially when combined with sequencing, is a often used and highly reproducible method (Schauer et al. 2000, Diez et al. 2001) to trace changes within microbial communities on temporal and spatial scales (e.g. Riemann et al. 1999) or after various environmental perturbations like dinoflagellate bloom events (Fandino et al. 2001).

Conscious of the constraints mentioned above and potential PCR biases (e.g. selective/differential amplification, (von Wintzingerode et al. 1997) also affecting final ratios of PCR amplicons (Suzuki and Giovannoni 1996), quantitative interpretations using relative DGGE band intensities should be done rather cautiously (discussed in Schauer et al. 2003).
For that reason I never attempted to give absolute values for the presence of the different phylogenetic groups, and for comparison of community similarity I conservatively focused mainly on the presence and absence of bands, although integrating relative values did not

change general patterns (not shown). Nevertheless, in order to further elucidate community dynamics, the additional consideration of relative OTU frequencies can be useful.

Meanwhile, many studies include relative band frequencies (Massana et al. 2001, Massana and Jürgens 2003, Schauer et al. 2003, Larsen 2004), argue that according to the high reproducibility of DGGE fingerprints (Schauer et al. 2000) changes in band intensities are likely due to relative changes in the abundances of the corresponding populations. Furthermore, several authors have shown a correlation between the abundance of morphologically distinct organisms and the corresponding DGGE signal e.g. (Nübel et al. 1999 and some even yielded better correlations with environmental variables (Muylaert et al. 2002).

Before all analyses, I reduced potential biases as much as possible and highly standardised the method (equal template amount in PCR, equal amount of PCR product in DGGE, automated image analysis, multi-band-standard in the gel), which I expect to make the analyses of relative frequency changes over time and along gradients more reliable.

Richness, diversity and taxonomic composition

Although total microbial diversity is estimated to be very high, a compilation of current research demonstrated the consistent finding of a low or moderate diversity in the marine environment (Pinhassi and Hagström 2000, Hagström et al. 2002). Samples of pelagic bacterioplankton mostly yielded astonishingly low richness estimates and small numbers of dominant taxa for a given time and space, at least when fingerprinting and no cloning approaches were applied.

The number of OTUs detected in spring and summer mesocosm experiments corresponds well to richness values documented by studies from other marine systems (Riemann and Middelboe 2002, Massana and Jürgens 2003), Larsen 2004,12 bands). Many investigations from coastal marine habitat report 2-15 numerically dominant bacterial taxa (in Pinhassi and Hagström 2000). Also values for Shannon's diversity index H´ of 1.8-2.4 in spring and 2.1-2.4 (1.3-2.3 in appendicularian mesocosms) in summer were well within the range described from other aquatic habitats. While values were slightly lower than those reported from the north-western Mediterranean coast (2.5–3.1; Schauer et al. 2000) and higher than diversity indices reported from a seasonal study in a eutrophic lake (0.2–0.9; Höfle et al. 1999, based on direct analysis of 5S rRNA), they perfectly matched with other data for Norwegian coastal waters (1.8 to 2.4, Larsen 2004, based on DGGE).

The taxonomic/phylogenetic community composition found in the experiments could be considered typical for a coastal pelagic habitat. Dominant bacterial OTU groups in the experiments affiliated with the *Cytophaga-Flavobacterium* cluster of the *Bacteroidetes* (see Eilers et al. 2001) (27-28% mean relative frequency along spring and summer copepod

gradients) as well as the alpha-(26% spring, 38% summer) and gamma- (26% spring, 20% summer) Proteobacteria. Only a minor proportion of the communities was related to other groups like Actinobacteria and beta-Proteobacteria, showing higher frequencies in spring (3.2%, 11%) than in summer (1.4%, 4.7%), respectively.

Alpha-Proteobacteria comprise one of the largest fractions of heterotrophic marine bacteria (Hagström et al. 2002) and their dominant clades SAR11 and *Roseobacter* were found to be an abundant and ecologically important bacterioplankton component in coastal and near surface waters (Gonzalez and Moran 1997, Acinas et al. 1999, Morris et al. 2002, Selje et al. 2004). Also within North Sea bacterioplankton assemblages alpha-Proteobacteria were a predominant group (Zubkov et al. 2001, 2002). These were cells with high nucleic acid and high protein content were mainly affiliated to the genus *Roseobacter*.

OTUs related to the *Roseobacter* lineage were also dominant members of all assemblages in spring and summer. OTUs 5 and 16 (99-100% similarity with *Roseobacter* NAC11-3) could be even assigned to the newly discovered RCA (*Roseobacter*-clade-affiliated) cluster (Selje et al. 2004).

In terms of community composition, I found a higher average proportion of alpha-Proteobacteria under relatively nutrient-poor conditions in summer. Similar evidence comes from Pinhassi and Hagström (2000), who discovered a significant contribution of certain alpha-Proteobacteria (*Sphingomonas* and *Caulobacter* species) possessing the capacity to successfully cope with oligotrophic growth conditions and low phosphate concentrations. These community differences could indicate a different physiological predisposition of the dominant bacteria in both seasons probably leading to the observed differences when comparing spring and summer community dynamics.

Bacterial community patterns – stability versus dynamics

The overall qualitative changes within the investigated bacterial communities were comparably small and a high proportion of OTUs was present in all treatments thus indicating a stable bacterial community very robust towards changes in food web configurations and grazing pressure.

When comparing relative increases and decreases of bacterial diversity with those of microbial food web parameters in highest and lowest copepod density treatments between start and termination of the experiment (after 1week), amplitudes were remarkably smaller in terms of diversity in both spring and summer (Tab.5). Relative changes in abundances and activity (means of enclosure replicates) varied most strongly in spring (ciliates: –99 to +85%, HNF: –82 to +88%, bacteria: +1 to +46%, bacterial thymidine uptake: –33 to +188%) and were often even larger between the start and the midst of the experiment. Contrastingly, average bacterial diversity increases over this period were less distinct (approximately 32%).

As one central outcome of DGGE profile analysis, community stability was even enhanced in summer, showed further reduced amplitude in terms of community similarity and bacterial diversity increase (+5.4 to +7.2%) and thereby corresponded to a numerically less dynamic and less productive bacterial population (+1 to +30%, thymidine uptake –37 to +10%) and less fluctuations at the level of protists (ciliates: –7 and –98%, HNF: –17 to +32%; Chapter III).

		time period	Relative changes [%] CIL	HNF	BACT	THY	H´
SPRING	**L1**	**day 0 - 3**	+40	-24	-51	-73	+28
		day 0 - 6	+85	-82	+46	+188	+32
	L4	**day 0 - 3**	-99	+93	+48	-36	+28
		day 0 - 6	-99	+88	+0.4	-33	+33
SUMMER	**Cop1**	**day 1 - 4**	-7	-58	-0.8	-45	+9.7
		day 1 - 7	-37	-17	+30	+10	+7.2
	Cop5	**day 1 - 4**	-93	+73	-39	-66	+5.4
		day 1 - 7	-98	+48	+1	-37	+5.4

Table 5:
Relative changes [%] of important food web parameters described in chapter III (ciliates, heterotrophic nano-flagellates, bacterial abundance and thymidine uptake) in comparison to changes in bacterial diversity (Shannon index H´) over different time intervals.

A considerable proportion of OTU diversity increase in both seasons could be attributed to non-bacterial OTUs (plastid DNA), concurrent with a strong increase in autotrophic nanoflagellates (chapter III). The reason for a more dampened density dependent response of the summer bacterial community could result from a pre-adaptation to higher ambient copepod and thus nanoplankton abundances, but also reduced nutrient availability. The overall community stability might also be interpreted as a consequence of the fact that the best adapted bacterial taxa with a certain ecological flexibility already prevailed from the beginning and conditions were not changed drastically enough by experimental manipulations to provide niches for new species, thereby contrasting to previous freshwater experiments with *Daphnia* (Jürgens et al. 1999a, Langenheder and Jürgens 2001).

These findings of a fairly robust bacterial assemblage are consistent with the notion of only moderate changes within the bacterial community during the spring bloom (Norwegian coast, Larsen 2004) and a minimal temporal (48 h) and small scale spatial (a few km) variation in community composition of Mediterranean waters (Acinas et al. 1999). In a study in Danish coastal waters Riemann and Middelboe (2002) found stable microbial community compositions and suggested that changes in microbial abundance and activity were not associated with successions in bacterial communities discernible by the applied DGGE protocol. However, contrasting to respective experiments with freshwater microbial communities, recent chemostat experiments with North Sea microbial assemblages, containing bacteria and bacterivorous protists (Massana and Jürgens 2003), have demonstrated that protist grazing did not result in significant changes in BCC.

Furthermore, in seawater enclosures Larsen et al. (2001) observed that the total bacterial number decreased markedly during the flagellate bloom but the community composition was maintained and the diversity remained high, as found in the highest density copepod treatments. With respect to the impact of higher trophic levels, Zubkov and Lopez-Urrutia (2003) also found a robustness of the bacterioplankton community structure in short-term experiments. The presence of both crustacean and gelatinous mesozooplankton did not affect bacterioplankton composition (as analysed by group-specific oligonucleotide probes) but showed opposite effects on bacterioplankton growth. This stability is in contrast to many food web manipulation effects in freshwater (e.g. Šimek et al. 1999, Jürgens et al. 1999a).

Nevertheless, despite a marked qualitative stability (mainly in summer) I revealed copepod-mediated time- as well as density-dependent bacterial community changes and dynamics. Thereby, it can not be completely excluded that indirect and non-predatory effects (DOC release by copepods, fishing of controls) might have stimulated bacterial growth or contributed to community changes.

Interestingly, two aspects of community dynamics and treatment-dependent changes were distinguishable, on one hand the degree to which communities separated dependent on copepod density after one week of experiment, on the other hand the extent to which assemblages changed in the first half of the experiment (diversity maintenance or switch-back).

In terms of community similarity, the highest density copepod treatments (L4, S4, Cop5) were always most separated after one week of experiment, over time and across gradients. Furthermore, a higher level of diversity was found in those treatments, that at the same time contained highest numbers of HNF grazers. Diversity increase was slower but persisted longest in those highest density mesocosms. Mainly in spring, very high HNF densities (and potentially high diversity of HNF feeding strategies) caused lower bacterial abundances and might have stimulated bacterial diversity by avoiding the predominance of single species. So, this could suggest that the predatory cascade from high copepod to low ciliate and high HNF abundances triggered microbial community changes.

In general, temporal dynamics and relative incline in diversity with diversity peaks on day 3 (4) were higher in controls and lowest density treatments in both seasons. Here, high ciliate and very low HNF abundances prevailed (and in addition potential stimulation by copepod fishing in controls and DOC by copepods). Concomitant with a clear temporal diversity increase in lowest density treatments and controls I observed even pronounced inclines in production and very high single-cell activities of bacteria in all experiments, probably indicating fast growing cells to take advantage of a potentially good substrate supply (grazing-derived DOC) and constantly low HNF abundances (see chapter III). Especially for the summer experiment with high ambient natural copepod abundances in the Fjord (28-55

ind.L^{-1}, Saage 2003), one might assume that an experimental initial reduction in copepod densities in lowest density treatments and controls caused a stronger disturbance and larger changes in grazing pressure regime than an increase in copepod density. Few other studies determined whether the phylogenetic succession is accompanied by detectable changes in metabolic activity both at the bulk community level and at the level of single cells as at present it is still difficult to assess the level of activity of the various phylogenetic groups directly (del Giorgio and Bouvier 2002).

Moreover, the present study revealed differential and heterogeneous (increase/decrease) responses of community subgroups. Correlations with other community parameters and environmental variables thus give support to other recent studies that found a covariance of bacterioplankton composition and environmental variables and a partitioning of ecological roles at the genus or species level (Stepanauskas et al. 2003). Horner-Devine et al. (2003) showed for the first time certain response patterns of richness of whole freshwater bacterial subgroups to a gradient of primary productivity, with a U-shaped relationship for alpha- and a hump-shaped one for beta-Proteobacteria. In contrast, Zubkov et al. (2002) found that the distribution of bacterioplankton groups did not correlate with the distribution of either chlorophyll *a* (chl *a*), or phytoplankton groups or physical parameters such as temperature and salinity. The analyses revealed various correlations between the relative frequencies of OTU subgroups to gradients of HNF-grazing resulting, for example, in a dominance of some alpha-Proteobacteria (mainly *Roseobacter*-affiliated OTUs) under very high HNF densities (e.g. L4 mesocosms). This could be an indication for a reduced susceptibility to HNF grazing. Other investigations also found evidence for selective grazing on taxonomically different components of the bacterial community and alpha-Proteobacteria showing less grazing susceptibility (Lebaron et al. 2001b, Beardsley et al. 2003).

However, it is still hard to discriminate between the ultimate factors of bacterial community shifts and to obtain an even better resolution of the interaction of factors governing the distribution of bacterial taxa. So, correlations remain speculative to a certain extent and trends along copepod gradients might not only be a result of direct grazing, but of course might be influenced additionally by the occurrence of certain taxa of algae, the abundance of host-specific viruses or the selective favouring of bacterial subgroups by in- or decreasing substrate diversity and concentrations of inorganic nutrients and DOC/DON excreted by copepods. More information on the phenotypic and physiological properties of the different bacterial taxa is needed to understand their dynamics.

Aspects of predominance – *Oikopleura dioica* versus copepods

This study not only provided clear evidence for changes in bacterial community composition and diversity due to copepod-triggered trophic cascades, but furthermore also for bacterial

assemblage alterations caused by a community shifts at higher trophic levels from appendicularian to copepod dominance. The observed blooming event of the appendicularian *Oikopleura dioica* reached its maximum on experimental day 9 and persisted until day 12, resulting in a strong reduction of bacterial abundance and production (see chapter III). The initial species-poor community increased in abundance until day 9, paralleled by increasing numbers and sizes of *Oikopleura*.

During this first period, *Oikopleura* only weakly affected BCC as shown by high similarity in NMDS analysis. Besides, some increase in Shannon's diversity index H´ and an increase in evenness until the peak of the appendicularian bloom was observed, indicating that filter-feeding zooplankton could enhance community homogeneity. These results obtained from the first experimental week could therefore provide larger-scale field evidence for a weak impact of *Oikopleura dioica* on BCC, supporting findings from short-term bottle experiments (Zubkov and Lopez-Urrutia 2003).

The second period between days 9 and 12, was characterized by a marked shift in mesozooplankton community. While the *Oikopleura* population already decreased, copepod increases from below 10 to approx. 30 per liter (Sommer et al. 2003) were noted. This experimental phase caused considerable alterations in BCC (see Figs.8, 9) with a further increase in diversity and new bacterial taxa, which became detectable and gained in relative frequency. Probably a combination of underlying mechanisms interacts, which are difficult to resolve. Still high numbers of now larger *Oikopleura* individuals caused a significant quantitative reduction of bacteria and could have affected bacterial diversity by favouring fast growing bacteria, maybe supported by a continuous increase in the percentage of HNA bacteria (see chapter III). On the other hand, increasing copepods reduced appendicularian and ciliate grazing pressure and potentially provided more substrate diversity. Subsequent changes (until day 18) were comparably small again, characterized mainly by an increase in chloroplast-derived OTUs due to increasing densities in autotrophic nanoplankton.

A very similar phenomenon became obvious under nutrient-enriched conditions, and patterns in BCC change could be linked to succession periods of mesozooplankton (Fig. 10). Microbial community shifts turned out to be most pronounced during the transition period of strongly increasing copepods, whereas striking changes in *Oikopleura* density caused no alterations of similar intensity. This could suggest that copepods more distinctly shape microbial community composition by reducing ciliate predators and by indirectly enhancing HNF densities.

Despite the same initial nutrient addition, bacterial community shifts differed according to deviating mesozooplankton successions. However, the degree to which nutrient regime accounted for bacterial community development remained speculative. Øvreås et al. (2003) found only moderate changes within the bacterial community in response to manipulation of

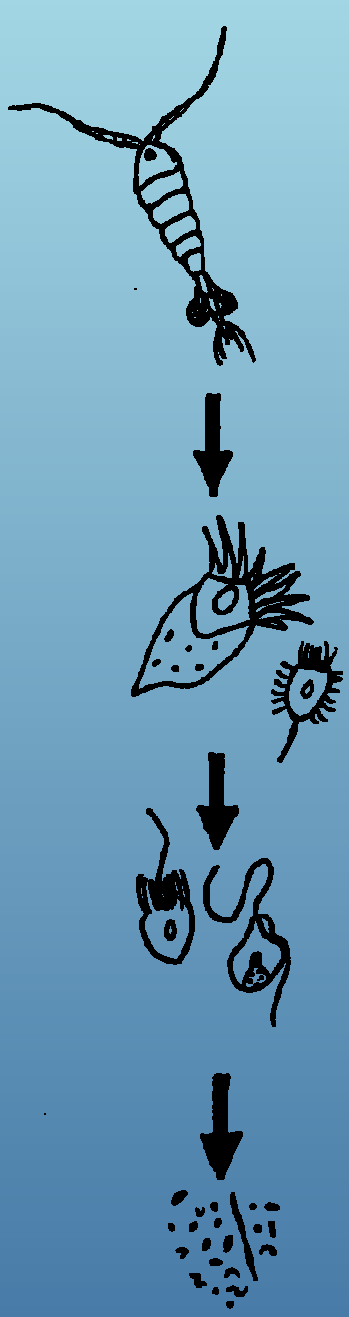
inorganic nutrients, which became more pronounced when glucose was added additionally. Also protein addition can trigger marked community changes (Pinhassi et al. 1999). Nevertheless, changes in relative frequencies of some dominant OTUs were much more intense under enriched conditions (EUAPP, Fig.11) than in non-enriched mesocosms with *Oikopleura*, although both mesocosm sets had comparable background densities of copepods during the first week before strong copepod inclines (on average approx. 8 ind.L^{-1}). Possibly, high and even increasing densities of ciliates (data not shown) could have been responsible for changes in EUAPP during that period. Alternatively, an earlier appearance of *Oikopleura dioica* in the non-eutrophicated mesocosms, compared with the delayed bloom in EUAPP (roughly 2 weeks after start), could have exerted an equalising effect on the bacterial community allowing for only very modest community changes.

In general, bacterioplankton communities proved quite well-buffered across a wide gradient of food web scenarios, but at the same time several temporal and copepod density-dependent trends in trophically induced changes of BCC were elucidated in the present study. Bacterial community shifts could be assigned to changes in mesozooplankton configurations and copepod-mediated cascading effects became visible at bacterial level and caused increasing trends in taxa richness and diversity. Correlations with food web parameters were observed and differential responses noted in various bacterial taxa. Finally, according to more pronounced food web dynamics in spring also bacterial communities displayed larger response amplitudes.

