Selected interactions between phytoplankton, zooplankton and the microbial food web: Microcosm experiments in marine and limnic habitats

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften

Dr. rer. nat.

der Fakultät für Biologie der Ludwig-Maximilians-Universität München

by

Alexis Katechakis

München 2005

Selected interactions between phytoplankton, zooplankton and the microbial food web: Microcosm experiments in marine and limnic habitats

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften

Dr. rer. nat.

der Fakultät für Biologie der Ludwig-Maximilians-Universität München

by

Alexis Katechakis

Eingereicht am: 24. November 2005

1. Gutachter: PD Dr. Herwig Stibor

2. Gutachter: Prof. Dr. Sebastian Diehl

Tag der mündlichen Prüfung: 17. März 2006

A L'ANNA

CONTENTS

Attachments

- **Paper reprints**
- **Personal notes**
  Curriculum vitae – Publication list / Grants – Acknowledgements – Declaration

SUMMARY

The experiments presented in this thesis elucidate selected interactions between the phytoplankton, the zooplankton and the microbial food web in aquatic ecosystems. The objective is to provide a mechanistic understanding of classic general ecology topics including competition, predator-prey relations, food web structure, succession, and transfer of matter and energy. Special relevance is attributed to the role of mixotrophic organisms, marine cladocerans, and gelatinous mesozooplankton. Although they may contribute substantially to plankton composition they have thus far been neglected in common ecosystem models. All experiments were based on enrichment with nutrients and organic compounds. Enrichment with nutrients and organic compounds that influence overall system productivity is one of the most pervasive human alterations of the environment and profoundly affects species composition, food web structure, and ecosystem functioning. In order to predict the consequences of such enrichment, a better understanding of the impact that trophic structure has on community dynamics and ecosystem processes is required.

The presented thesis consists of two studies. The first study includes three experiments in which I investigated the role copepods, cladocerans and doliolids play in plankton interactions. Copepods, cladocerans and doliolids are major mesozooplankton groups in marine systems. The first experiment (Katechakis et al. 2004) showed that copepods, cladocerans and doliolids have different food size spectra and different assimilation efficiencies. According to my experiment, copepods actively select for larger food items, whereas cladocerans and doliolids passively filter medium-sized and small food items, respectively, with doliolids being the only group that feeds efficiently on bacteria and picoplankton. The results illustrate that food niche separation enables copepods, cladocerans and doliolids to coexist. In addition, they emphasize the fact that doliolids are favored in low nutrient environments, characterized by small food items, whereas cladocerans and copepods have competitive advantages at moderate and high nutrient supplies, respectively. Furthermore, copepods obviously utilize ingested food best, gauged in terms of produced biomass, followed by cladocerans and doliolids, which suggests that the different mesozooplankton have different impacts on energy transfer efficiency within the food web.

In the second experiment (Katechakis et al. 2002), I investigated how copepods, cladocerans and doliolids directly influence the phytoplankton and the microbial food web over a longer period of time by grazing. Furthermore, I investigated how they indirectly influence the system's nutrient dynamics through "sloppy feeding" and their excretions. According to my experiment, in the long run, doliolids and cladocerans promote the growth of large algae whereas copepods shift the size spectrum towards small sizes with different consequences for food chain length. Doliolids, cladocerans and copepods also affect the microbial food web in different ways. Size-selective grazing may lead to differences in the nanoplankton concentrations. These in turn can affect bacterial concentrations in a trophic cascade. My findings offered the first experimental evidence for the occurrence of top-down effects in marine

systems. Although top-down explanations of phytoplankton size structure had been acknowledged for limnic systems before, they had not been attempted for marine systems.

In the last experiment of this series (Katechakis and Stibor 2004) I sought to complement the knowledge about the feeding behavior of marine cladocerans. Marine cladocerans are difficult to cultivate in the laboratory. Therefore, the three cladoceran genera found in marine systems, *Penilia*, *Podon* and *Evadne*, had never before been compared under similar conditions. Existing studies with single cladoceran genera were to some extent contradictory. My experiments indicate similar feeding characteristics for *Penilia*, *Podon* and *Evadne*, that is to say, similar food size spectra, clearance and ingestion rates. However, *Evadne* obviously has problems feeding on motile prey organisms.

The results generated by my first study have been summarized and their importance has been hypothetically extended to ecosystem level by Sommer et al. (2002) and by Sommer and Stibor (2002).

My second study includes two experiments that refer to the ecological role of mixotrophs in aquatic systems. Mixotrophic organisms combine phototrophic and phagotrophic production dependent on the availability of light and nutrients. Although they are common in aquatic systems, their function for nutrient cycling and as a link to higher trophic levels has never before been examined.

In my first experiment (Katechakis et al. 2005) I investigated if mixotrophs influence energy transfer efficiency to higher trophic levels differently than predicted for purely phototrophic organisms. My results indicate that compared to phototrophic specialists mixotrophs may enhance transfer efficiency towards herbivores at low light conditions and in situations when limiting nutrients are linked to bacteria and to the picoplankton. Additionally, the results suggest that mixotrophs may have a stabilizing effect on variations in trophic cascade strength caused by perturbations to light and nutrient supply ratios.

My second experiment (Katechakis and Stibor 2005a) served as a first step towards analyzing if the results gained from the first experiment have any ecological relevance in situ, that is, if mixotrophs in nature-like communities can gain enough importance to relevantly influence transfer efficiency and system stability. Competition experiments revealed that mixotrophs may invade and suppress plankton communities that consist of purely phototrophic and purely phagotrophic specialists at low nutrient conditions while high nutrient supplies prevent mixotrophs from successfully invading such communities. In systems where mixotrophs suppressed their specialist competitors they indeed had a habitat-ameliorating effect for higher trophic levels, gauged in terms of plankton food quality.

In the meantime, the results gained from my experiments have inspired various other studies in marine and limnic systems.

KEY WORDS

aquatic food web • assimilation efficiency • autotrophy • bottom-up control • cladocerans • clearance rate • coexistence • competition • copepods • doliolids • ecological stoichiometry (ES) • effective food concentration (EFC) • energy transfer efficiency • enrichment • eutrophication • feeding selectivity • food niche separation • food quality • food quantity • food web dynamics • food web model • food web theory • gelatinous plankton • generalist • grazing • herbivory • heterotrophy • indirect effects • ingestion rate • invasion • light-nutrient hypothesis (LNH) • limnic food web • marine food web • mechanistic resource competition theory • microbial food web • microcosm • mixotrophy • niche overlap • nutrient stoichiometry • omnivory • pelagic food web • phagotrophy • phototrophy • phytoplankton • plankton composition • plankton ecology • plankton size structure • predation • primary production • productivity • secondary production • specialization • top-down control • trophic cascade • trophic structure • tunicates • zooplankton

CHAPTER 1 – INTRODUCTION

Pelagic food webs are the most common type of food webs on earth and planktonic organisms involved in pelagic food webs may possibly be the most abundant on earth. Regarding solely protozoa, more than 1500 million tons of them exist in the Southern Ocean alone. In contrast, all vertebrates together, including fish, penguins, seals and whales make up only 16 million tons (Beaumont 2003). Hence, it is not surprising, that the dynamics of planktonic food webs have powerful impacts on important issues such as world climate (e.g. Beaumont et al. 1998, Toole and Siegel 2004), global biogeochemical cycling (e.g. Dachs et al. 2002, Valdes et al. 2004) and the world food production (e.g. Meadows et al. 2004). For example, plankton influences climate and biogeochemical cycling by absorbing carbon dioxide (e.g. Beaumont 1998), releasing cloud-forming compounds such as dimethylsulfoniopropionate (DMSP) (Toole and Siegel 2004) and drawing huge amounts of nitrogen from the air (Capone and Carpenter 1982). Drastic changes in plankton abundances affecting food web production have recently been reported from the waters of Northern California. Oceanic plankton have largely disappeared there, followed by a general decline in near-shore oceanic life, with far fewer fish, birds and marine mammals. Reasons for the absence of the plankton have yet to be fully understood. However, a recent study indicates that it may be a long term phenomenon linked to global warming (Gregg et al. 2003), that may, on the other hand, enhance plankton growth in other regions of the world (Goes et al. 2005).

These few examples demonstrate that despite their critical relevance for our planet, we are still only in the early stages of understanding the interactions in planktonic food webs that take place among species at different trophic levels and under changing environmental conditions. The main difficulty lies in that even relatively simple food webs have such complicated structures thus one cannot intuitively understand how a change in one variable might ultimately affect each of the others. Therefore, ecosystem models play an ever more important role in the understanding of applied and theoretical problems in food web ecology. The question regarding what features should be incorporated into these models is fundamental for improving them. Information on physiological and community structuring properties of functional key species are essential within this context.

1.1. Thesis objectives, approach and outline

In this thesis I focus on selected interactions such as competition, predator-prey relations, and the transfer of matter and energy between the phytoplankton, the zooplankton and the microbial food web. Through laboratory microcosm experiments, partly related to mesocosm studies, I specifically addressed these interactions. Micro- and mesocosm experiments provide a bridge between abstracted mathematical models and the full complexity of nature. Special relevance is attributed to the role of mixotrophic organisms, marine cladocerans, and gelatinous mesozooplankton. Although they may contribute substantially to plankton composition they have thus far been neglected in aquatic ecosystem models. All experiments were based on enrichment with nutrients and organic compounds. Enrichment with nutrients and organic compounds that influence overall system productivity is one of the most pervasive human alterations of the environment and profoundly affects species composition, food web structure, and ecosystem functioning. In order to predict the consequences of such enrichment, a better understanding of the impact that trophic structure has on community dynamics and ecosystem processes is required.

The thesis consists of two studies, A and B. The first study includes three experiments (A1, A2, A3) in which I investigated the ecological role of copepods, cladocerans and doliolids, which are major mesozooplankton groups in marine systems. They form a bottleneck in the pelagic food web as they distribute the organic matter synthesized by autotrophs towards higher trophic levels. Yet, the feeding properties, especially of marine cladocerans and doliolids were practically unknown when I began my experiments. Similarly, nothing was known about the role these organisms play in structuring plankton communities and in transferring energy to higher trophic levels. Papers A1, A2 and A3 refer to these topics.

The second study includes two experiments (B1, B2) that refer to the ecological role of mixotrophs in aquatic systems. Mixotrophic organisms combine phototrophic and phagotrophic production dependent on the availability of light and nutrients. Although they are common in aquatic systems, their function for nutrient cycling and as a link to higher trophic levels has never been examined before. Papers B1 and B2 deal with these questions.

In the following sub-chapters, I will briefly explain the structure of the pelagic food web to better illustrate the positions copepods, cladocerans, doliolids and mixotrophs take. Additionally, I describe the major flows of energy, matter and control that appear within pelagic food webs and how enrichment with nutrients influences them.

Chapter 2 provides more detailed backgrounds about the two studies, Chapter 3 summarizes the papers attached to this thesis, Chapter 4 concludes the findings, Chapter 5 shows how they have

already influenced other investigations and offers suggestions regarding next steps to be taken. References are provided in Chapter 6.

Throughout the thesis I follow the generally accepted plankton size classifications: picoplankton (<2 μm), nanoplankton (2 – 20 μm), microplankton (20 – 200 μm), mesoplankton (200 μm – 2 mm), macroplankton (2 mm – 2 cm), megaplankton (>2 cm). Phytoplankton are not represented in the megaplankton size range, zooplankton not in the picoplankton size range, and metazoans not in the pico- and nanoplankton size ranges.

1.2. The pelagic food web

During the last few decades, the notion of the pelagic food web has undergone drastic changes. The classic view was a simple food chain with phytoplankton (mainly diatoms) at the base, herbivorous mesozooplankton (mainly copepods in the sea and cladocerans in lakes) at the second trophic level, planktivorous fish at the third trophic level, and piscivorous fish at the forth trophic level (Fig. 1).