Up to now, this study comprises the most detailed investigations in this field and enables to judge changes within the bacterial community in relation to trophic interactions.
Further examinations of the degree of interaction between top-down and bottom-up determinants are needed in order to understand the linkage between zooplankton-induced bacterial community shifts, bacterial substrate utilisation patterns and dynamics at the species level and their implications for functional redundancy and biogeochemical cycles on a larger scale.

V

Chapter 5



Trophic cascades

–

Synoptical remarks

Trophic cascades affect aquatic microorganisms

- Synoptical remarks -

ABSTRACT

Microorganisms (bacteria, fungi, protists) rule the most important processes and matter fluxes within the ocean as well as in lakes, and their biogeochemical activity mediates element cycling and climate on a global scale (Cotner and Biddanda 2002, del Giorgio and Duarte 2002). Even though recent research in aquatic microbial ecology has been successful in identifying key players and novel metabolic pathways in bacterial communities, the impact of biotic interactions on microbial assemblages is only poorly understood. The importance of top-down regulation and cascading trophic interactions for primary producers and herbivores has been demonstrated in marine and freshwater systems (Brett and Goldman 1996, Micheli 1999). Here I show that similar mechanisms are effective in structuring aquatic heterotrophic microbial communities. Mesocosm experiments, performed during different seasons at one freshwater and one marine site, revealed that copepods, as a dominant zooplankton group, trigger a community-level trophic cascade which strongly affects the microbial food web structure. Despite variability in the physico-chemical conditions and the dominating copepod species, a similar general pattern could be observed which included an inverse relationship of mesozooplankton to microzooplankton (ciliates) and an inverse relationship of microzooplankton to nanoplankton (bacterivorous flagellates). The copepod predation impact propagated even to the bacterial level and affected bacterial abundance, production and diversity in all systems. However, the bacterial response was more dampened and variable between the different experiments, indicating the existence of stabilising feed-back mechanisms.

INTRODUCTION

Within aquatic pelagic habitats, predation of mesozooplankton on microbial food web organisms can be considered the most direct form of their interaction. In various studies, the importance of filter-feeding cladocerans, and here mainly of the genus *Daphnia*, for top-down regulation of lower trophic levels has been emphasised (e.g. Jürgens 1994, Langenheder and Jürgens 2001, Degans et al. 2002). Also in the present thesis, the trophic effects of daphnids on ciliates, nanoflagellates, auto- and heterotrophic bacterioplankton were analyzed in detail. Moreover, I investigated their ability to affect bacterial composition, activity patterns, substrate turnover and even their potential to suppress bloom events of filamentous bacteria and thus extended the view of their overall impact (Chapter I and II).

Analogous to their freshwater counterparts, marine filter-feeders like the appendicularian *Oikopleura dioica* (Chapter III) proved to significantly reduce at least the picoplankton size-class. Due to their rather small body size and a background population of simultaneously feeding copepods (see Chapter III) their impact on protists was difficult to estimate, but probably not as effective as freshwater cladocerans. Furthermore, appendicularian blooming events are rather short-lived scenarios and their grazing impact thus is not as long-lasting.

In the study of food web interactions, the discovery of trophic cascades has rendered substantial popularity and the concept is still intensely debated in community and ecosystem ecology (Shurin et al. 2002, Chase 2003). A trophic cascade, as one of the most prominent indirect interactions, can be defined as the inverse patterns of abundance or biomass across more than one trophic link in a food web (Pace et al. 1999). Trophic cascades have first been described for marine intertidal (Paine 1980) and lake plankton communities (Carpenter et al. 1985) but are meanwhile documented for a variety of marine and terrestrial ecosystems from the tropics to the Arctic (Pace et al. 1999). Generally, carnivores, herbivores and primary producers are involved in trophic cascades. Prominent examples in which food web effects propagate across four trophic levels were documented for freshwater lakes where piscivorous fish reduce planktivores which in turn allow abundant large-size zooplankton to control phytoplankton biomass and improve water clarity (Carpenter et al. 1985). Besides community organisation, trophic cascades and food web structures can impact on a variety of ecosystem functions such as community respiration and total ecosystem production (Carpenter et al. 2001, Pace et al. 1999).

The importance of cascades is, however, quite variable among different ecosystems and within a given ecosystem trophic interactions do not always cascade (Strong 1992). This is also true for the pelagic fish-zooplankton-phytoplankton food chain, for which the largest number of studies exist. Meta-analysis of published experiments suggested that cascading effects of fish on phytoplankton are uncommon in marine pelagic (Micheli 1999) but common

in freshwater plankton (Brett and Goldman 1996). Predatory effects might not cascade due to buffering mechanisms such as omnivory, substitutability of species and development of inedible prey (Strong 1992, Persson 1999). Moreover, the strength of trophic cascades may decrease along a gradient of productivity (Chase 2003). To account for the heterogeneity within trophic levels, recent studies differentiate trophic cascades into “species cascades”, whereby changes in predators affect only a subset of the community, and “community-level cascades” in which the biomass of whole trophic levels is changed (Polis 1999). Only community-level cascades are expected to cause significant changes in biomass distribution and effects on ecosystem processes. It is argued that such “true” trophic cascades are rather the exception than the rule, and are found mainly in aquatic ecosystems (Power 1992, Strong 1992).

Trophic cascades should be of particular importance when microorganisms are affected because their activity mediates most of the important biogeochemical processes. Heterotrophic and autotrophic prokaryotes constitute a significant biomass in planktonic ecosystems and have been recognized as an integral component of aquatic food webs. Multiple trophic levels (bacteria, autotrophic picoplankton, flagellates, ciliates) are organised within a microbial food web, in which a major part of planktonic nutrient recycling, respiration and production is taking place (e.g. Blackburn et al. 1996, Rivkin and Legendre 2001, del Giorgio and Duarte 2002. The main predators on planktonic prokaryotes are small heterotrophic and mixotrophic protists (flagellates, ciliates) which in turn are grazed by metazoan zooplankton. Therefore it is conceivable that cascading predatory effects can propagate from mesozooplankton via protists to bacteria. Such a cascade should have profound effects on the general structure and function of microbial communities because bacterial assemblages respond rapidly to changes in predation regime by shifts at the taxonomical, morphological and physiological level.

Copepods constitute the majority of the mesozooplankton in the oceans and temporarily also in many lakes, and they are the most important link between phytoplankton and fish production in marine systems. Although copepods depend mainly on phytoplankton as prey, they are generally omnivorous and have evolved mechanosensory systems to detect and efficiently capture motile prey such as ciliates (Tiselius and Jonsson 1990, Kiørboe 1997). It has been demonstrated in various systems that copepod populations have the capability to control planktonic ciliates, thereby linking the microbial food web to higher trophic levels (Stoecker and Capuzzo 1990, Levinsen et al. 2000, Zöllner et al. 2003). Cascading predation effects exerted by planktonic copepods should be a widespread and important factor for structuring pelagic food webs and influencing microbially mediated processes.

METHODS

In order to examine copepod-mediated trophic cascades a number of mesocosm experiments were performed in one marine and one freshwater system and during two different seasons (spring, summer). The mesozooplankton community was experimentally manipulated in order to obtain a strong dominance of copepods (> 90 % of mesozooplankton biomass) at 4-5 different population densities. In total, 7 independent copepod gradients were analyzed (2 in the lake, 5 in the marine system). Details on experimental set-up, and procedures of sampling and enumeration as well as measurements and DGGE-analyses have been addressed in chapters I and II for the freshwater, and in chapters III and IV for the marine experiments.

RESULTS

Two functionally different trophic levels of the microbial food web were consistently affected by increasing copepod densities: Ciliates of the microzooplankton size fraction (>20 µm) are important omnivorous grazers of pico- and nanoplankton in pelagic systems and often the dominating herbivores in marine plankton (Sherr and Sherr 2002). Heterotrophic nanoflagellates (HNF), mainly in the size range 3-5 µm, are specialized bacterivores and adapted to thrive on suspended planktonic bacteria (Fenchel 1986).

Despite differences in dominating copepod species, different temperatures and nutrient concentrations between the experiments (Tab. 1), a similar, general pattern in the development of the microbial components became visible after about one week of incubation which clearly revealed a community-wide cascade across three trophic levels (Fig. 1, Tab.1).

Microciliates were reduced 2- to 6-fold in freshwater and 10- to 1055-fold in the marine experiments (comparing means of mesocosm replicates with lowest and highest copepod densities after 1 week). In contrast, nanoplanktonic organisms increased significantly with increasing copepod biomass in all experiments. The increase in HNF was quite variable (2–35-fold increase) as well and particularly pronounced in the marine experiments in spring.

Table 1:
Parameters specifying experimental conditions are shown, dominant copepod taxa *Eudiaptomus gracilis* and *E. graciloides* (E), *Temora longicornis* (T) *Centropages* sp. (C) *Pseudocalanus elongatus* (P), *Calanus finmarchicus* (Cf) and *Oithona* sp. (O) are given in the order of numerical importance. In ** (copepods 250-500 µm, S-gradient) the mean proportion of Cf is 59 % with copepodite stages I-III, whereas in * (copepods >500 µm, L-gradient) the mean proportion of Cf is 67 % with copepodite stages >III. Positive (+) or negative (-) effects of copepods are indicated (a). Ranges of dependent parameters (as mean values of replicate enclosures) are shown for lowest – highest copepod density (b). Under (c) the type of regression model is given: linear (1): f=y0+a*x; second order polynomial (2): $f=y0+a*x+b*x^2$; exponential-linear combination (3): f=y0+a*exp(-b*x+c*x). Regressions are further specified as non-significant (n.s., p>0.05) or significant by values of r^2 and *p*. # indicates, that control mesocosms were omitted from regressions, ~ means log-transformed to achieve normality, *** indicates significance when total nanoplankton was used in regression.

Table 1 Copepod impact on ciliates, HNF and bacteria in different aquatic habitats

system		Lake	Lake	Fjord	Fjord	Fjord	Fjord	Fjord
month		May	August	July	April	April	May	May
water temperature °C		11.7-14.5	18.2-20.5	12.6-13.5	7.8-8.4	7.8-8.4	7.8-9.1	7.8-9.1
initial PO_4^{3-} [µg L^{-1}]		0.27 ± 0.21	29.20 ± 1.10	< 2.48	2.96 ± 0.63	2.92 ± 0.44	8.26 ± 0.30	8.44 ± 0.28
initial NO_3^- [µg L^{-1}]		13.58 ± 4.09	1.20 ± 0.84	< 1.82	3.02 ± 1.85	3.00 ± 1.99	41.82 ± 1.87	42.22 ± 2.05
chlorophyll a [µg L^{-1}]		5.18 ± 1.39	0.94 ± 0.19	1.56 ± 0.24	2.14 ± 0.37	2.25 ± 0.35	2.11 ± 0.19	1.96 ± 0.22
nominal densities [ind L^{-1}]		0-80	0-160	0-80	0-8.1	0-27	0-10	0-40
dominant taxa		E	E	T, C, P	Cf *, C, O	Cf **, C, O	Cf, O	Cf, P, T, O
microciliates [cells ml^{-1}]	a	-	-	-	-	-	-	-
	b	2.5-0.45	15-7.2	2.3-0.21	21.1-0.02	12.5-0.2	14.5-0.04	16.4-1.6
	c	1	2	2	1	1	#1	#1
	p	0.001	0.006	0.002	0.005	0.004	0.045	0.040
	R^2	0.67	0.64	0.74	0.64	0.67	0.51	0.53
HNF [x10^3 ml^{-1}]	a	+	+	+	+	+	+	+
	b	4.92-10.29	1.5-3.34	6.30-4.27	0.56-19.41	1.32-10.15	2.03-9.64	1.32-5.76
	c	1	1	no	1	2	1	1
	p	0.004	0.0001	n.s.	<0.0001	<0.0001	<0.0001	0.0005
	R^2	0.59	0.75	***	0.95	0.99	0.88	0.80
Bacteria								
abundance [x10^6 ml^{-1}]	a	+	n.s. (+)	-	-	-	n.s. (-)	-
	b	2.13-3.77	5.12-5.57	4.19-3.77	1.82-1.16	1.75-0.87	0.66-0.41	1.13-0.47
	c	2	/	3	#2	2	/	#1
	p	0.020	/	0.001	0.025	0.016	/	0.029
	R^2	0.58	/	0.85	0.77	0.70	/	0.58
total OTU richness	a	n.s.	n.s.	+	+	+	+	+
	b	14-14	13-13	18-19	13.5-18	13.5-18.5	15-18	15.5-22
	c	/	/	2~	2	1	1	1
	p	/	/	0.0003	0.0029	0.0001	0.0292	0.0006
	R^2	/	/	0.84	0.81	0.85	0.47	0.79
^{3}H-thymidine uptake	a	n.s. (+)	n.s.(+)	-	-	-	n.s.(-)	-
[µg C L^{-1} d^{-1}]	b	0.62-1.46	8.58-15.38	8.84-5.52	11.91-6.83	9.30-3.48	4.87-2.12	13.40-2.12
	c	/	/	3	2	1	/	#1
	p	/	/	0.0012	0.0465	0.0306	/	0.0393
	R^2	/	/	0.85	0.58	0.46	/	0.53

The observed response pattern is in accordance with the trophic cascade theory and a clear evidence for an indirect cascading predation effect of copepods on nanoplankton. Copepods efficiently controlled medium-sized, mainly oligotrichous ciliates, similar as shown in previous studies. Ciliates are voracious feeders on nanoplankton, which can not effectively be ingested by many copepods (Adrian and Schneider-Olt 1999). Thus, due to ciliate grazing pressure relief, nanoplanktonic organisms increased significantly with increasing copepod

density in all experiments. The increase in ciliates and decline in HNF with increasing zooplankton biomass is reflected in a change of the prey-predator ratios between HNF and ciliates as a function of copepod density (Fig.1A). The slopes of the regressions are consistently higher for the marine systems as a result of a stronger reduction of ciliates and a steeper increase in HNF, particularly during the spring experiments. The overall range of this ratio spans more than four orders of magnitude.

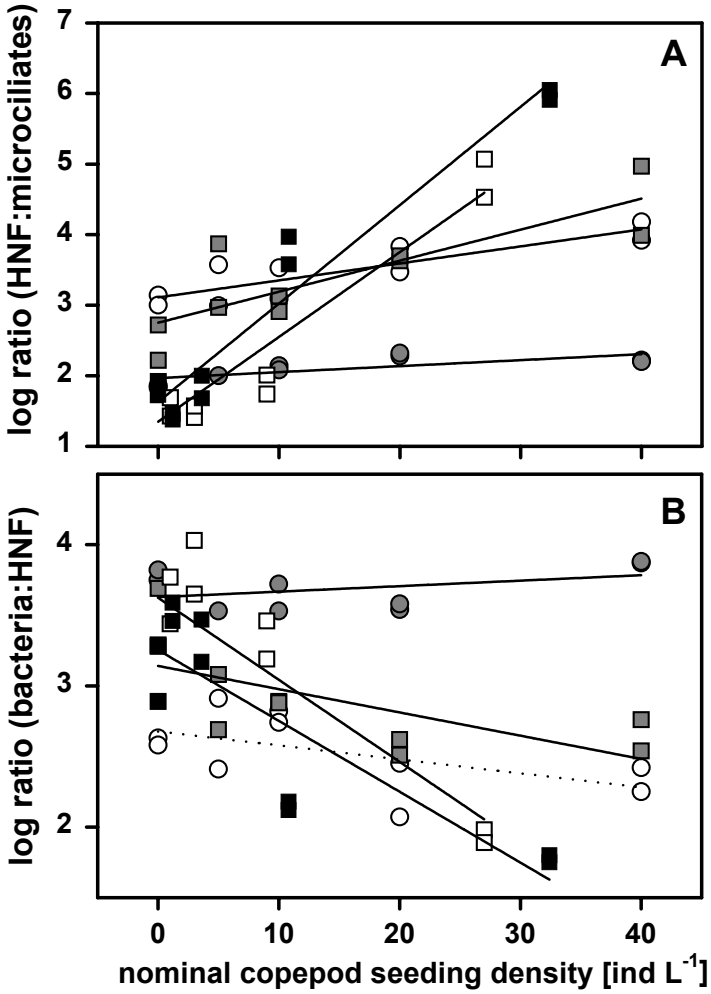


Figure 1:
Logarithmic ratios of heterotrophic nanoflagellates (HNF) to micro-sized ciliates (> 20 µm) (A) and of bacteria to HNF (B) for a copepod density range best overlapping in all investigated systems (0-40 individuals per liter). This range corresponds to naturally occurring copepod densities in the respective systems. In order to standardize scales, nominal densities of the large *Calanus finmarchicus* (0-10 individuals per liter) was taken times four, as its biomass is assumed to be approx. four times higher. Lake data are shown as circles (grey = summer situation, empty = spring situation). Marine examples are presented as squares (grey = summer situation, empty = spring situation, black = *Calanus* gradient, L). May-gradients were excluded for reasons of clarity.

Significant regression functions for (A) are:
Lake (summer): f=1.97+0.01x, R^2=0.55, p<0.05; Lake (spring): f=3.12+0.03x, R^2=0.75, p<0.05, Fjord (summer): f=2.76+0.05x, R^2=0.70, p<0.01; Fjord (spring I, S-gradient): f= 1.35+0.12x, R^2= 0.91, p< 0.0001; Fjord (spring, L-gradient): f=1.63+0.14x, R^2=0.95, <0.0001.
Significant regression functions for (B) are:
Lake (summer): f=3.63+0.004x, R^2=0.16, p>0.05; Lake (spring): f=2.68-0.01x, R^2=0.32, 0.05<p<0.10, Fjord (summer): f=3.14-0.02x, R^2=0.44, p<0.05; Fjord (spring, S-gradient): f= 3.62-0.06*x, R^2= 0.73, p< 0.01; Fjord (spring, L-gradient): f=3.25-0.05x, R^2=0.75, p<0.01.