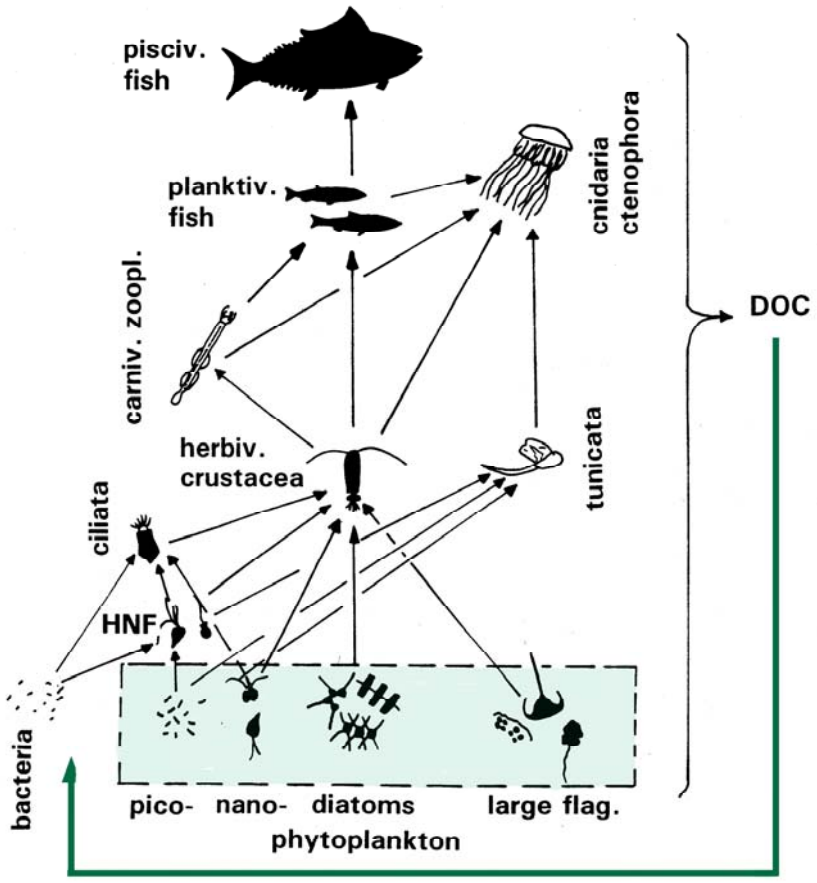

Fig. 1 Simplified pelagic food web; DOC: Dissolved Organic Carbon

The existence of second trophic zooplankton groups other that crustacea, for example protozoa and tunicates, had been acknowledged but considered negligible. Then, some decades ago, the traditional view was challenged by the discovery of the so-called microbial food web, composed of bacteria, nanoflagellates and microzooplankton, with the latter as key organisms that link the microbial food web to the classical food chain (Pomeroy 1974, Azam et al. 1983) (lower, left-hand portion of Fig. 1).

The bacteria within the microbial food web play an important role in the decomposition of waste materials and tissues of dead organisms, that is the remineralization of bound elements which thus

become available for the uptake by autotrophic organisms again. On the other hand, it gradually became clear that bacteria are often not only remineralizers of mineral nutrients but rather compete with phytoplankton for the uptake of dissolved mineral nutrients (Rothhaupt and Güde 1992). The subsequent debate centered on the question of whether the microbial food web was a "link" or a "sink" in the energy transfer to higher trophic levels. The answer is quite straightforward. The microbial food web is a link to the extent that organic carbon consumed by bacteria is made available to higher trophic levels. However, it is a sink to the extent, that primary production by pico- and nanoplankton is consumed by protozoans instead of metazoans.

A further extension of the traditional food web was revealed in the early 1980's, when it became clear that gelatinous zooplankton also play a larger role than anticipated at least in marine systems (e.g. Alldredge and Madin 1982, Bone 1998), with pelagic tunicates taking the position of crustacean zooplankton groups while jellyfish (Cnidaria and Ctenophora) take the position of planktivorous fish (right-hand side of Fig. 1). These organisms have a much higher water and lower protein content in their fresh biomass than crustaceans and fish have. Therefore, the food chain tunicates-jellyfish has also been called "jelly food chain" as opposed to the "crustacean-" or "muscle food chain" that leads from crustaceans to fish. Gelatinous zooplankton are usually considered a dead end in the pelagic food web (Verity and Smetacek 1996) since their low nutritional value makes them a minor food item for higher trophic levels (although there are some minor exceptions, e.g. the moon fish *Mola mola* and sea turtles). Consequently, most their biomass is remineralized in the water column thus fueling the microbial food web.

With the discovery of the microbial food web and the gelatinous realm, the classic four-link food chain from primary producers to piscivorous fish was forced open. Triggered by the availability and stoichiometry of dissolved inorganic nutrients, food chain length may vary up to seven links (picoplankton – heterotrophic nanoflagellates, HNF – ciliates – zooplankton – predatory zooplankton – planktivorous fish – piscivorous fish) or energy may be channeled away from fish if tunicates harvest most of the primary production (Fig. 1). Both food chain elongation and dead ends in energy flow have drastic impacts on the ratio of fish production to primary production, as energy is lost from the food chain on each transfer to the next higher trophic level (Sommer et al. 2002).

Mixotrophic organisms have yet to be considered within this context. Mixotrophs combine phototrophic and phagotrophic production dependent on the availability of light and nutrients (e.g. Sibbald and Albright 1991, Raven 1997). Hence, they act as both consumers and producers of organic carbon. Although they may contribute substantially to plankton biomass in marine (e.g. Arenovski et al. 1995, Havskum and Riemann 1996, Pitta and Giannakourou 2000) and freshwater (e.g. Sandgren 1988, Isaksson 1998, Sanders 1991) systems, their contribution to aquatic ecosystems is not yet fully understood.

1.3. Bottom-up vs. top-down control

The different community components within a food chain directly affect their neighbours through trophic interactions thereby indirectly influencing other components with which they are integrated. Effects of component interactions can travel through the food chain, although the effects may be dampened. Such a flow of controlling influence can start from the bottom of the food chain induced by the availability of resources to higher trophic levels (bottom-up control) or can flow downward induced by the impact of predators or grazers on lower trophic levels (top-down control). Today it is commonly accepted that, within the physical constraints of, for example, mixing, stratification, temperature and light conditions, plankton communities are structured by the simultaneous impact of bottom-up and top-down effects. However, the strength of each of the two flows will vary between ecosystems, over time and with the spatial scale of observation (e.g. Worm 2000).

Mankind seriously influences both bottom-up and top-down effects. For instance, global fishery has fully exploited two thirds of all fish stocks (Botsford et al. 1997), with severe implications for lower food web structure and dynamics (e.g. Durán and Castilla 1989, Estes et al. 1998, Steneck 1998). In addition, humans heavily influence aquatic food webs bottom-up through changes in the supply of nutrient resources (to be explained in more detail in the following sub-chapter).

1.4. Natural vs. cultural nutrient enrichment

Enrichment with nutrients and organic compounds that limit primary or secondary production is one of the most pervasive human alterations of the environment and profoundly affects species composition, food web structure, and ecosystem functioning (DeAngelis 1992, Rosenzweig 1995, Polis et al. 1997). Nutrient-rich wastes and effluents are often directly disposed into coastal environments and lakes or reach them by rain runoff, river input and atmospheric transport (Peierls et al. 1991, Carpenter et al. 1998). Increasing supply of nutrients and organic matter (eutrophication) has caused changes in plankton abundance and species composition, including toxic microalgal blooms in aquatic systems around the world.

The reason for this phenomenon is not solely absolute nutrient concentration, rather nutrient stoichiometry. The ratios of the different plant nutrients, mainly phosphorus (P), nitrogen (N), and silicate (Si) determine the taxonomical composition of the phytoplankton community. Si is of extra importance because it determines the proportion of diatoms in the phytoplankton community (e.g. Tilman 1986, Escaravage et al. 1996, Egge and Jacobsen 1997). A certain proportion of diatoms seems to be essential for a high food web efficiency (e.g. Sommer et al. 2002, Stibor et al. 2002). Natural enrichment from deep waters, as it occurs in upwelling regions and during seasonal mixing, is characterized by high Si:N and Si:P ratios beneficial for the growth of diatoms. On the contrary, cultural eutrophication is usually characterized by an excess of N and P leading to low Si:N and Si:P ratios, respectively (e.g. Billen and Garnier 1997, Cloern 2001). These frequently support the growth of large flagellates (mainly dinoflagellates) (e.g. Cadée 1992, Radach et al. 1990, Cooper 1995), that generally form a minor food source for herbivorous mesozooplankton compared to diatoms (e.g. Lancelot et al. 2002). In addition, many of these flagellates are potentially toxic (Granéli et al. 1989, Smayda 1990, Honjo 1993). As a consequence, primary production may be channeled away from higher trophic levels that are also substantial for human nourishment. Instead the microbial food web, that finally remineralizes all inedible food items, is stimulated.

Although the problems related to human-induced eutrophication are a recognized issue, the present management of eutrophication suffers from an insufficient understanding of the response of enhanced nutrient supply to aquatic ecosystems, especially in marine systems. The central question here is: How does human-induced nutrient enrichment cause changes in the structure and function of nearshore coastal ecosystems? To properly answer this question information on physiological and community structuring properties of functional key species is essential, thus leading to the backgrounds related to my first study.

CHAPTER 2 – STUDY BACKGROUNDS

2.1. Backgrounds Study A

The experiments summarized in Study A were conducted during Marine Science and Technology (MAST) III-project COMWEB funded by the EU. COMWEB provided an experimental approach to increase our understanding of the process of harmful coastal eutrophication. The project examined the effects of variable nutrient supply on the entire pattern of food web components and flows in the lower pelagic food web. Fundamental information on physiological properties of functional key species in the pelagic food web was used to constrain flow estimations established by so-called inverse modeling procedures (see Olsen et al. 2001 for more details). The comparative case studies covered the Baltic, the NW-Mediterranean, the North Sea, and the NE-Atlantic. The objective of my experiments was to contribute data regarding three major mesozooplankton groups: copepods, cladocerans and doliolids.

As already indicated in the above chapters, the grazing behavior of mesozooplankton is one of the critical factors structuring pelagic food webs. Mesozooplankton distribute the organic matter synthesized by autotrophs towards higher trophic levels. While marine copepods are relatively well investigated within this context, only limited knowledge existed regarding the feeding behavior of marine cladocerans and practically nothing was known about the feeding behavior of doliolids when I began my experiments. Similarly, nothing was known about the role marine copepods, cladocerans and doliolids play in structuring plankton communities and how their assimilation efficiencies differ from each other, an important parameter necessary to predict energy transfer efficiency between trophic levels.

Pelagic copepods occur both in the sea and in freshwaters. Traditionally, copepods have been considered the prototype of marine zooplankton and indeed dominate the mesozooplankton guild in many marine areas by number and biomass. Copepods have complicated life cycles (obligate sexuality, larval nauplius stages and subadult copepodid stages). Slow somatic growth leads to long generation cycles and low birth rates. Fast-growing copepods like *Acartia clausi* need on an average one to two months until maturity, while annual life cycles (e.g. *Calanus finnmarchicus*) and even longer ones (*Calanus hyperboreus* in polar seas) are common as well.

Similar to copepods in marine systems, cladocerans have been considered the prototype of zooplankton in lakes, particularly the genus *Daphnia* spp.. Cladocerans have simple life cycles with a parthenogenetic reproduction through most of the year and without larval stages. Neonates are morphologically similar to adults, relatively large and grow to sexual maturity within a few days.

Pelagic tunicates (salps, appendicularians, pyrosomas and doliolids) are exclusively marine organisms and ubiquitous members of all marine pelagic systems, from coastal areas to the deep sea.

As already explained, they are also referred to as gelatinous zooplankton because of their extremely watery body tissue (Acuña 2001). The reproduction cycle is complex, including sexual and asexual generations with high birth rates. Under good food conditions tunicates exhibit population growth rates that rank at the top among metazoans and, therefore, may form large swarms mainly by asexual reproduction (Bone 1998). Traditional sampling with nets damage the soft bodies of tunicates so that they are not easily identified. Therefore, their abundance has been underestimated for a long period of time.

My research focused on the following main questions:

1. Which are the feeding characteristics of copepods, cladocerans and doliolids in the NW Mediterranean? (→ Paper A1)
2. Based on the results of experiment A1, how do copepods, cladocerans and doliolids structure the phytoplankton community and the microbial food web in the long run? (→ Paper A2)
3. Do sub-tropical and boreal marine cladocerans differ in their feeding habits? (→ Paper A3)

2.2. Backgrounds Study B

The experiments summarized in Study B were conducted within the frame of a German Research Council (DFG) project that studied the effects organisms that work on multiple trophic levels (omnivores and mixotrophs) have on planktonic food web structure and dynamics. The experiments presented sought to contribute data about mixotrophic organisms.