As HNF are the major grazers on bacteria we should expect that the cascading effects should be transferred to the bacterial level and result in decreasing bacterial biomass. Planktonic bacteria were indeed affected by the trophic interactions but the response was more complex and less uniform. In the freshwater systems bacterial biovolume even increased (1.5 to 2fold) along the copepod gradient. Conversely, in the marine systems

bacterial biomass did either not change (summer experiment) or decrease (spring experiments; 1.6 to 2.4fold) with increasing copepod density, thus indicating an even 4-link trophic cascade in spring. However, due to the strongly increasing HNF numbers, the prey-predator ratios between bacteria and HNF were significantly decreasing along the copepod gradient for the marine experiments whereas they remain rather constant for the lake experiments (Fig. 1B). Due to the dampened response of bacterioplankton the overall range of the values is much more narrow than for the HNF:microciliate ratio.

Changes in mesozooplankton abundance not only affected the numerical response of protists and bacteria but impacted also qualitative features of the bacterial assemblage. Bacterial production as well as bacterial taxa richness consistently changed as a function of copepod density but the effects again differed in the freshwater and the marine mesocosms (Tab.1): While in the lake increasing copepod densities caused a stimulation of bacterial production after 1 week, copepods negatively effected on bacterial uptake rates in the marine systems according to the reduction of bacterial numbers. OTU richness rather increased in the marine assemblages (all OTUs as well as sequenced bacterial OTUs, see Chapter IV) and showed no significant response in the freshwater communities.

DISCUSSION

The uniform pattern in the response of ciliates and HNF to increasing copepod predation suggests that the link between copepods, ciliates and HNF is a true community-level 3-link trophic cascade, common to marine and freshwater pelagic systems (Fig. 2).

Although there is emerging evidence that inter- and intraspecific variation in bacterivorous and herbivorous protists in terms of feeding strategies and differential digestion can be a significant factor for the analysis of food webs and trophic interactions (Weisse 2002), the present study revealed trophic cascades even at the community level. Underlying reasons for such a clear cascade are size-structured predator-prey interactions between meso-, micro- and nanoplankton, a rather simple heterotrophic food chain with functionally different trophic levels, a homogenous habitat and the fact that both ciliates and flagellates are relatively uniformly edible for their predators. Only few protist species have evolved efficient escape or resistance mechanisms (Wiackowski et al. 1994).

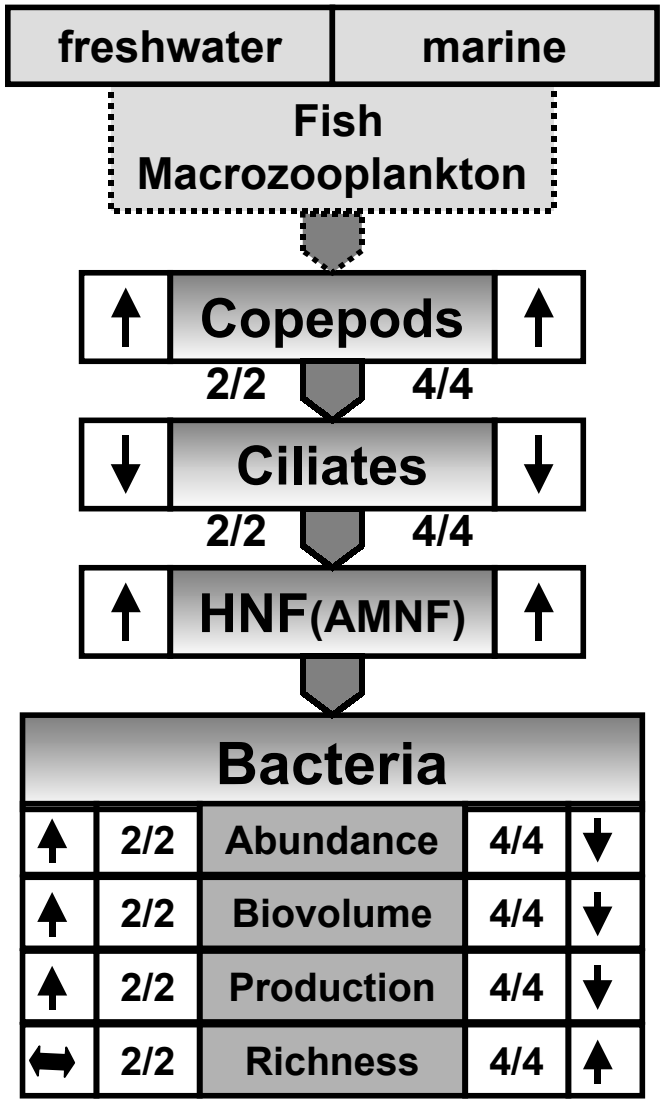


Figure 2:
Conceptual scheme of the copepod mediated trophic cascade: the observed responses (positive/negative) are indicated by arrows in independent marine (4) and freshwater (2) copepod gradients (after 1 week). Numbers indicate in how many of the 2-4 gradients positive or negative results were obtained.

One reason for the less distinct response pattern in bacteria might be the fact that the negative correlation between HNF and ciliates leads to a partial functional compensation at the bacterivory level, which is, in addition to that, a very complex level with many interactions. Since many ciliate taxa (e.g. Scuticociliates, some oligotrichs) are efficient grazers of aquatic bacteria, and under certain conditions their grazing impact on bacteria can exceed that of HNF (Šimek et al. 2000), one can assume a higher proportion of ciliate bacterivory at low copepod densities. This phenomenon of functional compensation was found to hold true also at the level of mesozooplankton as well as phytoplankton (Sommer et al. 2003b).

Another explanation might be that at the level of bacterioplankton the trophic cascade probably changes from a community-wide cascade to one which affects only parts of the assemblage. The response proved to be heterogeneous and more variable among the different experiments (Fig. 2). This is also confirmed by previous studies which demonstrated that predation on bacteria often results in stronger effects on species composition than on bacterial biomass (Jürgens et al. 1999a). Reasons for this are probably the high redundancy among bacterial communities and the potential to develop various resistance mechanisms against protist predation (Jürgens and Matz 2002). Furthermore, other factors such as nutrient and substrate availability might become increasingly important. When the population dynamics of distinct bacterial populations is followed, various species-level cascades might become apparent instead of a uniform trophic-level response. Similar to phytoplankton communities compensatory mechanisms might have resulted in the truncation of a true cascade at the bacterial level.

These results have important implications for our understanding of the regulation of pelagic microbial communities. The composition and activity of bacterial assemblages is not only a reflection of the actual substrate supply but also mediated by strong food web interactions which cascade from the highest to the lowest trophic levels of heterotrophic plankton organisms. The diversity and physiological potentials at the bacterial level are those which most strongly affect ecosystem processes such as nutrient uptake and mineralization rates, element and energy transfer, community respiration and organic matter decomposition. Whether the observed shifts in bacterial species richness and production due to predatory cascades imply also alterations in these biogeochemical processes depends on the functional overlap of the different coexisting species.

Zusammenfassung & Summary

SUMMARY

Mesozooplankton are a dominant component in freshwater and marine systems and directly link phytoplankton and the microbial food web with higher trophic levels like macrozooplankton and fish. Copepods, cladocerans and appendicularians as main mesozooplankton groups differ in terms of feeding selectivity and foraging strategy (filter-feeder versus selective particle catcher). From this circumstance, the main underlying hypothesis of the present thesis arose. Alterations in composition and densities of mesozooplankton should exert different direct and indirect trophic effects on freshwater and marine microbial food webs. This was investigated by experimental manipulation of mesozooplankton density and composition in five large-scale mesocosm experiments carried out in spring and summer in a mesotrophic lake (Schöhsee, Plön) and a fully marine site (Trondheim Fjord, Norway). The mesocosm experiments were accompanied by short-term bottle experiments in which grazing rates and DOC degradation were examined.

I analyzed the effects on microbial food webs by applying a variety of methods, such as epifluorescence and inverted microscopy, automated image analysis and flow cytometry. Furthermore, I determined bacterial uptake rates of radiolabeled substrates and protist grazing on fluorescently labeled bacteria. Bacterial community composition was analysed by Fluorescence-In-Situ-Hybridisation (FISH) and PCR-DGGE combined with sequencing.

I noted temporal and density-dependent structuring effects of mesozooplankton grazing on the microbial food webs of the investigated systems. The main results which were derived from the different mesocosm experiments are summarised in the following:

A) Lake, summer experiment

- The summer lake experiment revealed strong structuring effects of mesozooplankton on the planktonic microbial food webs with clear differences in the predation impact between the two zooplankton guilds cladocerans (*Daphnia* spp.) and copepods.
- The reduction of all ciliate groups, nanoflagellates and bacteria in *Daphnia*-treatments confirmed the important role of this unselective filter-feeder for controlling micro-, nano and picoplankton simultaneously.
- Selective grazing by copepods caused a clear trophic cascade via ciliates to nanoplankton: the proportional reduction of medium-sized (mainly oligotrichous) ciliates with increasing copepod biomass resulted in an increase of phototrophic and heterotrophic nanoflagellates and of nanociliates (prostomatides).
- Bacterial biomass and activity was either reduced (*Daphnia* treatments) or increased (copepod-treatments) with increasing zooplankton biomass; bacterial community

composition proved rather robust and it needed two weeks of experimental duration to see a treatment-coupled similarity clustering of the DGGE banding pattern.

B) Lake, spring experiment

- Like in the summer experiment, copepods triggered a trophic cascade, reduced micro-sized ciliates and enabled HNF to increase. Along a copepod density gradient, bacterial abundance and activity increased, whereas bacterial diversity was not significantly affected.
- In a longer-term study of a temporal succession of spring microbial food webs, *Daphnia* again top-down-regulated protists and bacteria and was identified as the main reason for the termination of a temporary bloom of filament-forming bacteria.
- The mass development of filamentous bacteria was likely favoured by selective protist grazing, but strongly influenced by other factors being discussed.
- By means of a newly designed oligonucleotide probe, up to 98% of all filaments could be assigned to the cosmopolitan LD2 cluster (LD2-739, almost identical 16S rRNA gene sequence types, belonging to the *Cytophaga-Flavobacteria*).
- These bacteria temporarily comprised more than 40% of total bacterial biomass, thereby likely constituting the most extreme case of dominance by a single bacterioplankton species ever reported from natural freshwaters. LD2-739 turned out to be relatively less susceptible to filter-feeding cladocerans.

C) Fjord, summer experiment

- Copepods likewise mediated a 3-link trophic cascade, significantly reduced all size classes of ciliates and thus gave rise to nanoplankton increases. This cascading predation pattern could be reproduced under nutrient enriched conditions, with even increased densities of mainly phototrophic nanoflagellates. Compared to picoplankton, these were selectively favoured by nutrient additions.
- Copepods triggered density-dependent patterns in bacterial DNA content, cell-specific activity and the proportion of actively respiring cells (CTC-positive).
- In systems with highest copepod densities and thus highest HNF abundances, indications of bacterial feedback mechanisms were observed.
- Bacterial community composition turned out to be generally stable. Nevertheless, with increasing copepod density, bacterial assemblages displayed increasing trends in diversity (Shannon's index H´) and OTU (operational taxonomic unit) richness and exhibited density-dependent patterns in overall community similarity.

- Temporal dynamics in bacterial diversity were most pronounced in mesocosms without copepods or low copepod densities, whereas communities under highest copepod densities showed most lasting shifts.
- Prominent bacterial community shifts could be assigned to periods with strongly increasing copepod densities.
- A temporary blooming event of the appendicularian *Oikopleura dioica* substantially reduced bacterial abundance and production (3- to 5-fold), but caused only very modest changes in bacterial community composition. Negative effects on protists were difficult to interpret due to treatment contamination with copepods.

D) Fjord, spring experiments

- Density gradients of both small copepods and larger copepods (>500 µm), dominated by the zooplankton key species *Calanus finmarchicus,* mediated 4-link trophic cascades. They caused substantial ciliate reductions and generated subsequent strong inclines in heterotrophic and phototrophic nanoplankton.
- Trophic impact of copepods altered protist grazing rates on bacteria and thus affected picoplanktonic assemblages manifold. Thereby, relative changes in microbial food web components and rates ranged from –99% to +188%.
- Treatments with high HNF densities provided indications of reduced grazing susceptibility in bacteria.
- According to more pronounced food web dynamics in spring in terms of abundances and activity, also bacterial community composition and diversity displayed larger response amplitudes than in summer. Again, copepods caused increasing trends in bacterial diversity and OTU richness.
- As in summer, eukaryotic OTUs contributed to a certain extent to deviations in community profiles, and higher temporal dynamics were registered in mesocosms without copepods or lowest copepod densities. Treatments with highest copepod abundances deviated with a generally elevated diversity and more persistent composition changes.
- Diverse trends and correlations between food web parameters and bacterial OTUs were observed. OTUs with a similarity to *Roseobacter*-turned out to be dominant members of the communities found under high copepod and thus HNF densities.

E) General patterns and differences between limnic and marine systems

- The comparison of 7 independent experimentally generated copepod density gradients in the investigated freshwater and marine systems yielded, despite considerable differences in the biotic and abiotic start conditions, a general pattern of microbial food web structuring.
- The different copepod communities investigated in both limnic and coastal marine food webs displayed a distinct preference for larger-sized prey items.
- This size-dependent food choice of copepods resulted in mostly community-level trophic cascades leading to a significant reduction of ciliate abundances and substantial increases in nanoplankton densities, sometimes comprising orders of magnitude. Thus, ciliates turned out to be an important component in copepod diet and a significant link between pico- and nanoplankton and higher trophic levels in all systems.
- Changes at mesozooplankton level cascaded down to bacterioplankton and triggered changes in bacterial abundance, activity, respiration, substrate turnover as well as phenotypic and genotypic community composition. Thereby, bacterial community composition showed an overall robustness towards changes at higher trophic levels in freshwater and marine food webs, although comparably large alterations in abundance and activity took place.
- In all systems, I observed a marked heterogeneity at the picoplankton level, likely accountable for most of the registered differences between the investigated limnic and marine food webs. Dampening mechanisms such as grazing resistance against protists occurred in bacterioplankton and bottom-up factors became increasingly important with trophic distance from the copepods.
- While the copepod-mediated predation cascade led to positive responses in freshwater bacterial communities with respect to biomass and activity, respective cascading effects caused negative trends in marine bacterioplankton. This fact as well as different modes of bacterial grazing resistance (filaments in the lake, small, curved and potentially highly motile bacteria in the fjord) might point at diverging stoichiometric constraints encountered within the different systems.
- Although temporary blooms of filter-feeding appendicularians can impose substantial negative impact on bacterioplankton biomass and activity, a trophic role similar to that of *Daphnia* in freshwater could not be attributed to *Oikopleura* in this study.

These changes in the structure of freshwater and marine microbial food webs mediated by strong trophic interactions and depending on alterations of mesozooplankton density and composition should have important implications for energy and matter fluxes in pelagic food webs.

ZUSAMMENFASSUNG

Mesozooplankton stellt eine dominierende Komponente in limnischen und marinen pelagischen Systemen dar sowie eine direkte Verknüpfung von Phytoplankton und mikrobiellem Nahrungsnetz mit höheren trophischen Ebenen wie Makrozooplankton und Fischen. Copepoden, Cladoceren und Appendikularien als wichtigste Mesozooplanktongruppen unterscheiden sich hinsichtlich ihrer Fraßselektivität und der Strategie ihres Nahrungserwerbs (Filtrierer, selektiver Beute-/Partikelgreifer). Aus dieser Tatsache ergab sich die dieser Arbeit zugrundeliegende Kernhypothese. Änderungen in der Zusammensetzung und Dichte des Mesozooplanktons sollten unterschiedliche direkte und indirekte trophische Effekte auf limnische und marine mikrobielle Nahrungsnetze ausüben.

Dieses wurde mittels experimenteller Manipulation der Mesozooplanktondichte und –zusammensetzung in fünf groß-dimensionierten Mesokosmos-Experimenten untersucht, die im Frühling und Sommer in einem mesotrophen See (Schöhsee, Plön) und einem voll marinen Standort (Trondheimsfjord, Norwegen) stattfanden. Die Mesokosmos-Experimente wurden begleitet von Kurzzeit-Flaschenexperimenten, in denen Fraßraten und DOC-Abbau untersucht wurden. Die Effekte auf mikrobielle Nahrungsnetze habe ich mittels einer Anzahl verschiedener Methoden analysiert, wie der Epifluoreszenz- und Umkehr-Mikroskopie, automatischer Bildanalyse und Durchfluss-Zytometrie. Weiterhin habe ich bakterielle Aufnahmeraten von radioaktiv markierten Substraten bestimmt sowie Fraßraten von Protisten mittels der Aufnahme fluoreszenzmarkierter Bakterien. Die Zusammensetzung der Bakteriengemeinschaft wurde mit Fluoreszenz-In-Situ-Hybridisierung (FISH) sowie einer Kombination von PCR-DGGE und Sequenzierung analysiert.

Ich habe zeitliche und dichteabhängige strukturierende Effekte der Mesozooplankton-Fraßaktivität (grazing) auf die mikrobiellen Nahrungsnetze der untersuchten Systeme festgestellt. Die wichtigsten aus den Experimenten stammenden Ergebnisse sind im folgenden zusammengefaßt:

A) See, Sommer-Experiment

- Das Sommer-Experiment im See brachte starke strukturierende Effekte des Mesozooplanktons auf das mikrobielle Nahrungsnetz zum Vorschein, mit deutlichen Unterschieden im Prädationseinfluss der beiden Zooplankton-Gilden Cladoceren (*Daphnia* spp.) und Copepoden.
- Die Reduktion aller Ciliatengruppen, von Nanoflagellaten und Bakterien in experimentellen Behandlungen („Treatments“) mit Daphnien bestätigte die wichtige Rolle dieses unselektiven Filtrierers für die gleichzeitige Kontrolle von Mikro-, Nano- und Pikoplankton.

- Selektiver Fraß der Copepoden verursachte eine deutliche trophische Kaskade über Ciliaten zum Nanoplankton: die entsprechende Reduktion mittelgroßer (hauptsächlich oligotricher) Ciliaten mit zunehmender Copepodenbiomasse resultierte in einem Anstieg von phototrophen und heterotrophen Nanoflagellaten sowie von Nanociliaten (Prostomatiden).
- Die bakterielle Biomasse wurde mit ansteigender Zooplankton-Biomasse entweder reduziert (Daphnien-Treatments) oder nahm zu (Copepoden-Treatments); die Zusammensetzung der Bakteriengemeinschaft erwies sich als eher robust and benötigte eine Experiment-Dauer von zwei Wochen, um eine der experimentellen Behandlung entsprechende Ähnlichkeitsgruppierung des DGGE-Bandenmusters erkennen zu lassen.

B) See, Frühjahrs-Experiment

- Wie im Sommer-Experiment, lösten die Copepoden ein trophische Kaskade aus, reduzierten Mikro-Ciliaten und ermöglichten es HNF zuzunehmen. Entlang des Copepoden-Dichtegradienten stiegen Bakterienabundanz und –aktivität an, wogegen die Bakteriendiversität nicht signifikant beeinflusst wurde.
- In einer längerfristigen Studie zur Sukzession mikrobieller Nahrungsnetze im Frühjahr, wurde eine top-down-Regulation von Protisten und Bakterien durch Daphnien festgestellt und Daphnien als Hauptursache für die Beendigung einer Blüte filamentbildender Bakterien ausgemacht.
- Diese Massenentwicklung filamentöser Bakterien wurde wahrscheinlich begünstigt durch selektiven Protozoenfraß, aber auch stark beeinflußt durch andere hier diskutierte Faktoren.
- Mit Hilfe einer neuentwickelten Oligonukleotidsonde konnten bis zu 98% dem kosmopolitisch verbreiteten LD2-Cluster zugeordnet werden (LD2-739, fast identische Typen von 16S rRNA Gensequenzen, zu *Cytophaga-Flavobacteria* gehörend).
- Diese Bakterien stellten zeitweise mehr als 40% der gesamten Bakterienbiomasse und bildeten dabei wahrscheinlich den extremsten Fall von Dominanz einer einzelnen Bakterienart, der jemals aus natürlichen Süßwasserhabitaten berichtet wurde. LD2-739 erwies sich als weniger anfällig gegenüber filtrierenden Cladoceren.

C) Fjord, Sommer-Experiment

- Copepoden vermittelten ebenfalls eine 3-stufige trophische Kaskade, reduzierten signifikant Ciliaten aller Größenklassen und ermöglichten somit starke Nanoplanktonzunahmen. Dieses kaskadenartige Prädationsmuster konnte unter nährstoffangereicherten Bedingungen reproduziert werden, mit sogar noch erhöhten Dichten von vor allem phototrophen Nanoflagellaten. Diese wurden im Vergleich zum Picoplankton selektiv durch die Nährstoffzugaben begünstigt.
- Copepoden lösten ebenfalls dichteabhängige Muster im Hinblick auf Bakterien-DNA-Gehalte, zellspezifische Aktivität und den Anteil aktiv respirierender Zellen (CTC-positiv) aus.
- In Systemen mit den höchsten Copepoden- und somit HNF-Dichten gab es Anzeichen von feedback-Mechanismen bei Bakterien.
- Die Zusammensetzung der Bakteriengemeinschaft stellte sich allgemein als stabil heraus. Dennoch zeigten die Bakteriengesellschaften mit zunehmender Copepodendichte ansteigende Trends bei der Diversität (Shannon Index H´) und der Anzahl der OTUs (operational taxonomic units) und offenbarten dichteabhängige Muster in der allgemeinen Ähnlichkeit der Gemeinschaften.
- Zeitliche Dynamiken der Bakteriendiversität waren stärker ausgeprägt in Mesokosmen ohne oder mit geringen Copepodendichten, wogegen die Bakteriengemeinschaften unter höchsten Copepodendichten nachhaltigste Änderungen zeigten.
- Deutliche Änderungen in der Bakteriengemeinschaft konnten Perioden mit einem starken Anstieg der Copepodendichte zugeordnet werden.
- Eine vorübergehende Massenentwicklung der Appendikularie *Oikopleura dioica* reduzierte Bakterienabundanz und –produktion substanziell (3- bis 5-fach), aber verursachte nur mäßige Änderungen der Zusammensetzung der Bakteriengemeinschaft. Negative Effekte auf Protisten waren aufgrund einer Kontamination der Mesokosmen mit Copepoden schwer zu interpretieren.

D) Fjord, Frühjahrs-Experimente

- Dichtegradienten von sowohl kleinen als auch großen Copepoden (> 500 µm), letztere dominiert von *Calanus finmarchicus*, lösten sogar 4-stufige trophische Kaskaden aus. Sie reduzierten Ciliaten beträchtlich und verursachten daran anschließende starke Zunahmen von heterotrophem und phototrophem Nanoplankton.

- Der trophische Einfluß der Copepoden veränderte den Protozoen-Fraßdruck auf Bakterien und beeinflusste so die Pikoplanktongemeinschaft auf vielfältige Weise. Dabei variierten relative Veränderungen bei Komponenten und Raten innerhalb des mikrobiellen Nahrungsnetzes zwischen –99% und +188%.
- Treatments mit hohen HNF-Dichten lieferten Anzeichen von reduzierter Fraßanfälligkeit bei Bakterien.
- Entsprechend der im Hinblick auf Abundanz und Aktivität stärker ausgeprägten Nahrungsnetz-Dynamik im Frühjahr, zeigte die Bakteriengemeinschaft auch hinsichtlich ihrer Zusammensetzung eine größere Reaktionsamplitude als im Sommer. Abermals verursachten die Copepoden ansteigende Trends bei bakterieller Diversität und OTU-Anzahl.
- Wie auch im Sommer trugen hier eukaryotische OTUs zu einem gewissen Grade zu den Abweichungen in den Gemeinschaftsprofilen bei, und in Mesokosmen ohne Copepoden oder mit geringer Copepodendichte wurde ebenfalls eine stärkere zeitliche Dynamik registriert. Bakteriengemeinschaften in Treatments mit höchster Copepodendichte wichen allgemein am stärksten ab, mit einer generell erhöhten Diversität sowie nachhaltigeren Änderungen der Zusammensetzung.
- Es wurden unterschiedliche Trends und Korrelationen zwischen Nahrungsnetzparametern und Bakterien-OTUs beobachtet. OTUs, die dem Taxon *Roseobacter* zugeordnet wurden, erwiesen sich als wichtige Mitglieder der Bakteriengemeinschaften und hohen Copepoden- und damit auch HNF-Dichten.

E) Allgemeine Muster und Unterschiede zwischen limnischen und marinen Systemen

- Der Vergleich von 7 unabhängigen, experimentell erzeugten Copepoden-Dichtegradienten in den untersuchten limnischen und marinen Systemen ergab, trotz beachtlicher Unterschiede in den biotischen und abiotischen Startbedingungen, ein allgemeines Muster der Strukturierung mikrobieller Nahrungsnetze.
- Die in sowohl limnischen als auch küstennahen marinen Nahrungsnetzen untersuchten unterschiedlichen Copepodengemeinschaften zeigten offensichtlich eine deutliche Präferenz für größere Beute-Partikel.
- Diese größenabhängige Nahrungswahl der Copepoden resultierte meist in einer die gesamte Gemeinschaft umfassenden trophischen Kaskade, führte zu signifikanten Reduktionen der Ciliatenabundanzen und substantiellen Zunahmen der Nanoplanktondichten, die zum Teil mehrere Größenordnungen umfassten. Somit erwiesen sich Ciliaten als wichtige Komponente im Nahrungsspektrum der Copepoden und als bedeutendes Bindeglied zwischen Piko- und Nanoplankton und höhreren trophischen Ebenen in allen Systemen.

- Änderungen auf der Ebene des Mesozooplanktons setzten sich bis auf die Bakterienebene fort und bewirkten Veränderungen der Abundanz, Aktivität, Respiration und Stoffumsetzung sowie der phänotypischen und genotypischen Zusammensetzung. Dabei zeigte die Bakteriengemeinschaftszusammensetzung in limnischen sowie marinen Nahrungsnetzen allerdings eine allgemeine Stabilität gegenüber Änderungen innerhalb der höheren trophischen Ebenen, obwohl vergleichsweise starke Veränderungen bei Abundanz und Aktivität stattfanden.
- In allen Systemen konnte ich eine deutliche Heterogenität innerhalb des Pikoplanktons beobachten, die wahrscheinlich für die meisten der festgestellten Unterschiede zwischen den untersuchten limnischen und marinen Nahrungsnetzen verantwortlich war. Abschwächende Mechanismen wie Fraßresistenz gegenüber Protozoen waren innerhalb des Bakterioplanktons festzustellen, und bottom-up-Faktoren wurden zunehmend wichtig mit trophischer Entfernung zu den Copepoden.
- Während die Copepoden-vermittelte trophische Kaskade die Biomasse und Aktivität der limnischen Bakteriengemeinschaft eher positiv beeinflusste, verursachten entsprechende Kaskadeneffekte negative Trends im marinen Bakterioplankton. Diese Tatsache sowie unterschiedliche Ausprägungsformen von (Protozoen-) Fraßresistenz bei Bakterien (Filamente im See, kleine gebogene und möglicherweise stark bewegliche Bakterien im Fjord) könnten auf die Relevanz voneinander abweichender stöchiometrischer Einschränkungen in den verschiedenen Systemen hindeuten.
- Obwohl vorübergehende Massenentwicklungen von filtrierenden Appendikularien einen beträchtlichen negativen Einfluss auf Bakterienbiomasse und –aktivität ausüben können, konnte *Oikopleura* anhand dieser Studie keine den Daphnien im limnischen Pelagial entsprechende trophische Stellung zugeschrieben werden.

Diese Änderungen in der Struktur von limnischen und marinen mikrobiellen Nahrungsnetzen, die durch starke trophische Interaktionen geprägt wurden und in Abhängigkeit von Änderungen in Mesozooplanktondichte und –zusammensetzung stattfanden, sollten wichtige Implikationen für Energie- und Stoffflüsse in pelagischen Nahrungsnetzen haben.

REFERENCES

Acinas, S. G., Anton, J. and Rodriguez-Valera, F. (1999). Diversity of free-living and attached bacteriain offshore western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. *Applied & Environmental Microbiology* 65 (2), 514-522

Acinas, S. G., Rodriguez-Valera, F. and Pedros-Alio, C. (1997). Spatial and temporal variation in marine bacterioplankton diversity as shown by RFLP fingerprinting of PCR amplified 16S rDNA. *FEMS Microbiology Ecology.* 24 (1), 27-40

Acuña, J. L. (2001). Pelagic tunicates: Why gelatinous? *American Naturalist* 158 (1), 100-107

Acuña, J. L. and Kiefer, M. (2000). Functional response of the appendicularian *Oikopleura dioica*. *Limnology and Oceanography* 45 (3), 608-618

Adrian, R. and Schneider-Olt, B. (1999). Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. *Journal of Plankton Research* 21 (1), 1-16

Adrian, R., Wickham, S. A. and Butler, N. M. (2001). Trophic interactions between zooplankton and the microbial community in contrasting food webs: the epilimnion and deep chlorophyll maximum of a mesotrophic lake. *Aquatic Microbial Ecology* 24 (1), 83-97

Alldredge, A. L. (1981). The Impact of Appendicularian Grazing on Natural Food Concentrations Insitu. *Limnology and Oceanography* 26 (2), 247-257

Amann, R. I., Ludwig, W. and Schleifer, K.-H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews* 59 143-169

Arndt, H. (1993). Rotifers as predators on components of the microbial web (bacteria, heterotrophicflagellates, ciliates) - a review. *Hydrobiologia* 255/256 231-246

Arndt, H., Dietrich, D., Auer, B., Cleven, E.-J., Gräfenhahn, T., Weitere, M. and Mylnikov, A. P. (2000). Functional diversity of heterotrophic flagellates in aquatic ecosystems. *In: Leadbeater, B.S.C. & Green, J.C.: The Flagellates: Unity, diversity and evolution (Systematics Association Special Volumes), pp. 239-268*

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. and Thingstad, F. (1983). The ecological role of water column microbes in the sea. *Marine Ecology Progress Series* 10 257-263

Bathmann, U., Bundy, M. H., Clarke, M. E., Cowles, T. J., Daly, K., Dam, H. G., Dekshenieks, M. M., Donaghay, P. L., Gibson, D. M., Gifford, D. J., Hansen, B. W., Hartline, D. K., Head, E. J. H., Hofmann, E. E., Hopcroft, R. R., Jahnke, R. A., Jonasdottir, S. H., Kiorboe, T., Kleppel, G. S., Klinck, J. M., Kremer, P. M., Landry, M. R., Lee, R. F., Lenz, P. H., Madin, L. P., Paffenhofer, G. A. and et al. (2001). Future marine zooplankton research - a perspective. *Marine Ecology Progress Series* 222 297-308

Beardsley, C., Pernthaler, J., Wosniok, W. and Amann, R. (2003). Are readily culturable bacteria in coastal North Sea waters suppressed by selective grazing mortality? *Applied and Environmental Microbiology* 69 (5), 2624-2630

Bell, R. T. (1993). Estimating Production of Heterotrophic Bacterioplankton via Incorporation ofTritiated Thymidine. *In: Handbook of Methods in Aquatic Microbial Ecology (Eds P.F.Kemp, B.F. Sherr, E.B. Sherr & J.J Cole), Lewis Publishers, Boca Raton, FL* 495-503

Bettarel, Y., Amblard, C., Sime-Ngando, T., Carrias, J. F., Sargos, D., Garabetian, F. and Lavandier, P. (2003). Viral lysis, flagellate grazing potential, and bacterial production in Lake Pavin. *Microbial Ecology* 45 (2), 119-127

Bettarel, Y., Dolan, J. R., Hornak, K., Lemee, R., Masin, M., Pedrotti, M. L., Rochelle-Newall, E., Simek, K. and Sime-Ngando, T. (2002). Strong, weak, and missing links in a microbial community of the NW Mediterranean Sea. *Fems Microbiology Ecology* 42 (3), 451-462

Beutler, M., Wiltshire, K. H., Meyer, B., Moldaenke, C., Luring, C., Meyerhofer, M., Hansen, U. P. and Dau, H. (2002). A fluorometric method for the differentiation of algal populations in vivo and in situ. *Photosynthesis Research* 72 (1), 39-53

Bianchi, M. (1989). Unusual bloom of a star-like prosthecate bacteria and filaments as a consequence of grazing pressure. *Microbial Ecology* 17 (2), 137-142

Björnsen, P. K. (1986). Automatic determination of bacterioplankton biomass by image analysis. *Applied & Environmental Microbiology* 51 (6), 1199-1204

Blackburn, N., Zweifel, U. L. and Hagstrom, A. (1996). Cycling of marine dissolved organic matter .2. a model analysis. *Aquatic Microbial Ecology* 11 (1), 79-90

Boenigk, J., Matz, C., Jürgens, K. and Arndt, H. (2001). Confusing selective feeding with differential digestion in bacterivorous nanoflagellates. *Journal of Eukaryotic Microbiology* 48 (4), 425-432

Bottrell, H. H., Duncan, A., Gliwicz, Z. M., Grygierek, E., Herzig, A., Hillbricht-Ilkowska, A., Kurasawa, H., Larsson, P. and Weglenska, T. (1976). A review of some problems in zooplankton production studies. *Norw. J. Zool.* 24 419-456

Brendelberger, H. (1991). Filter mesh size of cladocerans predicts retention efficiency for bacteria. *Limnology and Oceanography* 36 884-894

Brendelberger, H., Herbeck, M., Lang, H. and Lampert, W. (1986). Daphnias Filters Are Not Solid Walls. *Archiv Fur Hydrobiologie* 107 (2), 197-202

Brett, M. T. and Goldman, C. R. (1996). A meta-analysis of the freshwater trophic cascade. *Proceedings of the National Academy of Sciences of the United States of America* 93 (15), 7723-7726

Brett, M. T. and Goldman, C. R. (1997). Consumer versus resource control in freshwater pelagic foodwebs. *Science* 275 384-386

Brett, M. T., Wiackowski, K., Lubnow, F. S., Müller-Solger, A., Elser, J. J. and Goldman, C. R. (1994).Species-dependent effects of zooplankton on planktonic ecosystem processes in Castle Lake, California. *Ecology* 75 2243-2254

Broglio, E., Johansson, M. and Jonsson, P. R. (2001). Trophic interaction between copepods and ciliates: effects of prey swimming behavior on predation risk. *Marine Ecology-Progress Series* 220 179-186

Burns, C. W. and Gilbert, J. J. (1993). Predation on ciliates by freshwater calanoid copepods: Rates of predation and relative vulnerabilities of prey. *Freshwater Biology* 30 (3), 377-393

Burns, C. W. and Schallenberg, M. (1996). Relative impacts of copepods, cladocerans and nutrients on the microbial food web of a mesotrophic lake. *Journal of Plankton Research* 18 (5), 683-714

Burns, C. W. and Schallenberg, M. (1998). Impacts of nutrients and zooplankton on the microbial food web of an ultra-oligotrophic lake. *Journal of Plankton Research* 20 (8), 1501-1525

Burns, C. W. and Schallenberg, M. (2001a). Calanoid copepods versus cladocerans: Consumer effects on protozoa in lakes of different trophic status. *Limnology and Oceanography* 46 (6), 1558-1565

Burns, C. W. and Schallenberg, M. (2001b). Short-term impacts of nutrients, Daphnia, and copepods on microbial food-webs of an oligotrophic and leutrophic lake. *New Zealand Journal of Marine and Freshwater Research* 35 (4), 695-710

Calbet, A. (2001). Mesozooplankton grazing effect on primary production: A global comparative analysis in marine ecosystems. *Limnology and Oceanography* 46 (7), 1824-1830

Calbet, A. and Landry, M. R. (1999). Mesozooplankton influences on the microbial food web: Direct and indirect trophic interactions in the oligotrophic open ocean. *Limnology & Oceanography* 44 (6), 1370-1380

Caron, D. A., Goldman, J. C. and Dennett, M. R. (1988). Experimental demonstration of the roles of bacteria and bacterivorous protozoa in plankton nutrient cycles. *Hydrobiologia* 159 27-40

Carpenter, S. R., Cole, J. J., Hodgson, J. R., Kitchell, J. F., Pace, M. L., Bade, D., Cottingham, K. L., Essington, T. E., Houser, J. N. and Schindler, D. E. (2001). Trophic cascades, nutrients, and lake productivity: whole-lake experiments. *Ecological Monographs* 71 (2), 163-186

Carpenter, S. R., Kitchell, J. F. and Hodgson, J. R. (1985). Cascading trophic interactions and lake productivity. *Bioscience* 35 635-639

Carrick, H. J., Fahnenstiel, G. L., Stoermer, E. F. and Wetzel, R. G. (1991). The importance of zooplankton-protozoan trophic couplings in Lake Michigan. *Limnology & Oceanography* 36 (7), 1335-1345

Chase, J. M. (2003). Strong and weak trophic cascades along a productivity gradient. *Oikos* 101 (1), 187-195

Cho, B. C. and Azam, F. (1988). Major role of bacteria in biogeochemical fluxes in the ocean's interior. *Nature* 332 (6163), 441-443

Christaki, U., Jacquet, S., Dolan, J. R., Vaulot, D. and Rassoulzadegan, F. (1999). Growth and grazing on Prochlorococcus and Synechococcus by two marine ciliates. *Limnology and Oceanography* 44 (1), 52-61

Christoffersen, K., Bernard, C. and Ekebom, J. (1996). A comparison of the ability of different heterotrophic nanoflagellates to incorporate dissolved macromolecules. *Ergebnisse der Limnologie* 0 (48), 73-84

Christoffersen, K., Riemann, B., Klysner, A. and Søndergaard, M. (1993). Potential role of fish predation and natural populations of zooplankton in structuring a plankton community in eutrophic lake water. *Limnology & Oceanography* 38 (3), 561-573

Chrzanowski, T. H. and Grover, J. P. (2001). The light : nutrient ratio in lakes: a test of hypothesized trends in bacterial nutrient limitation. *Ecol Letters* 4 (5), 453-457

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

Cochran-Stafira, D. L. and von Ende, C. N. (1998). Integrating bacteria into food webs - studies with *Sarracenia purpurea* inquilines. *Ecology* 79 (3), 880-898.