Mixotrophy is defined as the combination of photosynthesis and phagotrophy in the same individual (Sanders 1991). An immediate advantage of being a mixotroph instead of being a specialist phototroph or phagotroph is assumed to include a better survival of mixotrophs during periods of nutrient or light limitation. On the other hand, the strategy requires investment in both a photosynthetic and a phagotrophic cellular apparatus, and the benefits must outweigh these costs. Although the mixotrophic feeding mode was already recognized in the first half of this century (Pascher 1917, Biecheler 1936), interest in its ecological importance remained low until its re-discovery in the 1980's (Bird and Kalff 1986). In the meantime, mixotrophs have been found in several classes of single-celled plankton (flagellates, ciliates, and radiolarians) (see e.g. Jones 2000 for review) and it is well established that mixotrophs are important members of planktonic food webs in marine (e.g. Arenovski et al. 1995, Havskum and Riemann 1996, Pitta and Giannakourou 2000) and freshwater (e.g. Sandgren 1988, Isaksson 1998, Sanders 1991) systems.

The relative importance of the phototrophic and phagotrophic modes of nutrition in mixotrophs is species specific and varies as a function of environmental parameters like particle density (Rothhaupt 1996a), light (Sanders et al. 1990), inorganic nutrient concentration (Nygaard and Tobiesen 1993), pH (Sanders et al. 1990) dissolved organic carbon (DOC) (Bergström et al. 2003), and perhaps dissolved inorganic carbon (DIC) (Porter 1988, Sanders et al. 1990). However, most mixotrophic organisms seem to combine phagotrophy and phototrophy primarily dependent on the availability of light and nutrients and generally rely more on either one nutrition mode (Jones 1997).

Mixotrophs offer an interesting field of experimentation. Their ability to combine light, mineral nutrients, and prey as substitutable resources suggests that they have a different impact on nutrient cycling and energy transfer in pelagic food webs than purely phototrophic or purely phagotrophic organisms. Similarly, it is to assume that their competitive capabilities differ from their specialized counterparts.

Although mixotrophs recently have been included more intensively in ecological studies (e.g. Hitchman and Jones 2000, Sanders et al. 2000, Bergström et al. 2003, Tittel et al. 2003), good controlled experimental tests of competition between mixotrophs and specialist phototrophs or phagotrophs are surprisingly rare. Likewise, the importance of mixotrophs for energy transfer to higher trophic levels and for nutrient cycling has never before been examined.

My research focused on the following main questions:

1. Do mixotrophs influence energy transfer to higher trophic levels differently than their specialized counterparts? (→ Paper B1)
2. Can mixotrophs invade established plankton communities consisting of specialist phototrophs and specialist phagotrophs? In the case of a successful invasion, how do mixotrophs change food web structure? (→ Paper B2)

CHAPTER 3 – PAPER SUMMARIES

Paper A1

Feeding selectivities and food niche separation of *Acartia clausi*, *Penilia avirostris* (Crustacea) and *Doliolum denticulatum* (Thaliacea) in Blanes Bay (Catalan Sea, NW Mediterranean)

Journal of Plankton Research

The grazing behavior of mesozooplankton is one of the critical factors structuring pelagic food webs. There are many studies concerning grazing of marine copepods. In contrast, gelatinous mesozooplankton and marine cladocerans are poorly investigated, possibly because they are difficult to sample and difficult to cultivate in the laboratory. In Blanes Bay (Catalan Sea, NW Mediterranean) I had the opportunity to work with copepods, cladocerans and doliolids captured directly from the sea, shortly before I started my experiments.

I was interested in the feeding characteristics as such but also in the question why copepods, cladocerans and doliolids are able to coexist in the NW Mediterranean, although they obviously depend on the same resources and their carbon contents (Table III in Paper A1) and energy requirements per carbon unit (Ikeda 1985, Schneider 1992) are similar. My hypothesis was that copepods, cladocerans and doliolids must have different niche specifications regarding their dietary preferences and that niche specifications must be based on food size, as size is the most important factor for feeding relationships in the pelagic (Sommer et al. 2002).

To test my hypothesis, I conducted short-time grazing experiments with the copepod *Acartia clausi*, the cladoceran *Penilia avirostris* and the doliolid *Doliolum denticulatum*. The plankton communities I used as food were grown at different nutrient supplies in off-shore mesocosms. The resulting average plankton sizes, proportion of diatoms and community densities were positively correlated with nutrient supply (Tables I, II, and Fig. 2 in Paper A1). The grazing-parameters I determined for each mesozooplankton group were food selectivity, clearance and ingestion rate, assimilation efficiency, food niche breadth and niche overlap.

My results show that *Acartia clausi*, *Penilia avirostris* and *Doliolum denticulatum* differ substantially in their food size selectivities, which helps explain their coexistence in the NW Mediterranean.

Although all grazers competed for food sizes between 7.5 and 100 µm (longest linear extension), none of the grazing spectra completely overlapped any of the others. Doliolids reached highest grazing coefficients for small food sizes between <1 and 15 µm, cladocerans for intermediate sizes between 15 and 70 µm, and copepods for large algae >70 µm (Fig. 1 in Paper A1).

Doliolum denticulatum and *Penilia avirostris* acted as passive filter-feeders that ingest food only as a result of anatomical constraints, whereas *Acartia clausi* actively selected beneficial prey. To actively grab beneficial prey has decisive advantages. First, it helps to choose food items that are especially nutritious thus helping to optimize metabolism. The assimilation efficiencies of copepods were indeed higher than those of cladocerans and doliolids, although the copepods' mean clearance and ingestion rates were lower (Figs. 4 and 5 in Paper A1). Secondly, it helps to avoid toxic or otherwise chemically unfavorable algae. Calculations of food-niche breadth sustain that in feeding *Acartia clausi* is indeed more specialized than *Penilia avirostris* and *Doliolum denticulatum* (Fig. 6 in Paper A1).

Although they coexist in the NW Mediterranean, my experiments revealed that copepods, cladocerans and doliolids are in principle best adapted to different kinds of pelagic environments.

Doliolids were the only mesozooplankton that fed efficiently on picoplankton and small nanoplankton. This ability is advantageous in low-nutrient environments that mainly support the growth of very small plankton (e.g. this paper, Sommer 2000). In contrast, cladocerans and copepods preferred medium-sized and large food items, which are promoted by moderate and high nutrient supplies, respectively. Correspondingly, doliolids met the highest proportion of usable food or "effective food concentration" (EFC) in plankton communities that were grown at low nutrient supplies, whereas cladocerans and copepods found a higher EFC in plankton communities that were grown at moderate and high nutrient supplies, respectively (Fig. 3 in Paper A1).

Furthermore, copepods and cladocerans were able to adjust their clearance rates to changing food concentrations, and thus to keep their ingestion rates stable over a wide range of food densities (Fig. 4 in Paper A1). This skill is especially useful in environments subject to fluctuating food densities, such as those regularly occuring in coastal environments. In contrast, doliolids reached much higher clearance rates than copepods and cladocerans, but could not adjust their filtration rates to changing food supplies. This caused their filtration apparatus to get blocked at higher food concentrations and suggests that they are better adapted to the open ocean where food concentrations are generally low but stable.

Two important conclusions can be derived from my experiments:

1. The possibility of metazoans to feed on protozoa and bacteria makes the trophic level of mesozooplankton more flexible than suggested by common models of pelagic food webs.
2. The fact that copepods, cladocerans and doliolids differ substantially in their feeding habits, suggests that they have different impacts on both lower food web structure and energy transfer efficiency to higher trophic levels.

Paper A2

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)

Based on the results derived from experiment A1, I wanted to know how copepods, cladocerans and doliolids would influence the phytoplankton and the microbial food web over a longer period of time, not only directly by grazing but also indirectly by influencing the system's nutrient dynamics through "sloppy feeding" and through their excretions. This question was especially interesting because top-down explanations have become accepted by the limnological scientific community since the 1980's (e.g. Oksanen 1981, Carpenter et al. 1985), whereas marine ecologists have almost never attempted top-down explanations of phytoplankton size structure (Shurin et al. 2002). At the time I conducted the following experiments, it was not yet clear whether the lack of data in marine systems is a consequence of a different research focus or of the absence of that phenomenon.

My hypothesis was that depending on which type of mesozooplankton grazer is predominant, phytoplankton and the microbial food web should be dominated by those size classes which are least edible. In addition, I expected the different grazer groups to cause different shifts in nutrient stoichiometry and, therefore, to influence competition between algal groups and the microbial food web differently. The tendency of zooplankton to minimize the excretion of the nutrient least abundant relative to demand in the body mass of their food (e.g. Hessen 1992, Elser and Urabe 1999) should accentuate already existing patterns of nutrient limitation or bias nutrient supply ratios.

To test my hypotheses, I invented circular two-stage chemostats (Fig. 1 in Paper A2). In the first stages I incubated plankton assemblages from the NW Mediterranean that included the microbial food web, as well as the natural phytoplankton community. In the second stages I incubated additionally either copepods, cladocerans or doliolids. To provide the grazers with food I regularly transferred a defined volume from the first stages to the second stages. Likewise, I returned a defined volume from the second stages to the first stages, together with uneaten food items and recycled nutrients. In this way, the plankton community in the first stages changed gradually depending on the direct and indirect impacts exerted by mesozooplankton grazing. The whole chemostat was fueled by nutrient supplies comparable to the in situ nutrient supply rate from natural terrestrial and human sources at the site.

My results show that, in the long run, copepods, cladocerans and doliolids alter the phytoplankton community in different ways.

Doliolids and cladocerans promoted the growth of large algae, whereas copepods shifted the size spectrum towards small sizes (Fig. 2 in Paper A2).

Doliolids, cladocerans and copepods also affected the microbial food web in different ways, both through direct grazing and indirectly via trophic cascades.

Doliolids showed a direct link with the microbial food web on the bacterial level, cladocerans on the flagellate level and higher. Copepods did not have a direct link to the microbial food web. Nevertheless, also copepods changed the structure of the microbial food web. Size-selective grazing caused differences in the concentrations of heterotrophic nanoflagellates (HNF). These in turn affected bacterial concentrations in a trophic cascade (Fig. 4 in Paper A2). As a consequence, paradoxically, bacterial abundances were lowest in treatments with copepods (Fig. 3 in Paper A2).

Furthermore, grazer-induced nutrient recycling led to shifts in dissolved nutrient stoichiometry. Phosphorus (P)-rich cladocerans and doliolids retained relatively more P and depressed nitrogen (N):P excretion ratios compared to more N-rich copepods. Similarly, all mesozooplankton grazers slowed down silicate (Si) cycling (Fig. 3 in Paper A2, right panel). However, these shifts were outweighed by the allochthonous input of nutrients to the chemostats (Fig. 3 in Paper A2, left panel). Hence, in my experiments, grazer-induced nutrient recycling was not powerful enough to influence phytoplankton competition. Nevertheless, grazer-induced nutrient recycling may influence phytoplankton composition in environments where nutrient supply is dominated by recycling. This, for example, being the case in nutrient-poor surface layers during summer stratification and in ultraoligotrophic regions of the open ocean.

Two important conclusions can be derived from my experiments:

1. In contrast to what was traditionally believed, top-down effects also appear in marine systems and are mediated differently by different consumers.
2. If mesozooplankton exert selective influence upon their food guild, then the dominant grazer group should decimate its own favored food while sparing the exclusive food sources of other mesozooplankton from grazing pressure and vice versa. Any such feedback would obscure bottom-up effects and dampen strongly expected shifts in mesozooplankton dominance along an eutrophication gradient (Sommer et al. 2002).

Paper A3

Feeding selectivities of the marine cladocerans
Penilia avirostris, Podon intermedius* and *Evadne nordmanni

Having over 600 recorded species, cladocerans are the dominant mesozooplankton in many lakes (Schram 1986). In contrast, only eight cladoceran species have been reported to be truly marine (Onbé 1977). These belong to the three genera *Penilia*, *Podon* and *Evadne*, which are mainly restricted to coastal waters. *Penilia* only exists in temperate waters (Della Croce and Venugopal 1973, Grahame 1976), while *Podon* and *Evadne* are mainly found in boreal oceans (Raymont 1983). Cladocerans play a less important role in marine pelagic systems compared to copepods (Raymont 1983, Egloff et al. 1997). Nevertheless, cladocerans sporadically consume a substantial portion of the primary production (Bosch and Taylor 1973, Turner et al. 1988, Kim et al. 1989).

Little is known about the feeding habits of marine cladocerans. At the time I conducted my experiments, the published studies were contradictory to some extent and did not include any feeding experiments with *Podon* or *Evadne* grazing on natural phytoplankton communities. During research stays in Spain and in Norway I had the opportunity to compare the feeding characteristics of representatives of all three cladoceran genera under similar experimental conditions.