Connon, S. A. and Giovannoni, S. J. (2002). High-throughput methods for culturing microorganisms in very-low-nutrient media yield diverse new marine isolates. *Applied and Environmental Microbiology* 68 (8), 3878-3885

Cotner, J. B. and Biddanda, B. A. (2002). Small players, large role: Microbial influence on biogeochemical processes in pelagic aquatic ecosystems. *Ecosystems* 5 (2), 105-121

Crump, B. C., Hopkinson, C. S., Sogin, M. L. and Hobbie, J. E. (2004). Microbial Biogeography along an Estuarine Salinity Gradient: Combined Influences of Bacterial Growth and Residence Time. *Applied & Environmental Microbiology* 70 (3), 1494-1505

Crump, B. C., Kling, G. W., Bahr, M. and Hobbie, J. E. (2003). Bacterioplankton community shifts in an arctic lake correlate with seasonal changes in organic matter source. *Applied and Environmental Microbiology* 69 (4), 2253-2268

Curtis, T. P., Sloan, W. T. and Scannell, J. W. (2002). Estimating prokaryotic diversity and its limits. *Proceedings of the National Academy of Sciences of the United States of America* 99 (16), 10494-10499

Daims, H., Bruhl, A., Amann, R., Schleifer, K. H. and Wagner, M. (1999). The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: Development and evaluation of a more comprehensive probe set. *Systematic and Applied Microbiology* 22 (3), 434-444

Dale, T. and Dahl, E. (1987). Mass Occurrence of Planktonic Oligotrichous Ciliates in a Bay in Southern-Norway. *Journal of Plankton Research* 9 (5), 871-879

Degans, H., Zöllner, E., Van der Gucht, K., De Meester, L. and Jürgens, K. (2002). Rapid *Daphnia*-mediated changes in microbial community structure: an experimental study. *Fems Microbiology Ecology* 42 (1), 137-149

Deibel, D. and Lee, S. H. (1992). Retention Efficiency of Submicrometer Particles by the Pharyngeal Filter of the Pelagic Tunicate *Oikopleura vanhoeffeni*. *Marine Ecology-Progress Series* 81 (1), 25-30

DelGiorgio, P. A. and Bouvier, T. C. (2002). Linking the physiologic and phylogenetic successions in free-living bacterial communities along an estuarine salinity gradient. *Limnology & Oceanography* 47 (2), 471-486

DelGiorgio, P. A., Cole, J. J. and Cimbleris, A. (1997). Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* 385 (N6612), 148-151

DelGiorgio, P. A. and Duarte, C. M. (2002). Respiration in the open ocean. *Nature* 420 (6914), 379-384

DelGiorgio, P. A., Gasol, J. M., Vaque, D., Mura, P., Agusti, S. and Duarte, C. M. (1996). Bacterioplankton community structure - protists control net production and the proportion of active bacteria in a coastal marine community. *Limnology & Oceanography* 41 1169-1179

DelGiorgio, P. A., Prairie, Y. T. and Bird, D. F. (1997). Coupling between rates of bacterial production and the abundance of metabolically active bacteria in lakes, enumerated using CTC reduction and flow cytometry. *Microbial Ecology.* 34 (2), 144-154

DelGiorgio, P. A., Bird, D. F., Prairie, Y. T. and Planas, D. (1996). Flow cytometric determination of bacterial abundance in lake plankton with the green nucleic-acid stain syto-13. *Limnology and Oceanography* 41 (N4), 783-789

DeMott, W. R. (1982). Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina*. *Limnology and Oceanography* 27 518-527

DeMott, W. R. (1988). Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnology & Oceanography* 33 (3), 397-408

Diez, B., Pedros-Alio, C., Marsh, T. L. and Massana, R. (2001). Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. *Applied & Environmental Microbiology* 67 (7), 2942-2951

Dolan, J. R. and Simek, K. (1998). Ingestion and digestion of an autotrophic picoplankter, *Synechococcus*, by a heterotrophic nanoflagellate, *Bodo saltans*. *Limnology and Oceanography* 43 (7), 1740-1746

Dollhopf, S. L., Hashsham, S. A. and Tiedje, J. M. (2001). Interpreting 16S rDNA T-RFLP data: Application of self- organizing maps and principal component analysis to describe community dynamics and convergence. *Microbial Ecology* 42 (4), 495-505

Domaizon, and al., e. (2003). Taxon-specific and seasonal variations in flagellates grazing on heterotrophic bacteria in the oligotrophic lake Annecy-importance of mixotrophy. *Fems Microbiology Ecology* 46, 317-329

Ducklow, H. (2000). Bacterial production and biomass in the oceans. *In: Kirchman D (ed) Microbial ecology of the*, 85-120.

Ducklow, H. W., Kirchman, D. L. and Anderson, T. R. (2002). The magnitude of spring bacterial production in the North Atlantic Ocean. *Limnology and Oceanography* 47 (6), 1684-1693

Ducklow, H. W., Kirchman, D. L. and Quinby, H. L. (1992). Bacterioplankton cell growth and macromolecular synthesis in seawater cultures during the North Atlantic spring phytoplankton bloom, May, 1989. *Microbial Ecology* 24 (2), 125-144.

Eilers, H., Pernthaler, J., Peplies, J., Glockner, F. O., Gerdts, G. and Amann, R. (2001). Isolation o novel pelagic bacteria from the German bight and their seasonal contributions to surface picoplankton. *Applied & Environmental Microbiology* 67 (11), 5134-5142

Engström-Öst, J., Koski, M., Schmidt, K., Viitasalo, M., Jonasdottir, S. H., Kokkonen, M., Repka, S and Sivonen, K. (2002). Effects of toxic cyanobacteria on plankton assemblage: community development during decay of *Nodularia spumigena*. *Marine Ecology Progress Series* 232 1-14

Fandino, L. B., Riemann, L., Steward, G. F., Long, R. A. and Azam, F. (2001). Variations in bacteria community structure during a dinoflagellate bloom analyzed by DGGE and 16S rDNA sequencing. *Aquatic Microbial Ecology* 23 (2), 119-130

Fenchel, T. (1986). The ecology of heterotrophic microflagellates. *Advances in Microbial Ecology* 9 57-97

Fenchel, T., Esteban, G. F. and Finlay, B. J. (1997). Local versus global diversity of microorganisms: Cryptic diversity of ciliated protozoa. *Oikos* 80 (2), 220-225

Fernandez, D. and Acuña, J. L. (2003). Enhancement of marine phytoplankton blooms by appendicularian grazers. *Limnology and Oceanography* 48 (2), 587-593

Fernandez, D., Lopez-Urrutia, A., Fernandez, A., Acuna, J. L. and Harris, R. (2004). Retention efficiency of 0.2 to 6 µm particles by the appendicularians *Oikopleura dioica* and *Fritillaria borealis. Marine Ecology Progress Series* Vol. 266: 89–101

Ferrari, V. C. and Hollibaugh, J. T. (1999). Distribution of microbial assemblages in the Central Arctic Ocean Basin studied by PCR/DGGE: analysis of a large data set. *Hydrobiologia* 401 55-68

Ferris, M. J. and Palenik, B. (1998). Niche adaptation in ocean cyanobacteria. *Nature* 396 (6708), 226-228

Finlay, B. J. (2002). Global dispersal of free-living microbial eukaryote species. *Science* 296 (5570), 1061-1063

Finlay, B. J., Esteban, G. F. and Fenchel, T. (1998). Protozoan diversity: Converging estimates of the global number of free-living ciliate species. *Protist* 149 (1), 29-37

Finlay, B. J., Maberly, S. C. and Cooper, J. I. (1997). Microbial diversity and ecosystem function. *Oikos* 80 (2), 209-213

Foissner, W. (1999). Protist diversity: Estimates of the near-imponderable. *Protist* 150 (4), 363-368 Foissner, W. (2000). A compilation of soil and moss ciliates (protozoa, ciliophora) from Germany, with new records and descriptions of new and insufficiently known species. *European Journal of Protistology* 36 (3), 253-283

Foissner, W. and Berger, H. (1996). A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater Biology* 35 (2), 375-482

Fuchs, B. M., Wallner, G., Beisker, W., Schwippl, I., Ludwig, W. and Amann, R. (1998). Flow cytometric analysis of the in situ accessibility of Escherichia coli 16S rRNA for fluorescently labeled oligonucleotide probes. *Applied and Environmental Microbiology* 64 (12), 4973-4982

Fuhrman, J. A. (1999). Marine viruses and their biogeochemical and ecological effects. *Nature* 399 (6736), 541-548

Fuhrman, J. A. (2002). Community structure and function in prokaryotic marine plankton. *Antonie van Leeuwenhoek International Journal of General & Molecular Microbiology* 81 (1-4), 521-527

Fuhrman, J. A., Griffith, J. F. and Schwalbach, M. S. (2002). Prokaryotic and viral diversity patterns in marine plankton. *Ecological Research* 17 (2), 183-194

Fuhrman, J. A. and Noble, R. T. (1999). Causative Agents of Bacterial Mortality on the Consequences to Marine Food Webs. *Proceedings of the 8th International Symposium on Microbial Ecology*

Fuhrman, J. A. and Ouverney, C. C. (1998). Marine microbial diversity studied via 16S rRNA sequences: cloning results from coastal waters and counting of native archaea with fluorescent single probes. *Aquatic Ecology* 32 3-15

Fussmann, G. (1996). The importance of crustacean zooplankton in structuring rotifer and phytoplankton communities: An enclosure study. *Journal of Plankton Research* 18 (10), 1897-1915

Gasol, J. M. (1994). A framework for the assessment of top-down vs bottom-up control of heterotrophic nanoflagellate abundance. *Marine Ecology-Progress Series.* 113 (3), 291-300

Gasol, J. M. and Del Giorgio, P. A. (2000). Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities. *Scientia Marina* 64 (2), 197-224

Gasol, J. M., Pedros-Alio, C. and Vaque, D. (2002). Regulation of bacterial assemblages in oligotrophic plankton systems: results from experimental and empirical approaches. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 81 (1-4), 435-452

Gasol, J. M., Zweifel, U. L., Peters, F., Fuhrman, J. A. and Hagstrom, A. (1999). Significance of size and nucleic acid content heterogeneity as measured by flow cytometry in natural planktonic bacteria. *Applied and Environmental Microbiology* 65 (10), 4475-4483

Geller, W. and Müller, H. (1981). The filtration apparatus of Cladocera: Filter mesh-sizes and their implications on food selectivity. *Oecologia* 49 316-321

Gilbert, J. J. (1994). Jumping behavior in the oligotrich ciliates *Strobilidium velox* and *Halteria grandinella*, and its significance as a defense against rotifer predators. *Microbial Ecology* 27 (2), 189-200

Giovannoni, S. J., Britschgi, T. B., Moyer, C. L. and Field, K. G. (1990). Genetic diversity in Sargasso Sea bacterioplankton. *Nature* 35 60-63

Giovannoni, S. J. and Rappé, M. S. (2000). Evolution, Diversity and Molecular Ecology of Marine Prokaryotes. *In: Microbial Ecology of the Oceans (Ed D.L. Kirchman).Jon Wiley & Sons, Inc.* 47-84

Glöckner, F. O., Fuchs, B. M. and Amann, R. (1999). Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Applied and Environmental Microbiology* 65 (8), 3721-3726

Glöckner, F. O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A. and Amann, R. (2000). Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Applied & Environmental Microbiology* 66 (11), 5053-+

Gocke, K. (1977). Comparison of methods for determining the turnover times of dissolved organic compounds. *Marine Biology* 42 (2), 131-142

Gonzalez, J. M. and Moran, M. A. (1997). Numerical dominance of a group of marine bacteria in the alpha-subclass of the class proteobacteria in coastal seawater. *Applied & Environmental Microbiology* 63 (11), 4237-4242

Gonzalez, J. M., Sherr, E. B. and Sherr, B. F. (1990). Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. *Applied & Environmental Microbiology* 56 (3), 583-589

González, J. M., Sherr, E. B. and Sherr, B. F. (1993). Differential feeding by marine flagellates on growing versus starving, and on motile versus nonmotile, bacterial prey. *Mar. Ecol. Progr. Ser.* 102 257-267

Graneli, E. and Turner, J. T. (2002). Top-down regulation in ctenophore-copepod-ciliate-diatom-phytoflagellate communities in coastal waters: a mesocosm study. *Marine Ecology Progress Series* 239 57-68

Grossart, H. P., Riemann, L. and Azam, F. (2001). Bacterial motility in the sea and its ecologica implications. *Aquatic Microbial Ecology* 25 (3), 247-258

Güde, H. (1988). Direct and indirect influences of crustacean zooplankton on bacterioplankton of Lake Constance. *Hydrobiologia* 159 63-73

Guixa-Boixereu, N., Vaque, D., Gasol, J. M. and Pedros-Alio, C. (1999). Distribution of viruses and their potential effect on bacterioplankton in an oligotrophic marine system. *Aquatic Microbial Ecology* 19 (3), 205-213

Hagström, Å., Pommier, T., Rohwer, F., Simu, K., Stolte, W., Svensson, D. and Zweifel, U. L. (2002). Use of 16S ribosomal DNA for delineation of marine bacterioplankton species. *Applied and Environmental Microbiology* 68 (7), 3628-3633

Hahn, M. W. and Hofle, M. G. (2001). Grazing of protozoa and its effect on populations of aquatic bacteria. *Fems Microbiology Ecology* 35 (2), 113-121

Hahn, M. W., Lunsdorf, H., Wu, Q. L., Schauer, M., Hofle, M. G., Boenigk, J. and Stadler, P. (2003). Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater habitats in Europe and Asia. *Applied and Environmental Microbiology* 69 (3), 1442-1451

Hahn, M. W., Moore, E. R. B. and Höfle, M. G. (1999). Bacterial filament formation, a defense mechanism against flagellate grazing, is growth rate controlled in bacteria of different phyla. *Applied & Environmental Microbiology* 65 (1), 25-35

Hansen, A. M. (2000). Response of ciliates and Cryptomonas to the spring cohort of a cyclopoid copepod in a shallow hypereutrophic lake. *Journal of Plankton Research* 22 (1), 185-203

Hansen, B. W., Hygum, B. H., Brozek, M., Jensen, F. and Rey, C. (2000). Food web interactions in a *Calanus finmarchicus* dominated pelagic ecosystem - a mesocosm study. *Journal of Plankton Research* 22 (3), 569-588

Havskum, H., Thingstad, T. F., Scharek, R., Peters, F., Berdalet, E., Sala, M. M., Alcaraz, M., Bangsholt, J. C., Zweifel, U. L., Hagstrom, A., Perez, M. and Dolan, J. R. (2003). Silicate and labile

DOC interfere in structuring the microbial food web via algal-bacterial competition for mineral nutrients: Results of a mesocosm experiment. *Limnology and Oceanography* 48 (1), 129-140.
Höfle, M. G., Haas, H. and Dominik, K. (1999). Seasonal dynamics of bacterioplankton community structure in a eutrophic lake as determined by 5S rRNA analysis. *Applied & Environmental Microbiology* 65 (7), 3164-3174
Holmquist, L. and Kjelleberg, S. (1993). Changes in viability respiratory activity and morphology of the marine vibrio-sp strain s14 during starvation of individual nutrients and subsequent recovery. *Fems* Microbiology Ecology 12 (4), 215-223
Hoppe, H. G. (1993). Use of Fluorogenic Model Substrates for Extracellular Enzyme Activity (EEA) Measurement of Bacteria. *In: Handbook of Methods in Aquatic Microbial Ecology (Eds P.F.Kemp, B.F. Sherr, E.B. Sherr & J.J Cole), Lewis Publishers, Boca Raton, FL* 423-431
Hoppe, H. G., Giesenhagen, H. C. and Gocke, K. (1998). Changing patterns of bacterial substrate decomposition in a eutrophication gradient. *Aquatic Microbial Ecology* 15 (1), 1-13
Horner-Devine, M. C., Leibold, M. A., Smith, V. H. and Bohannan, B. J. M. (2003). Bacterial diversity patterns along a gradient of primary productivity. *Ecology Letters* 6 (7), 613-622
Hugenholtz, P., Goebel, B. M. and Pace, N. R. (1998). Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity (vol 180, pg 4765, 1998). *Journal of Bacteriology* 180 (24), 6793
Huisman, J. and Weissing, F. J. (1999). Biodiversity of plankton by species oscillations and chaos. *Nature* 402 (6760), 407-410
Huisman, J. and Weissing, F. J. (2001). Biological conditions for oscillations and chaos generated by multispecies competition. *Ecology* 82 (10), 2682-2695
Hulot, F. D., Lacroix, G., Lescher-Moutoue, F. O. and Loreau, M. (2000). Functional diversity governs ecosystem response to nutrient enrichment. *Nature* 405 (6784), 340-344
Hygum, B. H., Petersen, J. W. and Søndergaard, M. (1997). Dissolved organic carbon released by zooplankton grazing activity - a high-quality substrate pool for bacteria. *Journal of Plankton Research* 19 97-111
Jack, J. D. and Gilbert, J. J. (1993). Susceptibilities of Different-Sized Ciliates to Direct Suppression by Small and Large Cladocerans. *Freshwater Biology* 29 (1), 19-29
Jack, J. D. and Gilbert, J. J. (1994). Effects of Daphnia on Microzooplankton Communities. *Journal of Plankton Research* 16 (11), 1499-1512
Jacquet, S., Havskum, H., Thingstad, T. F. and Vaulot, D. (2002). Effects of inorganic and organic nutrient addition on a coastal microbial community (Isefjord, Denmark). *Marine Ecology-Progress Series* 228 3-14
Jahnke, R. A. and Craven, D. B. (1995). Quantifying the role of heterotrophic bacteria in the carbon cycle - a need for respiration rate measurements. *Limnology & Oceanography* 40 (2), 436-441
Jannasch, H. W. and Jones, G. E. (1959). Bacterial Populations in Sea Water as Determined by Different Methods of Enumeration. *Limnology and Oceanography* 4 (2), 128-139
Jeppesen, E., Sortkjaer, O., Søndergaard, M. and Erlandsen, M. (1992). Impact of a trophic cascade on heterotrophic bacterioplankton production in two shallow fish-manipulated lakes. *Archiv für Hydrobiologie Beihefte Ergebnisse der Limnologie* 37 219-231
Joint, I., Henriksen, P., Fonnes, G. A., Bourne, D., Thingstad, T. F. and Riemann, B. (2002). Competition for inorganic nutrients between phytoplankton and bacterioplankton in nutrient manipulated mesocosms. *Aquatic Microbial Ecology* 29 (2), 145-159
Jürgens, K. (1994). Impact of *Daphnia* on planktonic microbial food webs - A review. *Marine Microbia Food Webs* 8 (1-2), 295-324
Jürgens, K., Arndt, H. and Rothhaupt, K. O. (1994a). Zooplankton-mediated changes of bacteria community structure. *Microbial Ecology* 27 (1), 27-42
Jürgens, K. and DeMott, W. R. (1995). Behavioral flexibility in prey selection by bacterivorous nanoflagellates. *Limnology and Oceanography* 40 1503-1507
Jürgens, K., Gasol, J. M., Massana, R. and Pedros-Alio, C. (1994b). Control of heterotrophic bacteria and protozoans by *Daphnia pulex* in the epilimnion of Lake Ciso. *Archiv für Hydrobiologie* 131 (1), 55-78
Jürgens, K., Gasol, J. M. and Vaque, D. (2000). Bacteria-flagellate coupling in microcosm experiments in the Central Atlantic Ocean. *Journal of Experimental Marine Biology & Ecology* 245 (1), 127-147
Jürgens, K. and Güde, H. (1991). Seasonal changes in the grazing impact of phagotrophic flagellates on bacteria in lake constance. *Marine Microbial Food Webs* 5 (1), 27-38
Jürgens, K. and Güde, H. (1994). The potential importance of grazing-resistant bacteria in planktonic systems. *Marine Ecology Progress Series* 112 169-188
Jürgens, K. and Jeppesen, E. (2000). The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake. *Journal of Plankton Research* 22 (6), 1047-1070