For this reason, I conducted short-time grazing experiments with *Penilia avirostris* from the NW Mediterranean and with *Podon intermedius* and *Evadne nordmanni* from the NE Atlantic. The plankton communities I used as food for *Penilia avirostris* were grown at different nutrient supplies in off-shore mesocosms. The resulting average plankton sizes, proportion of diatoms and community densities were positively correlated with nutrient supply (Tables 1, 2, and Fig. 2 in Paper A3). The plankton community I used as food for *Podon intermedius* and *Evadne nordmanni* was the natural plankton community found in summer in Hopavågen Fjord, Norway (Tables 1, 3, and Fig. 3 in Paper A3). The grazing-parameters I determined for each cladoceran species were food size selectivity and food taxon selectivity, as well as clearance and ingestion rate.

My results show that *Penilia avirostris* and *Podon intermedius* act as true filter feeders, while *Evadne nordmanni* feeds to some extent selectively.

Penilia avirostris and *Podon intermedius* reached highest grazing coefficients at similar food sizes ranging between 15 and 70 µm and between 7.5 and 70 µm (longest linear extension), respectively. *Evadne nordmanni* preferred organisms around 125 µm, but also showed high grazing coefficients for particles around 10 µm, while grazing coefficients for intermediate food sizes were

low (Fig. 1 in Paper A3). The latter indicates that *Evadne nordmanni* has difficulties capturing motile prey, as intermediate food sizes were attributed especially to motile plankton organisms.

All cladocerans fed relevantly on components of the microbial food web, that is ciliates and nanoflagellates. There is some evidence that *Podon intermedius* and *Evadne nordmanni* additionally fed on bacteria. However, for methodical reasons I could not ultimately verify grazing on bacteria.

Penilia avirostris was able to adjust its clearance rates to changing food concentrations thus keeping its ingestion rate stable over a wide range of food densities (Fig. 5 in Paper A3). This skill is especially useful in environments that are subject to fluctuating food densities, such as those regularly occuring in coastal environments. I did not offer different food densities to *Podon intermedius* and *Evadne nordmanni*. Therefore, future investigations will have to show how they handle different food abundances. However, mean clearance and ingestion rates were similar for all investigated cladocerans at similar food concentrations (Fig. 5 in Paper A3).

Penilia avirostris met the highest proportion of usable food or "effective food concentration" (EFC) in plankton communities that were grown at intermediate nutrient supplies (Fig. 4 in Paper A3).

Nutrient conditions in Hopavågen Fjord provided higher EFC for *Podon intermedius* than for *Evadne nordmanni*. This may be considered a competitive advantage for *Podon intermedius*, and could in part explain the general predominance of *Podon intermedius* over *Evadne nordmanni* in Hopavågen Fjord (Olav Vadstein, personal communication). On the other hand, clearance and ingestion rates were the same for both species.

Two important conclusions can be derived from my experiments:

1. On the whole, the feeding habits of marine cladocerans seem to be relatively similar.
2. However, further studies are necessary to investigate the functional responses of boreal cladocerans and to examine the importance of cladocerans in marine pelagic food webs in more detail.

Paper B1

Mixotrophic vs. obligately autotrophic algae as food for zooplankton – the light:nutrient hypothesis might not hold for mixotrophs

Limnology and Oceanography

Studies in aquatic productivity traditionally focused the role of food quantity. That is to say, high primary production and biomass would yield high secondary production and biomass of zooplankton and thus potentially also sustain a higher biomass of top predators (e.g. Begon et al. 1996). Recently, however, it has become clear that food quality in terms of elemental nutrient composition may be a key determinant to trophic efficiency in food webs (e.g. Hessen 1992, Gulati and DeMott 1997), and that food chain production varies with the degree of mismatch between the carbon (C):nutrient ratios of autotrophs and their consumers (e.g. Sterner et al. 1998, Hessen and Faafeng 2000).

The C:nutrient ratio of photoautotrophic primary producers is determined by the supply of light and nutrients. Global perturbations to solar insolation and to biogeochemical cycles are altering the inputs of light and nutrients to ecosystems thus influencing primary and secondary production (e.g. Lindroth et al. 1993, Schindler 1998). Recent ecological models predict an increasing decoupling of higher and lower trophic levels in lakes in the coming decades, especially because of an increasing mismatch in the C:phosphorus (P) ratios of autotrophs and their consumers (Sterner et al. 1997, 1998). In this context, algae with low C:P ratios are rated a better food quality for mesozooplankton than algae with high C:P ratios (e.g. Hessen and Faafeng 2000, Makino et al. 2002).

High C:P ratios in algae have been attributed to a joint effect of high light intensities and low P supplies. At high light:nutrient ratios, higher primary production may therefore, paradoxically, cause lower zooplankton production, due to a reduction in transfer efficiency caused by low food quality. On the other hand, at low light supply, food quantity may limit secondary production. These relationships have been summarized in the light:nutrient hypothesis (LNH) by Sterner et al. (1997), and seem well supported by recent theoretical (Andersen 1997, Loladze et al. 2000) and empirical studies (e.g. Urabe and Sterner 1996, Hessen et al. 2002, Urabe et al. 2002a).

However, the LNH is based on the assumption that purely photoautotrophic organisms constitute the base of the food chain. The role of mixotrophic organisms has thus far been neglected within this context, although they may contribute substantially to phytoplankton biomass (see Chapter 2.2.). Mixotrophic organisms combine phototrophic and phagotrophic production dependent on the availability of light and nutrients (e.g. Sibbald and Albright 1991, Raven 1997) and for the following reasons, I expected mixotrophs to have different effects on the algae–herbivore interface than predicted by the LNH:

- The ability to use alternative production pathways suggests that the stoichiometric composition of mixotrophs may be less affected by alterations in the supply with light and dissolved nutrients than the stoichiometry of phototrophic specialists.

- Potentially limiting nutrients, particularly P, are often several orders of magnitude more concentrated in the biomass of food organisms of mixotrophs (bacteria and bacterial-sized particulate matter) than in the dissolved phase (e.g. Vadstein 2000). Heterotrophic nutrition may therefore, entail low C:P ratios in mixotrophs, making them a nutrient-rich food source for mesozooplankton grazers even at high environmental light:nutrient ratios.
- Mixotrophic organisms may dominate phytoplankton biomass under low light conditions and in low nutrient environments (e.g. Riemann et al. 1995), that is exactly in those environments where, according to the LNH, secondary production may be restricted by autotroph food quantity and quality, respectively.

Based on these expectations I formulated the following two hypotheses:

1. The C:P ratios of mixotrophs are lower and much less dependent on external light:nutrient supply ratios than the C:P ratios of purely phototrophic algae.
2. Compared to photoautotrophic specialists, mixotrophs are a superior food source for mesozooplankton grazers at high light:nutrient supply ratios and in low light environments.

To test my hypotheses, I reared mixotrophic organisms (*Ochromonas tuberculata* and *Cryptomonas* sp.) and purely photoautotrophic algae (*Scenedesmus obliquus*) at different light:P supplies and compared their effects as food for zooplankton (*Daphnia magna*) in semicontinuous two-stage chemostats.

In accordance with the LNH, biomass and nutrient stoichiometry of *Scenedesmus obliquus* depended strongly on light:P supplies. As a consequence, *Daphnia magna* growth and fecundity were limited by food quantity at low light intensities and by stoichiometric food quality at high light intensities. In turn, P fertilization caused a transition from limitation by food quality to limitation by food quantity (Fig. 1 in Paper B1, circles).

In contrast to the LNH, biomass and nutrient stoichiometry of mixotrophs were almost unaffected by alterations in the supply of light and dissolved nutrients (Fig. 1 in Paper B1, open symbols). Bacterial counts suggest that mixotrophs compensated for light or P deficiency by heterotrophic nutrition (Fig. 3 in Paper B1). Compared to phototrophic specialists, a diet of *Cryptomonas* sp. therefore enabled a similar or higher and more stable secondary production at most light:nutrient supplies (Fig. 1 in Paper B1, triangles). *O. tuberculata*, however, appeared to be toxic.

Two important conclusions can be derived from my experiments:

1. My results show that compared to phototrophic specialists mixotrophs may enhance transfer efficiency towards herbivores at low light conditions and in situations when limiting nutrients are linked to bacteria and to the picoplankton.

2. Additionally, my results suggest that mixotrophs may have a balancing effect on variations in trophic cascade strength caused by perturbations to light and nutrient supply ratios.

Paper B2

The mixotroph *Ochromonas tuberculata* may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions

The following experiment served as a first step towards analyzing if the results gained from experiment B1 have any ecological relevance in situ, that is, if mixotrophs in nature-like communities can gain enough importance to relevantly influence transfer efficiency to higher trophic levels and system stability.

Mixotrophs combine light, mineral nutrients, and prey as substitutable resources. This nutritional flexibility may be advantageous against phototrophic specialists when dissolved nutrients are limiting and may be advantageous against phagotrophic specialists when prey like bacteria and picoplankton are limiting. On the other hand, mixotrophic organisms must invest in the synthesis and maintenance of both a photosynthetic apparatus and in the mechanisms for prey uptake and its subsequent digestion. These energetic costs may lower a mixotroph's resource use efficiency and may lower photosynthetic performance, resulting in a reduced maximum growth rate compared with a phototrophic or heterotrophic specialist. A mixotroph is therefore expected to be inferior if the environmental conditions sufficiently satisfy the demands of purely phototrophic and phagotrophic specialist, respectively (e.g. Rothhaupt 1996a, Raven 1997, Jones 2000). However, good controlled experimental tests of competition between mixotrophs and specialist phototrophs or phagotrophs are surprisingly rare and the mechanisms underlaying the succession or possible invasion of mixotrophs in aquatic systems are hardly known.

In the present study I tested the ability of mixotrophs to invade established plankton communities. I was interested as to if and how invading mixotrophs would alter food web structure, species diversity, and nutrient cycling.

Based on the aforementioned explanations and the results gained from experiment B1, I formulated the following hypotheses:

1. The potential of mixotrophs to invade an existing plankton community decreases with nutrient enrichment.
2. The carbon (C):nutrient ratio of a plankton community decreases as the proportion of mixotrophs increases.

To test my hypotheses, I first assembled limnic planktonic food webs in semicontinuous chemostats at different supplies of dissolved inorganic nutrients and dissolved organic carbon (DOC). Food webs consisted of bacteria, specialist phagotrophs (heterotrophic nanoflagellates, HNF, and ciliates), and purely phototrophic algae (siliceous and non-siliceous) covering a wide range of plankton sizes from pico- to microphyto- and -zooplankton (Fig. 1 in Paper B2). After plankton communities had established themselves for two weeks, I let mixotrophic *Ochromonas tuberculata* invade the systems.

In accordance with my expectations, low nutrient supplies fascilated the invasion of mixotrophic organisms while high nutrient supplies prevented mixotrophs from successfully invading the food webs (Figs. 2 and 3 in Paper B2).

Ochromonas tuberculata obviously supplemented nutrient restriction by grazing bacteria and picophytoplankton at oligotrophic and mesotrophic conditions (Fig. 4 in Paper B2). As a consequence of combining alternative production pathways, *Ochromonas tuberculata* practically suppressed all other plankton species that were present at the beginning of the experiments. In addition mixotrophs made much better use of the given resources (in the sense of generating biomass) than purely auto- and heterotrophic specialists (Fig. 2 in Paper B2).

In systems where mixotrophs suppressed their specialist competitors they significantly changed seston C:nutrient ratios and had a habitat-ameliorating effect for higher trophic levels, gauged in terms of plankton food quality (see experiment B1). The overall C:phosphorus (P) ratio was considerably lower where mixotrophs were common (Fig. 5 in Paper B2). This speaks for a scarcity of dissolved inorganic P that was compensated by additional P uptake by mixotrophs using particular P from prey as P source. In this way, mixotrophs may make nutrient sources available for higher trophic levels, which would not be accessible to them otherwise. On the contrary, nitrogen (N) obviously was obtainable in excess in my experiments as C:N ratios remained unaffected.

According to the presented results, one would expect mixotrophs to be especially invasive in steady-state like situations where light is sufficient, but dissolved nutrients are limiting and overall productivity is rather low, as is the case in surface layers after a longer period of stratification (e.g. Havskum and Riemann 1996) and in low productive areas like the subtropical Atlantic Ocean (Mann and Lazier 1996). Under such conditions, external import of nutrients is low, and recycling is the primary source for mineral nutrients. Growth rates of pure autotrophs are well below their possible maxima, and mixotrophs might take full advantage of their strategy.

Two important conclusions can be derived from my experiments:

1. In contrast to ecology perception, specialization may not necessarily be the most successful strategy for survival under stable conditions. Rather, the use of several resources with lower efficiency can be an equally or even more successful tactic in nature.
2. When limiting nutrients are linked to the bacterio- and picophytoplankton, invading mixotrophs may have a habitat-ameliorating effect for higher trophic levels, gauged in terms of food quantity and quality. This may help explain why trophic transfer efficiency and food web strength are generally higher in low nutrient environments than in eutrophic systems (Carpenter and Kitchell 1984, McQueen et al. 1986). However, further investigation will be necessary to sustain these findings in situ.

CHAPTER 4 – CONCLUSIONS

Two main conclusions can be derived from my studies:

1. The results gained from **Study A** show that mesozooplankton grazers influence the microbial food web and the phytoplankton on multiple trophic levels, not only by direct grazing but also indirectly by grazing-induced nutrient cycling and via trophic cascades. In other words, the results indicate that the trophic level of mesozooplankton is much more flexible than conveyed by classic food web theory. Therefore, depending on their feeding habits, different mesozooplankton grazers may influence food chain length differently along an oligotrophic-eutrophic gradient. Filter feeding mesozooplankton (cladocerans, doliolids) are around 15 to 1500 times larger than their food, whereas copepods which may feed by individual particle capture are around 5 to 120 times larger. If size constraints were the only determinant for food chain length it would be easy to squeeze one or even two trophic levels. Furthermore, mesozooplankton grazers obviously have deviating assimilation efficiencies. The combined outcomes suggest that the transmission of energy from primary producers to top trophic levels is more complex than predicted by common pelagic ecosystem models that only treat mesozooplankton as a single box.

2. The results gained from **Study B** indicate that mixotrophs may be strong competitors against specialist phototrophs and specialist phagotrophs depending on light–nutrient supplies. Furthermore, mixotrophs may influence nutrient cycling, secondary production and food web stability differently than predicted by common models that are exclusively based on purely phototrophic algae.

CHAPTER 5 – RESEARCH OUTLOOK

In the meantime, my experiments have influenced and inspired various other studies.

- The information on the feeding properties especially of marine cladocerans and doliolids have promoted extensive studies by other working groups in the NW Mediterranean (Albert Calbet and Dacha Atienza, personal communication) and have contributed to a better understanding of the role cladocerans play in marine systems compared to copepods (Ulrich Sommer, personal communication).
- The insight that trophic cascades also exist in marine systems has been extended to the upper pelagic food web. In experimental mesocosm studies we showed that top-down control in marine pelagic systems can be of the same strength as in trophic cascades in lakes (Stibor et al. 2004).
- The definition of mixotrophy as a special kind of omnivory (defined as feeding on more than one trophic level) and the work with differently enriched systems led me to the question how productivity, omnivory and mixotrophy influence food chain length and food web stability in pelagic systems – a question that is discussed controversially in regard to both productivity (e.g. Rosenzweig 1971, Kaunzinger and Morin 1998) and omnivory (e.g. Polis and Strong 1996, Diehl and Feissel 2000). Two experiments I recently finished indicate that the combination of high nutrient levels and the presence of omnivores may have a destabilizing impact on pelagic food webs leading to shorter food chains than expected from nutrient conditions alone (Katechakis and Stibor 2005b). Mixotrophs again shortcut the food chain in the lower food web but lengthen it towards higher trophic levels by making nutrients available to consumers that would get lost for secondary production in the absence of mixotrophs (Katechakis and Stibor 2005c).
- Finally, the information about the feeding habits of the investigated mesozooplankton coupled with the realization that mixotrophs may influence the flow of nutrients, material and energy differently than indicated by classical food chain theory, already have implications for pelagic food web modeling (Dag Hessen and Tom Andersen, personal communication).

Nevertheless, the results and hypotheses put forward by my work, certainly merit further exploration. From my findings I recommend the following future research topics:

1. **Does the crustacean to gelatinous ratio change along nutrient gradients?**

   Based on my results and the investigation of other authors we hypothesized in 2002 that the emphasis of either the "crustacean food chain" or the "jelly food chain" (see Chapter 1.2.) may depend substantially on the present nutrient regime (Sommer et al. 2002). In our article we give rise to the concern that the jelly food chain may be an alternative stable pathway in the

marine pelagic food web that once established may resist change and that cultural eutrophication may be an initiatory factor in this context. Since gelatinous zooplankton are usually considered a dead end in the pelagic food web (Verity and Smetacek 1996), a long-term shift from the the crustacean- to the jelly food chain and, thus, from fish to gelatinous megazooplankton will have drastic impacts on the ratio fish production to primary production as well as human nutrition. Despite observations in the field that sustain this assumption (Shushkina and Vinogradov 1991, Gove and Breitburg 2005), increased research efforts are needed for tunicates, cnidaria and ctenophores within this context.

To address the question whether the crustacean to gelatinous ratio changes along nutrient gradients, I suggest a combination of mesocosm studies and meta-analyses based on published data on the distribution of gelatinous zooplankton in the ocean.

2. **Does the impact of mixotrophic organisms change along nutrient gradients in situ?**

I used laboratory experiments to approach the question how mixotrophs influence planktonic food webs. It is important to recognize the limitation of this approach. Microcosm experiments are essential in highlighting distinctive interactions that cannot be resolved in field experiments. However, they do not capture the full range of species interactions, seasonal changes and natural variability of biotic and abiotic factors. Field studies are necessary to test my results under realistic conditions.

I suggest a comparative study in lakes subject to different nutrient supply rates. No such study has been thus far reported in the literature. Furthermore, in most standard plankton analyses potentially mixotrophic organisms are not even distinguished from purely phototrophic algae.

Basic research questions should include:

- Does the abundance of mixotrophs and their occurrence in relation to other nano- and microplankton (phototrophic and phagotrophic) depend on the trophic status of a lake?
- Does the proportion of mixotrophs change seasonally?
- To which extent do phago- and phototrophy contribute to mixotrophic production
  - at different nutrient supplies?
  - along vertical light gradients?
  - seasonally, that means depending on the combined influence of changing light- and nutrient-supplies?

Further research topics may include

- the question if seston stoichiometry varies with the abundance of mixotrophs and
- the determination of the trophic position of mixotrophs in planktonic food webs with the help of stable isotope measurements.

3. **Do mixotrophic organisms react differently to elevated pCO_2-pressure than purely phototrophic algae?**

It has become obvious that anthropogenic emissions of carbon dioxide (CO_2) have strong effects on the growth physiology of terrestrial plants across the earth (e.g. Körner 2000, Reich et al. 2001). Still, it is far from clear how increasing concentrations of CO_2 will influence aquatic food webs. Two recent studies indicate that an increased partial CO_2-pressure (pCO_2) may stimulate primary production in specialist phototrophic algae (Burkhart 1998, Urabe et al. 2003). On the other hand, the study of Urabe et al. (2003) also suggests that increases in pCO_2 may have a detrimental effect on secondary production by dilution of other essential elements by carbon. Such aspects have yet to be explored in mixotrophs. Based on the insight that mixotrophs may have a balancing effect on variations in light to nutrient supply ratios (Paper B1), I hypothesize that where mixotrophs are common they likewise may dampen perturbations to pCO_2.

I suggest to first test this hypothesis using chemostat experiments in the laboratory. In combination with the data received on the abundances of mixotrophs in natural systems (see Research outlook 2.), mathematically interpolating of the gained results to a larger scale should be possible.

4. **Does the biochemical composition of mixotrophs change depending on the contribution of phagotrophy to overall production?**

In some of my experiments I observed a declining secondary production in mesozooplankton feeding on mixotrophs, although the mixotrophs' stoichiometrical composition should have been perfect to sustain a high secondary production (Fig. 1 in Paper B1, open triangles). At the same time I found indications for a decreasing contribution of phagotrophy to overall mixotrophic production (Fig. 3 in Paper B1, open triangles). I assume that the lowered intake of bacteria had an impairing effect on the mixotrophs' food quality beyond nutrient stoichiometry. Recent publications show that besides nutrient stoichiometry biochemical compounds (and here especially essential fatty acids, EFAs) are a pivotal factor for the efficiency with which biomass and energy are transferred across the plant-animal interface (e.g. Müller-Navarra et al. 2004). I therefore hypothesize that the biochemical make-up of mixotrophs changes with the contribution of phagotrophy to overall mixotrophic production.

I suggest that this hypothesis be addressed using two-stage chemostat experiments similar to the experiments presented in Paper B1 but with a special focus on EFA-composition in purely phototrophic and mixotrophic organisms. In order to quantify the contribution of phagotrophy to overall mixotrophic production I recommend complementary experiments with fluorescent labeled bacteria (FLBs) or algae (FLAs) (e.g. Havskum and Riemann 1996, Sanders et al. 2000).

CHAPTER 6 – REFERENCES

Acuña JL (2001) Pelagic tunicates: why gelatinous? Am Nat 158:100-107

Alldredge AL, Madin LP (1982) Pelagic tunicates: unique herbivores in the marine plankton. Bioscience 32:655-663

Andersen T (1997) Grazers as sources and sinks for nutrients. Springer, Berlin Heidelberg

Arenovski AL, Lim EL, Caron DA (1995) Mixotrophic nanoplankton in oligotrophic surface waters of the Sargasso Sea may employ phagotrophy to obtain major nutrients. J Plankton Res 17:801-820

Azam F, Fenchel T, Field JG, Meier-Reil LA, Thingstad F (1983) The ecological role of water column microbes in the sea. Mar Ecol Progr Ser 10:257-263

Beaumont KL (2003) Planktonic interaction and particulate flux in Ellis Fjord, East Antarctica. PhD Thesis, Univ Tasmania, pp 247

Beaumont KL, Hosie GW, Swadlin KM, Davidson AT, Ritz DA, Trull T (1998) The impact of grazing and faecal production by *Oncaea curvata* (Poecilostomatoida, Copepoda) on carbon flux in coastal waters, eastern Antarctica. New Zeal Nat Sci 23:11-18

Begon M, Harper J, Townsend C (1996) Ecology: Individuals, Populations and Communities. Blackwell, Oxford

Bergström AK, Jansson M, Drakare S, Blomqvist P (2003) Occurrence of mixotrophic flagellates in relation to bacterioplankton production, light regime and availability of inorganic nutrients in unproductive lakes with differing humic contents. Freshwat Biol 48:868-877

Biecheler B (1936) Observation de la capture th la digestion des proies chez un péridien vert. Comptes rendus des séances de la Société de Biologie 122:1173-1175

Billen G, Garnier J (1997) The Phison river plume: coastal eutrophication in response to changes in land use and water management in the watershed. Aquat Microb Ecol 13:3-17

Bird DF, Kalff J (1986) Bacterial grazing by planktonic lake algae. Science 231:493-495

Bone Q (ed) (1998) The biology of pelagic tunicates. Oxford Univ Press, Oxford

Bosch HF, Taylor WR (1973) Distribution of the cladoceran *Podon polyphemoides* in the Chesapeake Bay. Mar Biol 19:161-171

Botsford LW, Castilla JC, Peterson CH (1997) The management of fisheries and marine ecosystems. Science 277:509-515

Burkhart S, Zondervan I, Riebesell U (1999) Effect of CO_2 concentration on C:N:P raio in marine phytoplankton: a species comparison. Limnol Oceanogr 44:683-690

Cadée GC (1992) Phytoplankton variability in the Marsdiep, the Netherlands. ICES Mar Sci Symp 195:213-222

Capone DG, Carpenter EJ (1982) Nitrogen fixation in the marine environment. Science 217:1140-1142

Carpenter SR, Caracao NF, Corell DL, Howarth RW, Sharpley A, Smith VH (1998) Non-point pollution of surface waters with phosphorus and nitrogen. Ecol Applic 8:559-568

Carpenter SR, Kitchell JF (1993) The trophic cascade in lakes. Cambridge University Press, Camebridge

Carpenter SR, Kitchell JF, Hodgson JR (1985) Cascading trophic interactions and lake productivity. BioScience 35:634-639

Cloern JE (2001) Our evolving conceptual model of the coastal eutrophication problem. Mar Ecol Prog Ser 210:223-253

Cooper SR (1995) Chesapeake Bay watershed historical land use: impact on water quality and diatom communities. Ecol Appl 5:703-723