Jürgens, K. and Matz, C. (2002). Predation as a shaping force for the phenotypic and genotypic composition of planktonic bacteria. *Antonie van Leeuwenhoek International Journal of General & Molecular Microbiology* 81 (1-4), 413-434.

Jürgens, K., Pernthaler, J., Schalla, S. and Amann, R. (1999a). Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. *Applied & Environmental Microbiology* 65 (3), 1241-1250

Jürgens, K., Skibbe, O. and Jeppesen, E. (1999b). Impact of metazooplankton on the composition and population dynamics of planktonic ciliates in a shallow, hypertrophic lake. *Aquatic Microbial Ecology* 17 (1), 61-75

Jürgens, K. and Stolpe, G. (1995). Seasonal dynamics of crustacean zooplankton, heterotrophic nanoflagellates and bacteria in a shallow, eutrophic lake. *Freshwater Biology.* 33 (1), 27-38

Jürgens, K., Wickham, S. A., Rothhaupt, K. O. and Santer, B. (1996). Feeding rates of macro- and microzooplankton on heterotrophic nanoflagellates. *Limnology & Oceanography* 41 (8), 1833-1839

Karner, M., Ferrierpages, C. and Rassoulzadegan, F. (1994). Phagotrophic Nanoflagellates Contribute to Occurrence of Alpha-Glucosidase and Aminopeptidase in Marine Environments. *Marine Ecology-Progress Series* 114 (3), 237-244

Katechakis, A., Stibor, H., Sommer, U. and Hansen, T. (2002). Changes in the phytoplankton community and microbial food web of Blanes Bay (Catalan Sea, NW Mediterranean) under prolonged grazing pressure by doliolids (Tunicata), cladocerans or copepods (Crustacea). *Marine Ecology Progress Series* 234 55-69

Kerkhof, L. J., Voytek, M. A., Sherrell, R. M., Millie, D. and Schohfield, O. (1999). Variability in bacterial community structure during upwelling in the coastal ocean. *Hydrobiologia* 401 139-148

King, C. H., Sanders, R. W., Shotts, E. B., Jr. and Porter, K. G. (1991). Differential survival of bacteria ingested by zooplankton from a stratified eutrophic lake. *Limnology & Oceanography* 36 (5), 829-845

King, K. R., Hollibaugh, J. T. and Azam, F. (1980). Predator-prey interactions between the larvacean *Oikopleura dioica* and bacterioplankton in enclosed water columns. *Marine Biology* 56 49-57

Kiørboe, T. (1997). Population regulation and role of mesozooplankton in shaping marine pelagic food webs. *Hydrobiologia* 363 13-27

Kirchman, D. L. (1993). Leucine incorporation as a measure of biomass production by heterotrophic bacteria. *In: Handbook of Methods in Aquatic Microbial Ecology (Eds P.F.Kemp, B.F. Sherr, E.B. Sherr & J.J Cole), Lewis Publishers, Boca Raton, FL* 509-512

Kisand, V. and Zingel, P. (2000). Dominance of ciliate grazing on bacteria during spring in a shallow eutrophic lake. *Aquatic Microbial Ecology* 22 (2), 135-142

Kleppel, G. S. (1993). On the Diets of Calanoid Copepods. *Marine Ecology-Progress Series* 99 (1-2), 183-195

Kogure, K., Simidu, U. and Taga, N. (1979). Tentative Direct Microscopic Method for Counting Living Marine-Bacteria. *Canadian Journal of Microbiology* 25 (3), 415-420

Koshikawa, H., Harada, S., Watanabe, M., Kogure, K., Ioriya, T., Kohata, K., Kimura, T., Sato, K. and Akehata, T. (1999). Influence of plankton community structure on the contribution of bacterial production to metazooplankton in a coastal mesocosm. *Marine Ecology-Progress Series* 186 31-42

Koshikawa, H., Harada, S., Watanabe, M., Sato, K. and Akehata, K. (1996). Relative contribution of bacterial and photosynthetic production to metazooplankton as carbon-sources. *Journal of Plankton Research* 18 (N12), 2269-2281

Lampert, W., Fleckner, W., Rai, H. and Taylor, B. E. (1986). Phytoplankton control by grazing zooplankton: a study on the clear water phase. *Limnology & Oceanography* 31 478-490

Lampert, W. and Taylor, B. E. (1985). Zooplankton grazing in an eutrophic lake: quantitative and qualitative implications of diel vertical migration. *Ecology* 66 68-82

Langenheder, S. and Jürgens, K. (2001). Regulation of bacterial biomass and community structure by metazoan and protozoan predation. *Limnology & Oceanography* 46 (1), 121-134

Langenheder, S., Kisand, V., Wikner, J. and Tranvik, L. J. (2003). Salinity as a structuring factor for the composition and performance of bacterioplankton degrading riverine DOC. *Fems Microbiology Ecology* 45 (2), 189-202.

Larsen, A. (2004). Spring phytoplankton bloom dynamics in Norwegian coastal waters:... *Limnology and Oceanography* 49 (1), 180-190

Larsen, A., Castberg, T., Sandaa, R. A., Brussaard, C. P. D., Egge, J., Heldal, M., Paulino, A., Thyrhaug, R., van Hannen, E. J. and Bratbak, G. (2001). Population dynamics and diversity of phytoplankton, bacteria and viruses in a seawater enclosure. *Marine Ecology Progress Series* 221 47-57

Laybourn-Parry, J. and Parry, J. (2000). Flagellates and the microbial loop. *In: Leadbeater, B.S.C. & Green, J.C.: The Flagellates: Unity, diversity and evolution (Systematics Association Special Volumes), pp. 216-239*

Lebaron, P., Servais, P., Agogue, H., Courties, C. and Joux, F. (2001a). Does the high nucleic acid content of individual bacterial cells allow us to discriminate between active cells and inactive cells in aquatic systems? *Applied & Environmental Microbiology* 67 (4), 1775-1782

Lebaron, P., Servais, P., Troussellier, M., Courties, C., Muyzer, G., Bernard, L., Schafer, H., Pukall, R., Stackebrandt, E., Guindulain, T. and Vives-Rego, J. (2001b). Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in abundances, activity and composition. *Fems Microbiology Ecology* 34 (3), 255-266

Levinsen, H. and Nielsen, T. G. (2002). The trophic role of marine pelagic ciliates and heterotrophic dinoflagellates in arctic and temperate coastal ecosystems: A cross-latitude comparison. *Limnology & Oceanography* 47 (2), 427-439

Levinsen, H., Turner, J. T., T.G., N. and B.W., H. (2000). On the trophic coupling between protists and copepods in arctic marine ecosystems. *Marine Ecology Progress Series* 204 65-77

Li, W. K. W. (1995). Composition of Ultraphytoplankton in the Central North-Atlantic. *Marine Ecology-Progress Series* 122 (1-3), 1-8

Lindström, E. S. (2000). Bacterioplankton community composition in five lakes differing in trophic status and humic content. *Microbial Ecology* 40 (2), 104-113

Liu, W. T., Marsh, T. L., Cheng, H. and Forney, L. J. (1997). Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16s rRNA. *Applied & Environmental Microbiology* 63 (11), 4516-4522

Long, R. A. and Azam, F. (2001). Antagonistic interactions among marine pelagic bacteria. *Applied & Environmental Microbiology* 67 (11), 4975-4983

Lopez-Urrutia, A., Irigoien, X., Acuna, J. L., Harris, R. (2003). In situ feeding physiology and grazing impact of the appendicularian community in temperate waters.*Marine Ecology-Progress Series* 252:125-141

Lopez-Urrutia, A., Harris, R. P. and Smith, T. (2004). Predation by calanoid copepods on the appendicularian Oikopleura dioica. *Limnology & Oceanography* 49 (1), 303-307

Maar, M., Nielsen, T. G., Richardson, K., Christaki, U., Hansen, O. S., Zervoudaki, S. and Christou, E. D. (2002). Spatial and temporal variability of food web structure during the spring bloom in the Skagerrak. *Marine Ecology-Progress Series* 239: 11-29

Manz, W., Amann, R., Ludwig, W., Vancanneyt, M. and Schleifer, K. H. (1996). Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiology* 142 1097-1106

Maranger, R. and Bird, D. F. (1995). Viral abundance in aquatic systems - a comparison between marine and fresh waters. *Marine Ecology-Progress Series* 121 (1-3), 217-226

Marchessault, P. and Mazumder, A. (1997). Grazer and nutrient impacts on epilimnetic ciliate communities. *Limnology & Oceanography* 42 (5), 893-900

Massana, R., Gasol, J. M., Björnsen, P. K., Blackburn, N., Hagström, A., Hietanen, S., Hygum, B. H., Kuparinen, J. and Pedros-Alio, C. (1997). Measurement of bacterial size via image analysis of epifluorescence preparations - description of an inexpensive system and solutions to some of the most common problems. *Scientia Marina* 61 397-407

Massana, R., Guillou, L., Diez, B. and Pedros-Alio, C. (2002). Unveiling the organisms behind novel eukaryotic ribosomal DNA sequences from the ocean. *Applied & Environmental Microbiology* 68 (9), 4554-4558

Massana, R. and Jürgens, K. (2003). Composition and population dynamics of planktonic bacteria and bacterivorous flagellates in seawater chemostat cultures. *Aquatic Microbial Ecology* 32 (1), 11-22

Massana, R., Pedros-Alio, C., Casamayor, E. O. and Gasol, J. M. (2001). Changes in marine bacterioplankton phylogenetic composition during incubations designed to measure biogeochemically significant parameters. *Limnology & Oceanography* 46 (5), 1181-1188

Matz, C. (2001). Mechanisms of grazing resistance in aquatic bacteria. *Dissertation, University of Kiel*

Matz, C., Boenigk, J., Arndt, H. and Jürgens, K. (2002). Role of bacterial phenotypic traits in selective feeding of the heterotrophic nanoflagellate *Spumella* sp. *Aquatic Microbial Ecology* 27 (2), 137-148

Matz, C. and Jürgens, K. (2001). Effects of hydrophobic and electrostatic cell surface properties o bacteria on feeding rates of heterotrophic nanoflagellates. *Applied & Environmental Microbiology* 67 (2), 814-820

Matz, C. and Jürgens, K. (2003). Interaction of nutrient limitation and protozoan grazing determinesthe phenotypic structure of a bacterial community. *Microbial Ecology* 45 (4), 384-398

Micheli, F. (1999). Eutrophication, fisheries, and consumer-resource dynamics in marine pelagic ecosystems. *Science* 285 (5432), 1396-1398

Middelboe, M., Hagström, A., Blackburn, N., Sinn, B., Fischer, U., Borch, N. H., Pinhassi, J., Simu, K. and Lorenz, M. G. (2001). Effects of bacteriophages on the population dynamics of four strains of pelagic marine bacteria. *Microbial Ecology* 42 (3), 395-406

Moeseneder, M. M., Arrieta, J. M., Muyzer, G., Winter, C. and Herndl, G. J. (1999). Optimization of terminal-restriction fragment length polymorphism analysis for complex marine bacterioplankton communities and comparison with denaturing gradient gel electrophoresis. *Applied & Environmental Microbiology* 65 (8), 3518-3525

Møller, E. F. and Nielsen, T. G. (2001). Production of bacterial substrate by marine copepods: Effect of phytoplankton biomass and cell size. *Journal of Plankton Research* 23 (5), 527-536

Møller, E. F., Thor, P. and Nielsen, T. G. (2003). Production of DOC by *Calanus finmarchicus*, *C-glacialis* and *C-hyperboreus* through sloppy feeding and leakage from fecal pellets. *Marine Ecology-Progress Series* 262 185-191

Montagnes, D. J. S., Poulton, A. J. and Shammon, T. M. (1999). Mesoscale, finescale and microscale distribution of micro- and nanoplankton in the Irish Sea, with emphasis on ciliates and their prey. *Marine Biology* 134 (1), 167-179

Moon-van der Staay, S. Y., De Wachter, R. and Vaulot, D. (2001). Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. *Nature* 409 (6820), 607-610

Moon-van der Staay, S. Y., van der Staay, G. W. M., Guillou, L., Vaulot, D., Claustre, H. and Medlin, L. K. (2000). Abundance and diversity of prymnesiophytes in the picoplankton community from the equatorial Pacific Ocean inferred from 18S rDNA sequences. *Limnology and Oceanography* 45 (1), 98-109

Moore, L. R., Post, A. F., Rocap, G. and Chisholm, S. W. (2002). Utilization of different nitrogen sources by the marine cyanobacteria Prochlorococcus and Synechococcus. *Limnology and Oceanography* 47 (4), 989-996

Morris, R. M., Rappe, M. S., Connon, S. A., Vergin, K. L., Siebold, W. A., Carlson, C. A. and Giovannoni, S. J. (2002). SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* 420 (6917), 806-810

Müller, H., Schöne, A., Pinto Coelho, R. M., Schweizer, A. and Weisse, T. (1991). Seasonal succession of ciliates in Lake Constance. *Microbial Ecology* 21 (2), 119-138

Murray, A. E., Preston, C. M., Massana, R., Taylor, L. T., Blakis, A., Wu, K. and DeLong, E. F. (1998). Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. *Applied and Environmental Microbiology* 64 (7), 2585-2595

Muylaert, K., Van der Gucht, K., Vloemans, N., De Meester, L., Gillis, M. and Vyverman, W. (2002). Relationship between bacterial community composition and bottom-up versus top-down variables in four eutrophic shallow lakes. *Applied & Environmental Microbiology* 68 (10), 4740-4750

Muyzer, G., Brinkhoff, T., Nübel, U., Santegoeds, C., Schäfer, H. and Wawer, C. (1998). Denaturing gradient gel electrophoresis (DGGE) in microbial ecology. *Molecular Microbial Ecology Manual* 3.4.4 1-17

Muyzer, G., de Waal, E. C. and Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology* 59 695-700

Muyzer, G. and Smalla, K. (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek International Journal of General & Molecular Microbiology* 73 (1), 127-141

Nagata, T. (2000). Production mechanisms of Dissolved Organic Matter. *In: Microbial Ecology of the Oceans (Ed D.L. Kirchman). Jon Wiley & Sons, Inc.* 121-152

Nakamura, Y., Suzuki, K., Suzuki, S. and Hiromi, J. (1997). Production of Oikopleura dioica (Appendicularia) following a picoplankton 'bloom' in a eutrophic coastal area. *Journal of Plankton Research* 19 (1), 113-124

Nee, S. (2003). Unveiling prokaryotic diversity. *Trends in Ecology & Evolution* 18 (2), 62-63

Nübel, U., Garcia-Pichel, F., Kühl, M. and Muyzer, G. (1999). Quantifying microbial diversity: Morphotypes, 16S rRNA genes, and carotenoids of oxygenic phototrophs in microbial mats. *Applied & Environmental Microbiology* 65 (2), 422-430

Ohman, M. D. and Hirche, H. J. (2001). Density-dependent mortality in an oceanic copepod population. *Nature* 412 (6847), 638-641

Olsen, L. M., Reinertsen, H. and Vadstein, O. (2002). Can phosphorus limitation inhibit dissolved organic carbon consumption in aquatic microbial food webs? A study of three food web structures in microcosms. *Microbial Ecology* 43 (3), 353-366

Ouverney, C. C. F., Jed A. (1999). Combined Microautoradiography - 16S rRNA Probe Technique for Determination of Radioisotope Uptake by Specific Microbial Cell Types In Situ. *Applied and Environmental Microbiology* 65 (4), 1746-1752.

Øvreås, L. (2000). Population and community level approaches for analysing microbial diversity in natural environments. *Ecology Letters* 3 (3), 236-251

Øvreås, L., Bourne, D., Sandaa, R. A., Casamayor, E. O., Benlloch, S., Goddard, V., Smerdon, G., Heldal, M. and Thingstad, T. F. (2003). Response of bacterial and viral communities to nutrient manipulations in seawater mesocosms. *Aquatic Microbial Ecology* 31 (2), 109-121

Pace, M. L. and Cole, J. J. (1994). Comparative and Experimental Approaches to Top-Down and Bottom-Up Regulation of Bacteria. *Microbial Ecology* 28 181-193

Pace, M. L. and Cole, J. J. (1996). Regulation of bacteria by resources and predation tested in whole-lake experiments. *Limnology & Oceanography* 41 (7), 1448-1460

Pace, M. L., Cole, J. J., Carpenter, S. R. and Kitchell, J. F. (1999). Trophic cascades revealed in diverse ecosystems. *Trends in Ecology & Evolution* 14 (12), 483-488

Pace, M. L. and Vaqué, D. (1994). The importance of Daphnia in determining mortality rates of protozoans and rotifers in lakes. *Limnology and Oceanography* 39 (5), 985-996

Pace, M. L. F., Elizabeth (1991). Regulation of planktonic microbial communities by nutrients and herbivores. *Ecology* 72 (3), 904-914

Pace, M. L. M., George B.; Findlay, Stuart E.G. (1990). Planktonic community structure determines the fate of bacterial production in a temperate lake. *Limnology and Oceanography* 35 (4), 795-808

Paine, R. T. (1980). Food web linkage, interaction strength and community infrastructure. *Journal of Animal Ecology* 49 667-685

Patterson, D. J. and Lee, W. J. (2000). Geographic distribution and diversity of free-living heterotrophic flagellates. *In: Leadbeater, B.S.C. & Green, J.C.: The Flagellates: Unity, diversity and evolution (Systematics Association Special Volumes), pp. 239-268*

Paul, J. and Myers, B. (1982). Fluorometric determination of DNA in aquatic microorganisms by use of Hoechst 33258. *Applied & Environmental Microbiology* 43 1393-1399

Pernthaler, J., Alfreider, A., Posch, T., Andreatta, S. and Psenner, R. (1997a). *In situ* classification and image cytometry of pelagic bacteria from a high mountain lake (Gossenköllesee, Austria). *Applied & Environmental Microbiology* 63 (12), 4778-4783

Pernthaler, J., Glockner, F. O., Schonhuber, W. and Amann, R. (2001). Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. *Methods in Microbiology, Vol 30, Academic Press, San Diego* 30 207-226

Pernthaler, J., Glöckner, F. O., Unterholzner, S., Alfreider, A., Psenner, R. and Amann, R. (1998). Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. *Applied & Environmental Microbiology* 64 (11), 4299-4306

Pernthaler, J., Posch, T., Simek, K., Vrba, J., Amann, R. and Psenner, R. (1997b). Contrasting bacterial strategies to coexist with a flagellate predator in an experimental microbial assemblage. *Applied and Environmental Microbiology* 63 (2), 596-601

Pernthaler, J., Sattler, B., Simek, K., Schwarzenbacher, A. and Psenner, R. (1996). Top-down effects on the size-biomass distribution of a freshwater bacterioplankton community. *Aquatic Microbial Ecology* 10 (3), 255-263

Pernthaler, J., Zöllner, E., Warnecke, F. and Jürgens, K. (2004). A bloom of filamentous bacteria in a mesotrophic lake: identity and potential controlling mechanism. *submitted to Applied and Environmental Microbiology*

Persson, L. (1999). Trophic cascades: abiding heterogeneity and the trophic level concept at the end of the road. *Oikos* 85 385-397

Pierce, R. W. and Turner, J. T. (1992). Ecology of planktonic ciliates in marine food webs. *Reviews in Aquatic Sciences* 6 (2), 139-181

Pinhassi, J., Azam, F., Hemphala, J., Long, R. A., Martinez, J., Zweifel, U. L. and Hagström, A. (1999). Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquatic Microbial Ecology* 17 (1), 13-26.

Pinhassi, J. and Hagström, A. (2000). Seasonal succession in marine bacterioplankton. *Aquatic Microbial Ecology* 21 (3), 245-256

Polis, G. A. (1999). Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. *Oikos* 86 (1), 3-15

Pomeroy, L. R. (1974). The ocean´s food web, a changing paradigm. *Bioscience* 9 499-504

Posch, T., Jezbera, J., Vrba, J., Simek, K., Pernthaler, J., Andreatta, S. and Sonntag, B. (2001). Size selective feeding in *Cyclidium glaucoma* (Ciliophora, Scuticociliatida) and its effects on bacterial community structure: A study from a continuous cultivation system. *Microbial Ecology* 42 (3), 217-227

Posch, T., Simek, K., Vrba, J., Pernthaler, J., Nedoma, J., Sattler, B., Sonntag, B. and Psenner, R. (1999). Predator-induced changes of bacterial size-structure and productivity studied on an experimental microbial community. *Aquatic Microbial Ecology* 18 235-246.

Power, M. E. (1992). Top-down and bottom-up forces in food webs do plants have primacy? *Ecology* 73 (3), 733-746

Rappé, M. S., Connon, S. A., Vergin, K. L. and Giovannoni, S. J. (2002). Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* 418 (6898), 630-633

Rappé, M. S. and Giovannoni, S. J. (2003). The uncultured microbial majority. *Annual Review of Microbiology* 57 369-394

Rappé, M. S., Vergin, K. and Giovannoni, S. J. (2000). Phylogenetic comparisons of a coastal bacterioplankton community with its counterparts in open ocean and freshwater systems. *Fems Microbiology Ecology* 33 (3), 219-232

Reid, P. C., Turley, C. M. and Burkill, P. H. (1991). Protozoa and their role in marine processes. *Springer Verlag, Heidelberg [NATO ASI 25 Ser G: Ecol Sciences])*

Riemann, B. (1985). Potential importance of fish predation and zooplankton grazing on natural populations of freshwater bacteria. *Applied & Environmental Microbiology* 50 (2), 187-193

Riemann, B. and Christoffersen, K. (1993). Microbial trophodynamics in temperate lakes. *Marine Microbial Food Webs* 7 (1), 69-100

Riemann, L. and Middelboe, M. (2002). Stability of bacterial and viral community compositions in Danish coastal waters as depicted by DNA fingerprinting techniques. *Aquatic Microbial Ecology* 27 (3), 219-232

Riemann, L., Steward, G. F., Fandino, L. B., Campbell, L., Landry, M. R. and Azam, F. (1999). Bacterial community composition during two consecutive NE Monsoon periods in the Arabian Sea studied by denaturing gradient gel electrophoresis (DGGE) of rRNA genes. *Deep-Sea Research Part Ii-Topical Studies in Oceanography* 46 (8-9), 1791-1811

Rivkin, R. B. and Legendre, L. (2001). Biogenic carbon cycling in the upper ocean: Effects of microbial respiration. *Science* 291 (5512), 2398-2400

Saage, A. (2003). Populationsentwicklungen des Zooplankton in Mesokosmosexperimenten. *Diplomarbeit Christian-Albrechts-Universität, Kiel, Germany*

Samuelsson, K. (2003). Mechanisms structuring the pelagic microbial food web. *Dissertation,* Umeå

Samuelsson, K., Berglund, J., Haecky, P. and Andersson, A. (2002). Structural changes in an aquatic microbial food web caused by inorganic nutrient addition. *Aquatic Microbial Ecology* 29 (1), 29-38

Sanders, R. W. and Wickham, S. A. (1993). Planktonic protozoa and metazoa: Predation, food quality and population control. *Marine Microbial Food Webs* 7 (2), 197-223

Sanders, R. W. C., David A.; Berninger, Ulrike-G. (1992). Relationships between bacteria and heterotrophic nanoplankton in marine and fresh waters: an inter-ecosystem comparison. *Marine Ecology Progress Series* 86 1-14

Sarnelle, O. (1993). Herbivore effects on phytoplankton succession in a eutrophic lake. *Ecological Monographs* 63 129-149

Sarnelle, O. (1997). Daphnia effects on microzooplankton: Comparisons of enclosure and whole-lake responses. *Ecology* 78 (3), 913-928

Schäfer, H., Bernard, L., Courties, C., Lebaron, P., Servais, P., Pukall, R., Stackebrandt, E., Troussellier, M., Guindulain, T., Vives-Rego, J. and Muyzer, G. (2001). Microbial community dynamics in Mediterranean nutrient-enriched seawater mesocosms: changes in the genetic diversity of bacterial populations. *Fems Microbiology Ecology* 34 (3), 243-253

Schauer, M., Balague, V., Pedros-Alio, C. and Massana, R. (2003). Seasonal changes in the taxonomic composition of bacterioplankton in a coastal oligotrophic system. *Aquatic Microbial Ecology* 31 (2), 163-174

Schauer, M., Massana, R. and Pedros-Alio, C. (2000). Spatial differences in bacterioplankton composition along the Catalan coast (NW Mediterranean) assessed by molecular fingerprinting. *Fems Microbiology Ecology* 33 (1), 51-59.

Scheffer, M., Rinaldi, S., Huisman, J. and Weissing, F. J. (2003). Why plankton communities have no equilibrium: solutions to the paradox. *Hydrobiologia* 491 (1-3), 9-18

Schwieger, F. and Tebbe, C. C. (1998). A new approach to utilize PCR-single-strand-conformation polymorphism for 16s rRNA gene-based microbial community analysis. *Applied and Environmental Microbiology* 64 (12), 4870-4876

Selje, N., Simon, M. and Brinkhoff, T. (2004). A newly discovered Roseobacter cluster in temperate and polar oceans. *Nature* 427 (6973), 445-448

Setälä, O. and Kivi, K. (2003). Planktonic ciliates in the Baltic Sea in summer: distribution, species association and estimated grazing impact. *Aquatic Microbial Ecology* 32 (3), 287-297

Shannon, C. E. (1948). A Mathematical Theory of Communication. *Bell System Technical Journal* 27 (3), 379-423

Sherr, B. F., del Giorgio, P. and Sherr, E. B. (1999). Estimating abundance and single-cell characteristics of respiring bacteria via the redox dye CTC. *Aquatic Microbial Ecology* 18 (2), 117-131.

Sherr, B. F., Sherr, E. B. and Fallon, R. D. (1987). Use of monodispersed fluorescently labeled bacteria to estimate in situ protozoan bacterivory. *Applied & Environmental Microbiology* 53 (5), 958-965

Sherr, E. B. and Sherr, B. F. (1987). High rates of consumption of bacteria by pelagic ciliates. *Nature* 325 (6106), 710-711

Sherr, E. B. and Sherr, B. F. (1996). Temporal offset in oceanic production and respiration processes implied by seasonal changes in atmospheric oxygen - the role of heterotrophic microbes. *Aquatic Microbial Ecology* 11 (1), 91-100

Sherr, E. B. and Sherr, B. F. (2002). Significance of predation by protists in aquatic microbial food webs. *Antonie van Leeuwenhoek International Journal of General & Molecular Microbiology* 81 (1-4), 293-308

Shikano, S., Luckinbill, L. S. and Kurihara, Y. (1990). Changes of traits in a bacterial population associated with protozoan predation. *Microbial Ecology* 20 75-84

Shurin, J. B., Borer, E. T., Seabloom, E. W., Anderson, K., Blanchette, C. A., Broitman, B., Cooper, S.D. and Halpern, B. S. (2002). A cross-ecosystem comparison of the strength of trophic cascades. *Ecology Letters* 5 (6), 785-791

Sieracki, M. E., Cucci, T. L. and Nicinski, J. (1999). Flow cytometric analysis of 5-cyano-2,3-ditolyl tetrazolium chloride activity of marine bacterioplankton in dilution cultures. *Applied & Environmental Microbiology* 65 (6), 2409-2417

Sime-Ngando, T. (1997). Viruses in aquatic ecosystems - a review. *Annee Biologique* 36 (3), 181-210

Šimek, K. and Chrzanowski, T. H. (1992). Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. *Applied & Environmental Microbiology* 58 (11), 3715-3720

Šimek, K., Jurgens, K., Nedoma, J., Comerma, M. and Armengol, J. (2000). Ecological role and bacterial grazing of Halteria spp.: small freshwater oligotrichs as dominant pelagic ciliate bacterivores. *Aquatic Microbial Ecology* 22 (1), 43-56

Šimek, K., Kojecka, P., Nedoma, J., Hartman, P., Vrba, J. and Dolan, J. R. (1999). Shifts in bacterial community composition associated with different microzooplankton size fractions in a eutrophic reservoir. *Limnology and Oceanography* 44 (7), 1634-1644

Šimek, K., Nedoma, J., Pernthaler, J., Posch, T. and Dolan, J. R. (2002). Altering the balance betweenbacterial production and protistan bacterivory triggers shifts in freshwater bacterial community composition. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology* 81 (1-4), 453-463

Šimek, K., Pernthaler, J., Weinbauer, M. G., Hornak, K., Dolan, J. R., Nedoma, J., Masin, M. and Amann, R. (2001). Changes in bacterial community composition and dynamics and viral mortality rates associated with enhanced flagellate grazing in a mesoeutrophic reservoir. *Applied and Environmental Microbiology* 67 (6), 2723-2733

Šimek, K., Vrba, J., Pernthaler, J., Posch, T., Hartman, P., Nedoma, J. and Psenner, R. (1997). Morphological and compositional shifts in an experimental bacterial community influenced by protists with contrasting feeding modes. *Applied and Environmental Microbiology* 63 (2), 587-595

Sipura, J., Lores, E, Snyder, R.A. (2003). Effect of copepods on estuarine microbial plankton in short-term microcosms. *Aquatic Microbial Ecology* Vol. 33: 181–190

Smith, E. M. (1998). Coherence of microbial respiration rate and cell-specific bacterial activity in a coastal planktonic community. *Aquatic Microbial Ecology* 16 (1), 27-35

Smith, E. M. and Prairie, Y. T. (2004). Bacterial metabolism and growth efficiency in lakes:The importance of phosphorus availability. *Limnology and Oceanography* 49 (1), 137-147

Smits, J. D. and Riemann, B. (1988). Calculation of cell production from tritiated thymidine incorporation with freshwater bacteria. *Applied & Environmental Microbiology* 54 (9), 2213-2219

Sommaruga, R., Krossbacher, M., Salvenmoser, W., Catalan, J. and Psenner, R. (1995). Presence of large virus-like particles in a eutrophic reservoir. *Aquatic Microbial Ecology* 9 (3), 305-308

Sommaruga, R. and Psenner, R. (1995). Permanent presence of grazing-resistant bacteria in a hypertrophic Lake. *Applied & Environmental Microbiology* 61 (9), 3457-3459

Sommer, F. (2003). A comparison of the impact of major mesozooplankton taxa on marine, brackish and freshwater phytoplankton during summer. *Ph.D. Thesis, University of Kiel*

Sommer, F., Hansen, T., Feuchtmayr, H., Santer, B., Tokle, N. and Sommer, U. (2003a). Do calanoid copepods suppress appendicularians in the coastal ocean? *Journal of Plankton Research* 25 (7), 869-871

Sommer, F., Santer, B., Jamieson, C., Hansen, T. and Sommer, U. (2003b). *Daphnia* population growth but not moulting is a substantial phosphorus drain for phytoplankton. *Freshwater Biology* 48 (1), 67-74

Sommer, U., Sommer, F., Santer, B., Jamieson, C., Boersma, M., Becker, C. and Hansen, T. (2001). Complementary impact of copepods and cladocerans on phytoplankton. *Ecology Letters* 4 (6), 545-550

Sommer, U., Sommer, F., Santer, B., Zöllner, E., Jürgens, K., Jamieson, C., Boersma, M. and Gocke, K. (2003b). *Daphnia* versus copepod impact on summer phytoplankton: functional compensation at both trophic levels. *Oecologia* 135 (4), 639-647

Sommer, U. and Stibor, H. (2002). Copepoda-Cladocera-Tunicata: The role of three major mesozooplankton groups in pelagic food webs. *Ecological Research* 17 (2), 161-174

Staley, J. T. and Konopka, A. (1985). Measurement of Insitu Activities of Non-photosynthetic Microorganisms in Aquatic and Terrestrial Habitats. *Annual Review of Microbiology* 39 321-346

Stepanauskas, R., Moran, M. A., Bergamaschi, B. A. and Hollibaugh, J. T. (2003). Covariance of bacterioplankton composition and environmental variables in a temperate delta system. *Aquatic Microbial Ecology* 31 (1), 85-98

Sterner, R. W., Elser, J. J., Fee, E. J., Guildford, S. J. and Chrzanowski, T. H. (1997). The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. *American Naturalist* 150 (6), 663-684

Stibor, H. and Sommer, U. (2003). Mixotrophy of a photosynthetic flagellate viewed from an optimal foraging perspective. *Protist* 154 (1), 91-98

Stoecker, D. K. and Capuzzo, J. M. (1990). Predation on protozoa its importance to zooplankton. *Journal of Plankton Research* 12 (5), 891-908

Strong, D. (1992). Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. *Ecology* 73 747-754

Suttle, C. A. and Chan, A. M. (1993). Marine cyanophages infecting oceanic and coastal strains of Synechococcus: Abundance, morphology, cross-infectivity and growth characteristics. *Marine Ecology Progress Series* 92 (1-2), 99-109

Suzuki, M. T. and Giovannoni, S. J. (1996). Bias caused by template annealing in the amplification of mixtures of 16s rRna genes by PCR. *Applied & Environmental Microbiology* 62 (2), 625-630

Tessier, A. J. and Woodruff, P. (2002). Cryptic trophic cascade along a gradient of lake size. *Ecology* 83 (5), 1263-1270

Thingstad, T. F. (2000a). Control of Bacterial Growth in Idealized Food Webs. *In: Microbial Ecology of the Oceans (Ed D.L. Kirchman). Jon Wiley & Sons, Inc.*

Thingstad, T. F. (2000b). Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnology and Oceanography* 45 (6), 1320-1328

Thingstad, T. F., Heldal, M., Bratbak, G. and Dundas, I. (1993a). Are Viruses Important Partners in Pelagic Food Webs. *Trends in Ecology & Evolution* 8 (6), 209-213

Thingstad, T. F. and Lignell, R. (1997). Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. *Aquatic Microbial Ecology* 13 (1), 19-27

Thingstad, T. F., Skjoldal, E. F. and Bohne, R. A. (1993b). Phosphorus Cycling and Algal-Bacterial Competition in Sandsfjord, Western Norway. *Marine Ecology-Progress Series* 99 (3), 239-259

Tiedje, J. M., Asuming-Brempong, S., Nusslein, K., Marsh, T. L. and Flynn, S. J. (1999). Opening the black box of soil microbial diversity. *Applied Soil Ecology* 13 (2), 109-122

Ting, C. S., Rocap, G., King, J. and Chisholm, S. W. (2002). Cyanobacterial photosynthesis in the oceans: the origins and significance of divergent light-harvesting strategies. *Trends in Microbiology* 10 (3), 134-142

Tiselius, P. and Jonsson, P. R. (1990). Foraging behavior of six calanoid copepods observations and hydrodynamic analysis. *Marine Ecology-Progress Series* 66 (1-2), 23-34.