Dachs J, Lohmann R, Ockenden WA, Eisenreich SJ, Jones KC (2002) Oceanic biogeochemical controls on global dynamics of persistent organic pollutants. Environ Sci Technol 36:4229-4237

DeAngelis DL (1992) Dynamics of nutrient cycling and food webs. Chapman and Hall, New York

Della Croce N, Venugopal P (1973) *Penilia avirostris* Dana in the Indian Ocean (Cladocera). Int Rev Gesamte Hydrobiol Hydrogr 58:713-721

Diehl S, Feissel M (2000) Effects of enrichment on three-level food chains with omnivory. Am Nat 155:200-218

Durán LR, Castilla JC (1989) Variation and persistance of the middle rocky intertidal community of central Chile, with and without human harvesting. Mar Biol 103:555-562

Egge JK, Jacobsen A (1997) Influence of silicate on particulate carbon production in phytoplankton. Mar Ecol Progr Ser 147:219-230

Egloff DA, Fofonoff PW, Onbé T (1997) Reproductive biology of marine cladocerans. Adv Mar Biol 31:79-167

Elser JJ, Urabe J (1999) The stoichiometry of consumer-driven nutrient cycling: theory, observations, and consequences. Ecology 80:735-751

Escaravage V, Prins TC, Smaal AC, Peeters JCH (1996) The response of phytoplankton communities to phosphorous input reductions in mesocosm experiments. J Exp Mar Biol Ecol 198:55-79

Ester JA, Tinker MT, Williams TM, Doak DF (1998) Killer whale predation on sea otters linking oceanic nad nearshore ecosystems. Science 282:473-476

Goes JI, Thoppil PG, Gomes HR, Fasullo JT (2005) Warming of the Eurasian Landmass is making the Arabian Sea more productive. Science 308:545-547

Grahame J (1976) Zooplankton of a tropical harbour: the numbers, composition and response to physical factors of zooplankton in Kingston Harbour, Jamaica. J Exp Mar Biol Ecol 25:219-237

Granéli E, Carlsson P, Olsson P, Sundström B, Granéli W, Lindahl O (1989) From anoxia to fish poisoning: the last ten years of phytoplankton blooms in Swedish marine waters. *In*: Cosper EM, Bricelj VM, Carpenter EJ (eds) Novel Phytoplankton Blooms. Springer, New York, pp 407-427

Gregg WW, Conkright ME, Ginoux P, O'Reily JE, Casey NW (2003) Ocean primary production and climate: global decadal changes. Geophys Res Letter 30:doi10.1029/2003GL016889

Grove M, Breitburg DL (2005) Growth and reproduction of gelatinous zooplankton exposed to low dissolved oxygen. Mar Ecol Progr Ser 301:185-198

Gulati RD, DeMott WR (1997) The role of food quality for zooplankton: remarks on the state-of-the-art, perspectives and priorities. Freshwat Biol 38:753-768

Havskum H, Riemann B (1996) Ecological importance of bacterivorous, pigmented flagellates (mixotrophs) in the Bay of Aarhus, Denmark. Mar Ecol Progr Ser 137:251-263

Hessen DO (1992) Nutrient element limitation of zooplankton production. Am Nat 140:799-814

Hessen DO, Faafeng BO (2000) Elemental ratios in freshwater seston: implications for community structure and energy transfer in food webs. Arch Hyrobiol Special Issues Advanced Limnol 55:349-363

Hessen DO, Færøvig P, Andersen T (2002) Light, nutrients and P:C-ratios in algae: grazer performance related to food quality and quantity. Ecology 83:1886-1898

Hitchman RB, Jones HLJ (2000) The role of mixotrophic protists in the population dynamics of the microbial food web in a small artificial pond. Freshwat Biol 43:231-241

Honjo T (1993) Overview on bloom dynamics and physiological ecology of *Heterosigma akashiwo*. *In*: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, Amsterdam, pp 33-41

Ikeda T (1985) Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. Mar Biol 85:1-11

Isaksson A (1998) Phagotrophic phytoflagellates in lakes – a literature review. Arch Hydrobiol Arch Hyrobiol Special Issues Adv Limnol 51:63-90

Jones HLJ (1997) A classification of mixotrophic species based on their behaviour. Freshwat Biol 37:35-43

Jones RI (2000) Mixotrophy in planktonic protists: an overview. Freshwat Biol 45:219-226

Katechakis A, Haseneder T, Kling R, Stibor H (2005) Mixotrophic vs. obligately autotrophic algae as food for zooplankton – the light:nutrient hypothesis may not hold for mixotrophs. Limnol Oceanogr 50:1290-1299

Katechakis A, Stibor H (2004) Feeding selectivities of the marine cladocerans *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni*. Mar Biol 145:529-539

Katechakis A, Stibor H (2005a) The mixotroph Ochromonas tuberculata may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions. Oecologia (submitted)

Katechakis A, Stibor H (2005b) Effects of productivity and omnivory on the stability of an experimental limnic planktonic food web. (in prep)

Katechakis A, Stibor H (2005c) Effects of productivity, omnivory and mixotrophy on an experimental limnic planktonic food web. (in prep)

Katechakis A, Stibor H, Sommer U, 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). Mar Ecol Progr Ser 234:55-69

Katechakis A, Stibor H, Sommer U, Hansen T (2004) Feeding selectivities and food niche separation of *Acartia clausi*, *Penilia avirostris* (Crustacea) and *Doliolum denticulatum* (Thaliacea) in Blanes Bay (Catalan Sea, NW Mediterranean). J Plankton Res 26:589-603

Kaunzinger CMK, Morin PJ (1998) Productivity controls food-chain properties in microbial communities. Nature 195:495-497

Kim SW, Onbé T, Yoon YH (1989) Feeding habits of marine cladocerans in the Inland Sea of Japan. Mar Biol 100:313-318

Körner C (2000) Biosphere responses to CO2 enrichment. Ecol Appl 10:1590-1619

Lancelot C, Martin JM, Panin N, Zaitsev Y (2002) The north-western Black Sea: a pilot site to understand the complex interaction between human activities and the coastal environment. Estuar Coast Shelf Sci 54:279-283

Lindroth RL, Kinney, KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO_2-productivity, phytochemistry, and insect performance. Ecology 74:763-777

Loladze I, Kuang Y, Elser JJ (2000) Stoichiometry in producer-grazer systems: linking energy flow and element cycling. Bull Math Biol 62:1137-1162

Makino W, Urabe J, Elser JJ, Yoshimizu C (2002) Evidence of phosphorus-limited individual and population growth of Daphnia in a Canadian Shield lake. Oikos 96:197-205

Mann KH, Lazier JRN (1996) Dynamics of marine ecosystems: biological-physical interactions in the ocean. Blackwell, Cambridge

McQueen DJ, Post JR, Mills EL (1986) Trophic relationships in freshwater pelagic ecosystems. Can J Fish Aquat Sci 43:1571-1581

Meadows DL, Randers J, Meadows DL (2004) Limits to growth: the 30-year update. Chalsea Green Publishing Cpmpany, Chalsea

Müller-Navarra DC, Brett MT, Park S, Chandra S, Ballantyne AP, Zortta E, CR Goldman CR (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427:69-72

Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Luchenco J, Mooney H, Troell M (2000) Effect of aquaculture on world fish supplies. Nature 405:1017-1024

Oksanen L, Fretwell SD, Arruda L, Niemela P (1981) Exploitation ecosystems in gradients of primary productivity. Am Nat 118:240-261

Olsen Y, Reinertsen H, Vadstein O, Andersen T, Gismervik I, Duarte C, Agusti S, Stibor H, Sommer U, Lignell R, Tamminen T, Lancelot C, Rousseau V, Hoell E, Sanderud KA (2001) Comparative analysis of food webs based on flow networks: effects of nutrient supply on structure and function of coastal plankton communities. Cont Shelf Res 21:2043-2053

Onbé T (1977) The biology of marine cladocerans in a warm temperate water. Proc Symp Warm Water Zoopl, Spec Publ, UNESCO/NIO (Goa), pp 383-398

Pascher A (1917) Flagellaten und Rhizopoden in ihren gegenseitigen Beziehungen. Archiv für Protistenkunde 38:1-87

Peierls B, Caraco N, Pace M, Cole J (1991) Human influence on river nitrogen. Nature 350:386-387

Pitta P, Giannakourou A (2000) Planktonic ciliates in the oligotrophic Eastern Mediterranean: vertical, spatial distribution and mixotrophy. Mar Ecol Prog Ser 194:269-282

Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Ann Rev Ecol Sys 28:289-316

Polis GA, Strong DR (1996) Food web complexity and community dynamics. Am Nat 147:813-846

Pomeroy LR (1974) The ocean foodweb, a changing paradigm. BioScience 24:499-504

Porter KG (1988) Phagotrophic phytoflagellates in microbial food webs. Hydrobiologia 159:89-97

Radach G, Berg J, Hagneier E (1990) Longterm changes of the annual cycles of meteorological, hydrographic, nutrient and phytoplankton time series at Helgoland and the LV ELBE 1 in the German Bight. Cont Shelf Res 10:305-328

Raven JA (1997) Phagotrophy in phototrophs. Limnol Oceanogr 42:198-205

Raymont JEG (1983) Plankton and productivity in the oceans: Zooplankton, vol 2. Pergamon, New York

Reich PB, Tilman D, Craine J (2001) Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO_2 and N availability regimes? A field test with 16 grassland species. New Phytol 150:435-448

Reynolds CS (1997) Vegetation processes in the pelagic: A model for ecosystem theory. Ecology Institute, Oldendorf/Luhe

Riemann B, Havskum H, Thingstad F, Bernard C (1995) The role of mixotrophy in pelagic environments. *In*: Joint I (ed), Molecular Ecology of Aquatic Microbes. NATO ASI Series G 38. Springer, New York, pp 87-114

Rosenzweig ML (1971) Paradox of Enrichment: destabilisation of exploitation ecosystems in ecological time. Science 171:385-387

Rosenzweig ML (1995) Species diversity in space and time. Camebridge Univ Press, Cambridge

Rothhaupt KO (1996a) Utilizations and substitutable carbon and phosphorus sources by the mixotrophic chrysophyte *Ochromonas* sp.. Ecology 77:706-715

Rothhaupt KO (1996b) Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. Ecology 77:716-724

Rothhaupt KO, Güde H (1992) The influence of spatial and temporal concentration gradients on phosphate partitioning between different size fractions of plankton: Further evidence and possible causes. Limnol Oceanogr 37:739-749

Sanders RW (1991) Mixotrophic protists in marine and freshwater ecosystems. J Protozool 38:76-81

Sanders RW, Berninger UG, Lim EL, Kemp PF, Caron DA (2000) Heterotrophic and mixotrophic nanoplankton predation on picoplankton in the Sargasso Sea and on Georges Bank. Mar Ecol Prog Ser 192:103-118

Sanders RW, Porter KG, Caron DA (1990) Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte *Proteriochromonas malhamensis*. Microb Ecol 19:97-109

Sandgren CD (1988) The ecology of chrysophyte flagellates: their growth and perennation strategies as freshwater phytoplankton. *In*: Sandgren CD (ed) Growth and Reproductive Strategies of Freshwater Phytoplankton. Cambridge University Press, Cambridge, pp 9-104

Schindler DW (1998) A dim future for boreal waters and landscapes: cumulative effects of climate warming, stratospheric ozone depletion, acid precipitation and other human activities. BioScience 48:157-164

Schneider G (1992) A comparison of carbon-specific respiration rates in gelatinous and non-gelatinous zooplankton: a search for general rules in zooplankton metabolism. Helgoländer Meeresuntersuchungen 46:377-388

Schram FR (1986) Crustacea. Oxford Univ Press, New York

Shurin JB, Borer ET, Seabloom EW, Anderson K, Blanchette CA, Broitman B (2002) A cross-ecosystem comparison of the strength of trophic cascades. Ecol Lett 5:785-791

Shushkina EA, Vinogradov MY (1991) Long-term changes in the biomass of plankton in open areas of the Black Sea. Oceanology 31:716-721

Sibbald MJ, Albright LJ (1991) The influence of light and nutrients on phagotrophy by the mixotrophic nanoflagellate *Ochromonas* sp.. Mar Microb Food Webs 5:39-47

Smayda TJ (1990) Novel and nuisance blooms in the sea: evidence for a global epidemic. *In*: Granéli E, Sundström B, Edler L, Anderson DM (eds) Toxic Marine Phytoplankton. Elsevier, Amsterdam, pp 29-41

Sommer U (2000) Scarcity of medium-sized phytoplankton in the northern Red Sea explained by strong bottom-up and weak top-down control. Mar Ecol Progr Ser 197:19-25

Sommer U, Stibor H (2002) Copepoda – cladocera – tunicata: the role of three major mesozooplankton groups in pelagic food webs. Ecol Res 17:161-174

Sommer U, Stibor H, Katechakis A, Sommer F, Hansen T (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. Hydrobiologia 484:11-20

Steneck RS (1998) Human influences on coastal ecosystems: does overfishing create trophic cascades? TREE 13:429-430

Sterner RW, Clasen J, Lampert W, Weisse T (1998) Carbon:phosphorus stoichiometry and food chain production. Ecol Lett 1:146-150

Sterner RW, Elser JJ, Fee EJ, Guilford SJ, Chrzanowski TH (1997) The light:nutrient ratio in lakes: the balance of energy and materials affects ecosystem structure and process. Am Nat 150:663-684

Sterner, R. W., Clasen, J., Lampert, W., and T. Weisse. 1998. Carbon:phosphorus stoichiometry and food chain production. Ecol. Lett. 1: 146-150.

Stibor H, Vadstein O, Diehl S, Gelzleichter A, Hansen T, Hantzsche F, Katechakis A, Lippert B, Løseth K, Peters C, Roederer W, Sandow M, Sundt-Hansen L, Olsen Y (2004) Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. Ecol Lett 7:321-328

Tilman D, Kiesling R, Sterner RW, Kilham SS, Johnson FA (1986) Green, blue-green and diatom algae: taxonomic differences in competitive ability for phosphorous, silicon and nitrogen. Arch Hydrobiol 106:473-485

Tittel J, Bissinger V, Zippel B, Gaedke U, Bell E, Lorke A, Kamjunke N (2003) Mixotrophs combine resource use to outcompete specialists: Implications for aquatic food webs. Proc Nat Acad Sci 100:12776-12781

Toole DA, Siegel DA (2004) Light-driven cycling of dimethylsulfide (DMS) in the Sargasso Sea: Closing the loop. Geophys Res Letter 31:doi10.1029/2004GI019581

Turner JT, Tester PA, Ferguson RL (1988) The marine cladoceran *Penilia avirostris* and the "microbial loop" of pelagic food webs. Limnol Oceanogr 33:245-255

Urabe J, Kyle M, Makino W, Yoshida T, Andersen T, Elser JJ (2002a) Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. Ecology 83:619-627

Urabe J, Sterner RW (1996) Regulation of herbivore growth by the balance of light and nutrients. Proc Natl Acad Sci USA 93:8465-8469

Urabe J, Togari J, Elser JJ (2003) Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. Global Change Biology 9:818-825

Vadstein O (2000) Heterotrophic planktonic bacteria and cycling phosphorus. Phosphorus requirements, competitive ability and food web interactions. *In*: Schink B (ed) Advances in Microbial Ecology. Kluwer, pp 115-167

Valdes L, Harris R, Ikeda T, McKinnell S, Peterson WT (eds) (2004) The role of zooplankton in global ecosystem dynamics: Comparative studies from the world oceans. ICES J Mar Sci 61:441-737

Verity PG, Smetacek V (1996) Organism life cycles, predation, and the structure of marine pelagic ecosystems. Mar Ecol Progr Ser 130:277-293

Worm B (2000) Consumer versus resource control in rocky shore food webs. PhD thesis, University Kiel, pp 147

PAPER REPRINTS

PAPER A1

Journal of
Plankton Research

Feeding selectivities and food niche separation of *Acartia clausi, Penilia avirostris* (Crustacea) and *Doliolum denticulatum* (Thaliacea) in Blanes Bay (Catalan Sea, NW Mediterranean)

Katechakis A, Stibor H, Sommer U, Hansen T (2004)
***Journal of Plankton Research* 26:589–603**

JOURNAL OF PLANKTON RESEARCH | VOLUME 26 | NUMBER 6 | PAGES 589–603 | 2004

Feeding selectivities and food niche separation of *Acartia clausi*, *Penilia avirostris* (Crustacea) and *Doliolum denticulatum* (Thaliacea) in Blanes Bay (Catalan Sea, NW Mediterranean)

ALEXIS KATECHAKIS*, HERWIG STIBOR, ULRICH SOMMER[1] AND THOMAS HANSEN[1]

DEPARTMENT BIOLOGIE II, LUDWIGS-MAXIMILIANS-UNIVERSITÄT, ABTEILUNG AQUATISCHE ÖKOLOGIE, KARLSTRAßE 23–25, 80333 MÜNCHEN, GERMANY AND[1] INSTITUT FÜR MEERESKUNDE, CHRISTIAN-ALBRECHTS-UNIVERSITÄT, ABTEILUNG EXPERIMENTELLE ÖKOLOGIE, DÜSTERNBROOKER WEG 20, 24105 KIEL, GERMANY

*CORRESPONDING AUTHOR: katechakis@zi.biologie.uni-muenchen.de

Received on September 9, 2002; accepted on March 1, 2004; published online on March 24, 2004

Selectivity-size spectra, clearance and ingestion rates and assimilation efficiencies of Acartia clausi (Copepoda), Penilia avirostris (Cladocera) and Doliolum denticulatum (Doliolida) from Blanes Bay (Catalan Sea, NW Mediterranean) were evaluated in grazing experiments over a wide range of food concentrations (0.02–8.8 mm^3 L^{-1} plankton assemblages from Blanes Bay, grown in mesocosms at different nutrient levels). Acartia clausi reached the highest grazing coefficients for large algae >70 μm (longest linear extension), P. avirostris for intermediate food sizes between 15 and 70 μm, and D. denticulatum for small sizes from 2.5 to 15 μm. Penilia avirostris and D. denticulatum acted as passive filter-feeders. Acartia clausi gave some evidence for a supplementary raptorial feeding mode. Effective food concentration (EFC) decreased linearly with increasing nutrient enrichment for D. denticulatum and followed domed curves for A. clausi and for P. avirostris with maximum values at intermediate and high enrichment levels, respectively. Clearance rates of crustacean species showed curvilinear responses with narrow modal ranges to increasing food concentration. Clearance rates of D. denticulatum increased abruptly and levelled into a plateau at low food concentrations. Mean clearance rates were 13.9, 25.5 and 64.1 mL ind.$^{-1}$ day^{-1}, respectively. No clearance could be detected for A. clausi at food concentrations <0.1 mm^3 L^{-1} and for P. avirostris at food concentrations ≤0.02 mm^3 L^{-1}. Ingestion rates indicate a rectilinear functional response for A. clausi and for P. avirostris and showed a sigmoidal curve for D. denticulatum. Mean ingestion rates were 1.3, 2.8 and 7.7 μg C μg C$_{ind.}$$^{-1}$ day^{-1}, respectively. Conversion of ingested carbon to tissue was 30–80% for the investigated crustaceans and 20–50% for doliolids. Food niche calculations suggest that food niche separation may explain the coexistence of the three species in summer in Blanes Bay.

INTRODUCTION

The grazing behaviour of mesozooplankton is one of the critical factors structuring pelagic food webs. Mesozooplankton distribute the organic matter synthesized by autotrophs towards higher trophic levels. There are many studies concerning grazing of marine copepods. Gelatinous mesozooplankton and marine cladocerans are poorly investigated, though seasonally they may dominate zooplankton communities (Alldredge and Madin, 1982; Deibel, 1982, 1998; Crocker *et al.*, 1991; Paffenhöfer *et al.*, 1991). Likewise, the calanoid copepod *Acartia clausi*, the cladoceran *Penilia avirostris* and the doliolid *Doliolum denticulatum* may dominate the mesozooplankton community in summer in Blanes Bay (Catalan Sea, NW Mediterranean) in terms of both abundance and biomass (Andreu and Duarte, 1996). Although they obviously depend on the same resources and their carbon contents (this article) and energy requirements per carbon

doi: 10.1093/plankt/fbh062, available online at www.plankt.oupjournals.org

unit (Ikeda, 1985; Schneider, 1992) are similar, they coexist. This indicates different niche specifications regarding their dietery preferences.

To study the feeding selectivities and potential niche overlap of *A. clausi*, *P. avirostris* and *D. denticulatum*, we conducted short-term grazing experiments with plankton assemblages of Blanes Bay, grown at different nutrient levels.

METHOD

Experiments were performed as batch cultures in 100 mL glass jars. The jars were placed randomly in a water bath at ~22°C. The *in situ* surface temperature in Blanes Bay was 25–26°C (measured with a WTW LF 20 temperature sensor). The jars were filled with the plankton assemblages, including bacterioplankton, protozoa and phytoplankton, found in summer in Blanes Bay. Plankton assemblages were grown in mesocosms 0.8 km off-shore at different nutrient levels. The mesocosm units (33 m^3) received N, P and Si at a stoichiometric ratio of 20 N:7 Si:1 P, at the normal nutrient loading rate at the site (5 mmol N m^{-2} day^{-1} and 0.25 mmol P m^{-2} day^{-1}), and at 0.5 to 16 times the normal nutrient loading rate [for more details see (Duarte *et al.*, 2000)]. Enrichment resulted in nine different food densities covering a biovolume range between 0.02 and 8.81 mm^3 L^{-1} (Table I) and a range of seston food sizes from <1 to 300 μm at the longest linear extension (Tables I and II). The higher the enrichment level, the higher the community's density and the bigger its size spectrum. Community 5 resembled the conditions *in situ* in summer in Blanes Bay.

To exclude extraneous metazoan grazers from the jars, water was filtered through a 100 μm mesh size plankton net. Filtration allowed sufficient needle-shaped algae >100 μm to pass, so that the plankton communities offered as food contained enough food species >100 μm (at the longest linear extension). For each of the nine food densities five adult copepods (*Acartia clausi* females), five adult cladocerans (*Penilia avirostris*), or three doliolids (solitary gonozooids of *Doliolum denticulatum*) were incubated once for 6 h in the dark. Grazers were collected with surface tows using a mesozooplankton net with a mesh size of 250 μm, and were returned to the laboratory within 1 h of collection inside a cooler. A plastic bag in the cod end of the net prevented the animals from being much damaged.

Experimental grazers were sorted with a wide-bore pipette. Between 24 and 36 individuals were measured, placed into filtered sea water and allowed to acclimate to the laboratory conditions for 1 h before being incubated for the experiments. The decision was made to incubate fewer doliolids than crustacean grazers because of different dry weights (Table III). Dry weights were determined by filtering batches of 19 to 39 animals on precombusted Whatman GF/C filters. The filters were dried before and after the filtration at 60°C for 24 h and weighed each time with a Sartorius microbalance to the nearest μg. Dry weights were obtained from calculating the weight differences. For the determination of particulate carbon and nitrogen the same filters were measured with

Table I: Densities, size spectra, main food size and relative biovolume of main food size of plankton communities from Blanes Bay (NW Mediterranean) offered as food in grazing experiments with Acartia clausi, Penilia avirostris and Doliolum denticulatum

Food plankton community	Concentration (mm^3 L^{-1})	Size spectrum (μm)	Main size class (interval means in μm)	Relative biovolume of main size class (%)
1	0.023	<1–50	2.5	35.96
2	0.074	<1–60	2.5	41.58
3	0.091	<1–85	10.25	59.53
4	0.363	<1–125	42.5	54.59
5	0.364	<1–125	85	35.12
6	1.290	<1–205	85	57.88
7	5.460	<1–250	125	86.88
8	5.940	<1–250	175	78.85
9	8.807	<1–300	>210	53.85

All food sizes are based on the longest linear extension of food organisms. Community 5 resembles the conditions *in situ* in summer in Blanes Bay.