Torrella, F. and Morita, R. (1981). Microcultural study of bacteria size changes and microcolony and ultramicrocolony formation by heterotrophic bacteria in seawater. *Applied and Environmental Microbiology* 41 518-527

Torsvik, V., Ovreas, L. and Thingstad, T. F. (2002). Prokaryotic diversity - Magnitude, dynamics, and controlling factors. *Science* 296 (5570), 1064-1066

Tuomi, P., Fagerbakke, K. M., Bratbak, G. and Heldal, M. (1995). Nutritional enrichment of a microbial community - the effects on activity, elemental composition, community structure and virus production. *Fems Microbiology Ecology* 16 (2), 123-134

Tuomi, P. and Kuuppo, P. (1999). Viral lysis and grazing loss of bacteria in nutrient- and carbon-manipulated brackish water enclosures. *Journal of Plankton Research* 21 (5), 923-937

Urban, J. L., McKenzie, C. H. and Deibel, D. (1992). Seasonal Differences in the Content of Oikopleura-Vanhoeffeni and Calanus-Finmarchicus Fecal Pellets - Illustrations of Zooplankton Food Web Shifts in Coastal Newfoundland Waters. *Marine Ecology-Progress Series* 84 (3), 255-264.

Vadstein, O. and al., e. (2003). Is phosphorus limitation of planktonic heterotrophic bacteria and accumulation of degradable DOC a normal phenomenon in phosphorus-limited systems ? A microcosm study. *Fems Microbiology Ecology* 46 307-316

van Hannen Erik, J., Van Agterveld Miranda, P., Gons Herman, J. and Laanbroek Hendrikus, J. (1998). Revealing genetic diversity of eukaryotic microorganisms in aquatic environments by denaturing gradient gel electrophoresis. *Journal of Phycology* 34 (2), 206-213

Vanni, M. J. and Temte, J. (1990). Seasonal patterns of grazing and nutrient limitation of phytoplankton in a eutrophic lake. *Limnology & Oceanography* 35 697-709

Vaqué, D. and Pace, M. L. (1992). Grazing on bacteria by flagellates and cladocerans in lakes of contrasting food-web structure. *Journal of Plankton Research* 14 (2), 307-321

Vargas, C. and Gonzalez, H. E. (2004a). Plankton community structure and carbon cycling in a coastal upwelling system. I. Bacteria, microprotozoans and phytoplankton in the diet of copepods and appendicularians. *Aquatic Microbial Ecology* Vol. 34: 151–164

Vargas, C. and Gonzalez, H. E. (2004b). Plankton community structure and carbon cycling in a coastal upwelling system. II. Microheterotrophic pathway. *Aquatic Microbial Ecology* Vol. 34 165–180

Vargas, C. A., Tonnesson, K., Sell, A., Maar, M., Moller, E. F., Zervoudaki, T., Giannakourou, A., Christou, E., Satapoomin, S., Petersen, J. K., Nielsen, T. G. and Tiselius, P. (2002). Importance of copepods versus appendicularians in vertical carbon fluxes in a Swedish fjord. *Marine Ecology-Progress Series* 241 125-138

Verity, P. G. and Smetacek, V. (1996). Organism life cycles, predation, and the structure of marine pelagic ecosystems. *Marine Ecology-Progress Series* 130 (1-3), 277-293

Von Wintzingerode, F., Göbel, U. B. and Stackebrandt, E. (1997). Determination of microbial diversity in environmental samples - pitfalls of PCR-based rRNA analysis. *Fems Microbiology Reviews* 21 (3), 213-229

Vörös, L., Callieri, C., K, V. B. and Bertoni, R. (1998). Freshwater picocyanobacteria along a trophic gradient and light quality range. *Hydrobiologia* 369-370 (0), 117-125

Vrba, J., Nedoma, J., Kohout, L. and al., e. (2003). Massive occurrence of heterotrophic filaments in acidified lakes: seasonal dynamics and composition. *Fems Microbiology Ecology* 46 281-294

Weinbauer, M. G. and Höfle, M. G. (1998). Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. *Applied & Environmental Microbiology* 64 (2), 431-438

Weisse, T. (1997). Growth and production of heterotrophic nanoflagellates in a meso-eutrophic lake. *Journal of Plankton Research* 19 703-722

Weisse, T. (2002). The significance of inter- and intraspecific variation in bacterivorous and herbivorous protists. *Antonie van Leeuwenhoek International Journal of General & Molecular Microbiology* 81 (1-4), 327-341

Weisse, T. and Frahm, A. (2002). Direct and indirect impact of two common rotifer species (Keratella spp.) on two abundant ciliate species (Urotricha furcata, Balanion planctonicum). *Freshwater Biology* 47 (1), 53-64

Whitman, W. B., Coleman, D. C. and Wiebe, W. J. (1998). Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences of the United States of America* 95 (12), 6578-6583

Wiackowski, K., Brett, M. T. and Goldman, C. R. (1994). Differential effects of zooplankton species on ciliate community structure. *Limnology & Oceanography* 39 (3), 486-492

Wickham, S. A. (1995). Trophic Relations between Cyclopoid Copepods and Ciliated Protists - Complex Interactions Link the Microbial and Classic Food Webs. *Limnology and Oceanography* 40 (6), 1173-1181.

Wickham, S. A. (1998). The direct and indirect impact of Daphnia and Cyclops on a freshwater microbial food web. *Journal of Plankton Research* 20 (4), 739-755

Woese, C. (1987). Bacterial evolution. *Microbiol. Rev.* 51 221-271

Wommack, K. E., Ravel, J., Hill, R. T., Chun, J. S. and Colwell, R. R. (1999). Population dynamics of Chesapeake bay virioplankton: Total-community analysis by pulsed-field gel electrophoresis. *Applied & Environmental Microbiology* 65 (1), 231-240

Wu, Q. L., Boenigk, J. and Hahn, M. W. (2004). Successful Predation of Filamentous Bacteria by a Nanoflagellate Challenges Current Models of Flagellate Bacterivory *Applied & Environmental Microbiology* 70 (1), 332-339

Yin, B., Crowley, D., Sparovek, G., De Melo, W. J. and Borneman, J. (2000). Bacterial functional redundancy along a soil reclamation gradient. *Applied and Environmental Microbiology* 66 (10), 4361-4365

Yoshida, T., Gurung, T. B., Kagami, M. and Urabe, J. (2001). Contrasting effects of a cladoceran (Daphnia galeata) and a calanoid copepod (*Eodiaptomus japonicus*) on algal and microbial plankton in a Japanese lake, Lake Biwa. *Oecologia* 129 (4), 602-610

Yu, Z. T. and Mohn, W. W. (2001). Bacterial diversity and community structure in an aerated lagoon revealed by ribosomal intergenic spacer analyses and 16S ribosomal DNA sequencing. *Applied & Environmental Microbiology* 67 (4), 1565-1574

Zöllner, E., Santer, B., Boersma, M., Hoppe, H. G. and Jürgens, K. (2003). Cascading predation effects of Daphnia and copepods on microbial food web components. *Freshwater Biology* 48 (12), 2174-2193

Zubkov, M. V., Fuchs, B. M., Archer, S. D., Kiene, R. P., Amann, R. and Burkill, P. H. (2001). Linking the composition of bacterioplankton to rapid turnover of dissolved dimethylsulphoniopropionate in an algal bloom in the North Sea. *Environmental Microbiology* 3 (5), 304-311

Zubkov, M. V., Fuchs, B. M., Tarran, G. A., Burkill, P. H. and Amann, R. (2002). Mesoscale distribution of dominant bacterioplankton groups in the northern North Sea in early summer. *Aquatic Microbial Ecology* 29 (2), 135-144

Zubkov, M. V. and Lopez-Urrutia, A. (2003). Effect of appendicularians and copepods on bacterioplankton composition and growth in the English Channel. *Aquatic Microbial Ecology* 32 (1), 39-46

Zubkov, M. V., Sleigh, M. A., Burkill, P. H. and Leakey, R. J. G. (2000). Bacterial growth and grazing loss in contrasting areas of North and South Atlantic. *Journal of Plankton Research* 22 (4), 685-711

Zwart, G., Crump, B. C., Agterveld, M. P. K.-v., Hagen, F. and Han, S. K. (2002). Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. *Aquatic Microbial Ecology* 28 (2), 141-155

Zwart, G., Hiorns, W. D., Methe, B. A., Van Agterveld, M. P., Huismans, R., Nold, S. C., Zehr, J. P. and Laanbroek, H. J. (1998). Nearly identical 16S rRNA sequences recovered from lakes in North America and Europe indicate the existence of clades of globally distributed freshwater bacteria. *Systematic and Applied Microbiology* 21 (4), 546-596

DANKSAGUNG - ACKNOWLEDGEMENTS

Eine Reihe von Personen und Institutionen haben mich in unterschiedlicher Weise unterstützt und damit direkt oder indirekt zum Gelingen dieser Doktorarbeit beigetragen. Ihnen allen gilt mein herzlicher Dank.

An erster Stelle möchte ich Prof. Klaus Jürgens sehr herzlich für die Betreuung dieser Doktorarbeit danken, für inspirierende Diskussionen, die freundschaftliche Arbeitsatmosphäre sowie die Freiheit, die er mir bei der Bearbeitung des Themas gelassen hat. Das inspirierende Umfeld seiner Arbeitsgruppe „mikrobielle Ökologie“ (insbesondere in deren goldenen Zeiten mit bis zu 11 Leuten im Labor), seine Unterstützung im Hinblick auf Tagungsbesuche und des Forschungsaufenthaltes in Barcelona und die Tatsache, dass er trotz seines Wechsels zum IOW Warnemünde und eines zeitraubenden neuen Tätigkeitsfeldes immer Zeit für seinen „alten“ Doktoranden gefunden hat, haben sehr zum Gelingen dieser Arbeit beigetragen.

Vielen Dank auch an Prof. Winfried Lampert für die Möglichkeit, im MPI für Limnologie diese Doktorarbeit anzufertigen und die finanzielle Unterstützung von Tagungsbesuchen. Die exzellenten Arbeitsbedingungen und die Förderung der materialaufwendigen Mesokosmos-Experimente und molekularen Methoden haben die Arbeit in dieser Form erst ermöglicht.

Weiterhin gilt ein herzlicher Dank allen Mitgliedern des Mesokosmos-Teams, allen voran dem Projektleiter Prof. Ulrich Sommer und meinen Mitdoktorand(inn)en Heidrun Feuchtmayr, Claes Becker, Daniela Brepohl und Frank Sommer. Ich danke Euch für die gute Zusammenarbeit und die nette Atmosphäre während der Enclosure-Experimente und besonders der Norwegenaufenthalte. Zusammen haben wir über 150 Mesokosmen gebaut und gefüllt sowie unzählige „grosse und kleine Probennahmen“ bei Wind und Wetter durchgestanden. An Heidrun und Frank an dieser Stelle nochmal ein Dankeschön für ihren Einsatz bei der logistischen Abwicklung der Norwegen-Expeditionen.

Einen ganz lieben Dank an meine „Norwegen-Hiwi“ Jennifer Netzer für ihre unermüdliche Hilfe im Labor und ihre ansteckend gute Laune, auch zu ungewöhnlichen Arbeitszeiten.

Ein herzlicher Dank gilt auch Thomas Hansen für die humorvollen Zeiten während der Experimente und des Stationslebens, für die exzellente Nährstoff-Analytik und natürlich die „Trucker-Romantik“ während der gemeinsamen LKW-Fahrten nach Norwegen.

Maarten Boersma, Barbara Santer, Prof. Hans-Georg Hoppe, Andrea Saage und Klaus Gocke danke ich außer für die Zusammenarbeit auch für wertvolle Kommentare zu Manuskripten und Statistik (Maarten) und die Bereitstellung von Rohdaten (Barbara, Andrea, Herr Hoppe). Vielen Dank auch an Jakob Pernthaler (MPI Bremen) für die nette Kooperation und die Bereitstellung von Daten.

Sehr herzlich danken möchte meinem ehemaligen Bürogenossen, Freund und jetzigem Wahlaustralier Carsten Matz für die stets heitere Stimmung in Büro 018a und interessante, nicht nur mikrobendominierte Gespäche. Vielen Dank auch an die anderen Mitglieder der alten und neuen Arbeitsgruppe, Peter Deines, Gundula Eller, Gianluca Corno und Merle Hanke sowie alle anderen, die mich mit Tips, Diskussionen und Aufmunterungen durch meine Doktorandenzeit in Plön begleitet haben.

Herzlich danken möchte ich ansonsten auch allen, die bei der erfolgreichen Durchführung der Probennahmen mitgewirkt und zur guten Arbeitsatmosphäre im „Feld“ und im Labor beigetragen haben. In Plön waren das vor allem Cornelia Burghardt, Heike Paul-Wardenga, Ines Schultz, Regine Koppe (IfM), Ilka Dankert, Nadine Ryk, Claudia Schleker, Colleen Jamieson und Sonja Dembeck. Während der Norwegenexperimente halfen zudem Friederike Funke und Jette Dröse (Sommer) sowie Julia Wohlers und Kerstin Maczassek (Frühjahr).

For interesting and instructive discussions and their hospitality during my stay in Barcelona I would like to thank my friends Ramon Massana and Pep Gasol (both Institut de Ciències del Mar, Barcelona) very much. Many thanks also go to Mike Zubkov (Southampton Oceanography Centre) and Hanne Degans (Laboratory of Aquatic Ecology, Leuven) for inspiration and the nice times during your stays in Plön.

Many thanks also to our Norwegian colleagues Prof. Yngvar Olsen, Andrea Neyts, Nils Tokle, Øystein Leiknes and „Kro-Jon“ for giving diverse valuable help and support during our stays in Norway. I further would like to acknowledge the support by the Trondheim Marine Systems Research Infrastructure and the European Commission´s Improving Human Potential Programme (Projekt COMEZOO) through contract HPRI-1999-CT-00060.

Der Deutschen Forschungsgemeinschaft (DFG) sei an dieser Stelle für die finanzielle Unterstützung (Projekte JU 367/4-1, 4-2) dieser Arbeit gedankt. Für weitere finanzielle Förderung dieser Arbeit sowie von Auslandsaufenthalten danke ich zudem der Max-Planck-Gesellschaft (MPG), dem Consejo Superior de Investigaciones Científicas (CSIC) und der Dr. Robert-Gedächtnisstiftung.

Nicht zuletzt möchte ich meiner Freundin Julia Wohlers und meiner Familie für Ihre nimmermüde Unterstützung und Aufmunterung sehr herzlich danken.

CURRICULUM VITAE

Name: Eckart Zöllner
Geboren: am 30.10.1971 in Kiel, deutsche Nationalität
Familienstand: ledig
Wohnhaft: Brückenstrasse 13, 24306 Plön

Schulausbildung

- Grundschule Bordesholm 1978 - 1982
- Alexander-von-Humboldt-Gymnasium Neumünster 1982 - 1991
- Abitur 1991

Wehrdienst

- Als Sanitäter und LKW-Fahrer 07/1991-07/1992

Studium

- Biologie (Diplom) an der Christian-Albrechts-Universität zu Kiel WS 1992/93
- Vordiplom WS 1994/95
- Hauptstudium: Zoologie (Hauptfach) ab WS 1994/95
  Nebenfächer Limnologie, Bodenkunde
  und Biologische Meereskunde (Zusatzfach)
- Diplomhauptprüfung 06/1998
- Diplomarbeit 07/1998-07/1999
  „*Bodenbiologische Untersuchungen im nordsibirischen Lena-Delta*“ im BMBF-Projekt
  “System Laptev Sea 2000”
- Erlangung des Titels „Diplom-Biologe“ 09/1999
- 1 Semester Fernstudium Umweltingenieurwesen 09/1999-07/2000
  Gutachtertätigkeit für das Umweltamt Neumünster
  Forschungsaufenthalte am Max-Planck-Institut für Limnologie
- Promotion am Max-Planck-Institut für Limnologie 08/ 2000-03/2004

Erklärung

Hiermit erkläre ich an Eides statt, dass die vorliegende Dissertation, abgesehen von der Beratung durch meine akademischen Lehrer, nach Inhalt und Form meine eigene Arbeit ist und ich keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Des weiteren versichere ich, dass die vorliegende Dissertation weder ganz noch zum Teil bei einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegen hat.

Plön, den 17. März 2004 **Eckart Zöllner**

Teilergebnisse dieser Dissertation wurden bereits wie folgt vorab veröffentlicht bzw. eingereicht:

Veröffentlicht:
Kapitel I:

- **Eckart Zöllner**, Barbara Santer, Maarten Boersma, Hans-Georg Hoppe, Klaus Jürgens (2003): Cascading predation effects of *Daphnia* and copepods on microbial food web components. FRESHWATER BIOLOGY 48, 2174–2193

Abgeleitete Datensätze von Kapitel I sind enthalten in:

- Ulrich Sommer, Frank Sommer, Barbara Santer, **Eckart Zöllner,** Klaus Jürgens, Colleen Jamieson, Maarten Boersma, Klaus Gocke (2003): *Daphnia* versus copepod impact on summer phytoplankton: functional compensation at both trophic levels. OECOLOGIA 135:639-647

Eingereicht:

- ***Teile von Kapitel II wurden eingereicht bei „Applied and Environmental Microbiology" :*** Jakob Pernthaler, **Eckart Zöllner**, Falck Warnecke and Klaus Jürgens (2004). A bloom of filamentous bacteria in a mesotrophic lake: identity and potential controlling mechanism.

Manuskripte fertig zum Einreichen:
(geplante Einreichung im April 2004 bei Marine Ecology Progress Series)

Eckart Zöllner et al.: Mesozooplankton (copepods, appendicularians) impact on microbial food web structure in a coastal marine system. I. Cascading predation effects on protists and bacteria

Eckart Zöllner et al.: Mesozooplankton (copepods, appendicularians) impact on microbial food web structure in a coastal marine system. II. Patterns of bacterial community composition and diversity

Plön, den 17. März 2004 **Eckart Zöllner**