Table II: Taxonomic list of all plankton offered as food in grazing experiments

	Longest extension (μm)	Biovolume (μm^3 cell^{-1})	Carbon content (pg C cell^{-1})
Picoplankton			
around 1 μm	1	0.52	0.05
Nanoplankton			
around 2.5 μm	2.5	8.2	0.82
around 5 μm	5	65	6.5
Cyanobacteria			
Filamentous	20	98	9.8
Bacillariophycaea			
Centrales			
Bacteriastrum sp.	30	9425	943
Coscinodiscus sp.	12.5–150	920–530 144	92–53 014
Chaetoceros socialis	17.5–52.5	221–663	22–66
Chaetocerus sp. A	25	295	30
Chaetocerus sp. B	75	2356	236
Rhizosolenia alata	60–125	1178–22 089	118–2209
Rhizosolenia fragilissima	125–250	22 089–44 178	2209–4418
Rhizosolenia shrubsolei	300	5890	589
Skeletonema costatum	32.5–195	1436–8616	144–862
Stephanopyxis sp.	120	85 765	8577
Thalassiosira sp.	42.5–255	7510–45 060	751–4506
Pennales			
Licmophora sp.	70–120	13 779–94 162	1378–9416
Navicula sp.	15	147	15
Nitzschia longissima	75–200	125–2344	13–234
Pleurosigma sp.	50	1094	109
Dinophyceae			
Dinophysiales			
Dinophysis rotundata	40	4712	471
Dinophysis sp.	25	2045	205
Peridiniales			
Ceratium lineatum	100	8357	836
Ceratium longipes	180	74 286	7429
Gymnodinium sp.	15	785	79
Heterocapsa triquetra	20	1178	118
Peridinium sp. A	15	1767	177
Peridinium sp. B	55	40 497	4050
Prorocentrales			
Prorocentrum micans	30	2209	221
Prymnesiophyceae			
Coccolithus sp.	7.5	221	22
Other flagellates			
ANF spp.	2.5–10	8.2–523	1.8–73
HNF spp.	2.5–10	8.2–523	1.8–73
Ciliata			
Ciliate sp. A	15	4712	660
Ciliate sp. B	25	29 452	4123

Biovolumes were calculated using the equations of Hillebrand *et al.* (Hillebrand *et al.*, 1999). Carbon contents were estimated after Nalewajko (Nalewajko, 1966) for phytoplankton, after Bøsheim and Bratbak (Bøsheim and Bratbak, 1987) for flagellates, and after DeBiase *et al.* (DeBiase *et al.*, 1990) for ciliates. ANF, autotrophic nanoflagellates; HNF, heterotrophic nanoflagellates.

Table III: Sizes, biovolumes, dry weights, biomasses and C:N ratios of Acartia clausi, Penilia avirostris and Doliolum denticulatum incubated in grazing experiments

	n	Size (μm)	Biovolume (mm^3 $ind.^{-1}$)	*n*	Animals on filters	Dry weight (mg $ind.^{-1}$)	Biomass (μg C $ind.^{-1}$)	Biomass (μg N $ind.^{-1}$)	C:N
D. denticulatum	24	1480 ± 127	1.07 ± 0.18	2	59	0.41 ± 0.03	2.73 ± 0.15	0.61 ± 0.05	4.50 ± 0.09
P. avirostris	25	680 ± 44	0.12 ± 0.02	3	87	0.25 ± 0.04	2.17 ± 0.06	0.39 ± 0.02	4.84 ± 0.39
A. clausi	36	920 ± 31	0.12 ± 0.01	3	92	0.23 ± 0.01	3.80 ± 0.34	0.84 ± 0.14	4.57 ± 0.43

Means ± SEM are based on *n* measurements of animals and filters, respectively (see text for details).

a Fisons CN-analyser (NA 1500N) using acetanilide (71.09% C, 10.30% N) as standard. Measurements were duplicated for doliolids and triplicated for copepods and cladocerans.

Swimming behaviour was observed at the beginning of the experiments and several times during the experimental terms as a way of checking whether the animals were intact. To prevent food plankton sedimentation, the vessels were also mixed gently on these occasions. In addition, mesozooplankton swimming caused some turbulence in the flasks. To account for possible changes in the species composition of the food guilds during the experiment, start samples were taken and compared with controls without mesozooplankton. After the incubation period, the experiments were terminated by addition of Lugol's iodine (5 g I_2 + 10 g KI in 100 mL aq. dest.) to all vessels.

To determine grazer-induced changes in abundance, the species composition, the biovolume and the biomass of the food guild, samples were counted using an inverted microscope (Leica DMIL) and settling chambers with a volume of 10 or 30 mL, depending on the food guild's density (Utermöhl, 1958). Sedimentation time was at least 24 h. If present, at least 400 cells were counted for each species to ensure an error <10% (Lund *et al.*, 1958). Biovolumes were calculated using the equations of Hillebrand *et al.* (Hillebrand *et al.*, 1999). For this purpose, the linear dimensions of 20 specimens were measured for each species. Carbon contents were estimated after Nalewajko (Nalewajko, 1966) for phytoplankton, after Bøsheim and Bratbak (Bøsheim and Bratbak, 1987) for flagellates, and after DeBiase *et al.* (DeBiase *et al.*, 1990) for ciliates (Table II).

For all following analyses the plankton community was subdivided into nine size classes with interval means of 1, 2.5, 5, 10.25, 42.5, 85, 125, 175 and 205 μm and into organisms >210 μm. All food sizes are based on the longest linear extension of food organisms. Colonial species were assigned to classes according to the longest linear extension of colonies. Pico-, nano- and microplankton are used to describe food sizes ranging from ~0.2 to 2 μm, from 2 to 20 μm and from 20 to 200 μm.

Selectivity coefficients and effective food concentrations

Grazing coefficients g (day^{-1}) of *A. clausi*, *P. avirostris* and *D. denticulatum* were measured by calculating the differences between food concentrations at the beginning and at the end of the experiments using the equations of Frost (Frost, 1972):

$$g = \mu - \frac{\ln C_1^* - \ln C_0^*}{t_1 - t_0} \quad \text{with} \quad \mu = \frac{\ln C_1 - \ln C_0}{t_1 - t_0},$$

where μ is the gross growth rate of food organisms, C_1 and C_0 are the food concentrations (mm^3 L^{-1}) at the end (t_1) and at the beginning (t_0) of the experiment in the controls, and C_1^* and C_0^* are the food concentrations in treatments with grazers.

The grazing coefficients, *g*, were used to study selectivity through the normalized selectivity coefficient W' defined by Vanderploeg and Scavia (Vanderploeg and Scavia, 1979) and modified after Vanderploeg *et al.* (Vanderploeg *et al.*, 1984):

$$W' = \frac{g_i}{g_{max}}$$

where g_i is the the grazing coefficient reached for food size class i and g_{max} is the grazing coefficient for the most preferred size class ($0 < W' < 1$).

According to Vanderploeg *et al.* (Vanderploeg *et al.*, 1984), these W' values were used to estimate the effective food concentrations (EFCs) for every grazer and every plankton community offered as food:

$$EFC = \sum_{i=1}^{n} W' \cdot X_i$$

where X_i is the concentration of food size class i and *n* is the total number of size classes.

Clearance rates and ingestion rates

Clearance rates F [mL individual (ind.)$^{-1}$ day^{-1}] and ingestion rates I (μg C ind.$^{-1}$ day^{-1}) were calculated according to Frost (Frost, 1972):

$$F = V \cdot \frac{g}{N_G} \quad \text{and} \quad I = F \cdot \bar{C},$$

where V is the jar volume (mL), g is the grazing coefficient (day^{-1}), N_G is the number of incubated animals, and $\bar{C}$ is the mean food concentration (μg C mL^{-1}) in the experimental vessel.

Assimilation efficiencies

A radioisotope technique was used to measure assimilation rates of *A. clausi*, *P. avirostris* and *D. denticulatum*. Tissue culture flasks (250 mL) were filled with the same plankton assemblages as used in the grazing experiments described above and then incubated with 20 μCi ^{14}C for 24 h. This ensured an even radioactive labelling of the included plankton. Twenty copepods, 20 cladocerans, or 20 doliolids were allowed to feed on each of the radioactive labelled plankton assemblages. After 8 min, the estimated time needed to fill the digestive tract, the grazers were removed from the flasks and washed in Whatman-GF/F-filtered sea water to remove the attached radioactivity. Half of the animals were put into scintillation vials, the other half were transferred to non-radioactive food of the same type and concentration. After allowing the animals to feed on non-radioactive food for 15 min, the animals were removed, assuming that they had cleared their digestive tracts of radioactive material. Afterwards all animals were radio-assayed.

Ingestion was calculated from the amount of radioactive material eaten before the animals started to reject the undigested remains of radioactively labelled food. Assimilation was calculated from the amount of radioactivity left in the animals after they had cleared their digestive tracts. For all radio-assays, animals were first dissolved with a tissue solubilizer (Soluene 350), and Hionic Fluor was added as a scintillation reagent. All measurements were made in triplicate and carried out with a Packard Tricarb 1800 scintillation counter. Replicates with animals that were damaged during the washing procedure or with animals that showed an abnormal swimming behaviour afterwards remained unaccounted for.

Food niche calculations

To quantify the different degrees of specialization of the investigated mesozooplankton grazers, their food niche breadths were measured, using Hurlbert's standardized niche breadth B'_A [(Hurlbert, 1978) *cf.* (Krebs, 1999)]:

$$B'_A = \frac{B' - a_{min}}{1 - a_{min}} \quad \text{with} \quad B' = \frac{1}{\sum_{i=1}^{n} (p_i^2 / a_i)}$$

where B' is Hurlbert's niche breadth, p_i is the proportion that size class i is of the total resources used by the studied grazer group ($\Sigma p_i = 1$), a_i is the proportion that size class i is of the total resources ($\Sigma a_i = 1$), and a_{min} is the smallest observed proportion of all size classes. B'_A can take values from 0 to 1; the higher the value, the broader the niche, the less selective the consumer.

To infer the degree of interspecific competition between the investigated grazers the extent of food size niche overlap was calculated with the Morosita–Horn index C_H [(Horn, 1966) *cf.* (Krebs, 1999)]:

$$C_H = \frac{2 \sum_{i=1}^{n} p_{ij}\, p_{ik}}{\sum_{i=1}^{n} p_{ij}^2 + \sum_{i=1}^{n} p_{ik}^2},$$

where p_{ij} and p_{ik} are the proportions that size class i is of the total resources used by the two grazer groups j and k, and n is the total number of size classes.

For statistical analysis SigmaStat 2.0 software was used.

RESULTS

Selectivity-size spectra, selectivity profiles and effective food concentrations

Figure 1 shows the selectivity-size spectra of *A. clausi*, *P. avirostris* and *D. denticulatum*, expressed as the selectivity coefficient W' of Vanderploeg and Scavia (Vanderploeg and Scavia, 1979), based on the grazing coefficients presented in Table IV. Values for W' are overall means calculated from the single experiments shown in Figure 2. As not all plankton communities offered as food covered the whole size range of all food size classes (Table I, Figure 2), calculation of means and standard errors are based on three to nine measurements (n in Table IV). All grazing coefficients measured are significantly different from zero at $P < 0.01$ (99% CI). All food sizes are based on the longest linear extension of food organisms. *Doliolum denticulatum* filtered the entire food size range from the smallest sizes detected by counting, ~1 μm, to large phytoplankton, ~75 μm. *Penilia avirostris* reached the highest grazing coefficients at intermediate food sizes between 15 and 70 μm. Lower size limits were >2.5 μm, thus including nanoflagellates

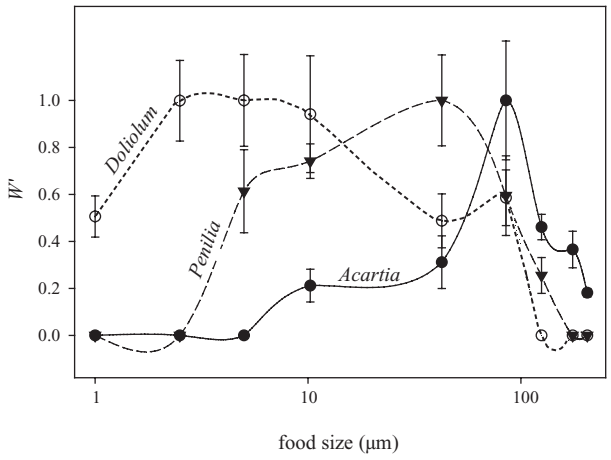

Fig. 1. Food niche separation and niche overlap of *Acartia clausi*, *Penilia avirostris* and *Doliolum denticulatum*, based on the selectivity coefficients *W′* of Vanderploeg and Scavia (Vanderploeg and Scavia, 1979) presented in Table IV. All food sizes are based on the longest linear extension of food organisms. Data points are means of three to nine measurements (see text for details). Error bars represent ± SE of the means. All grazing coefficients are significantly different from zero at $P < 0.01$ (99% CI). Note logarithmic scale of food size axis.

and ciliates, and upper ones were ~100 μm. *Acartia clausi* did not ingest particles <7.5 μm and showed the highest values for large diatoms ≥70 μm. The upper size limit was 210 μm.

The selectivity profiles of *D. denticulatum* and *P. avirostris* followed the food size patterns only at low total food concentrations (TFCs ≤0.09 and ≤0.36 mm^3 L^{-1}, respectively) (Figure 2). *Doliolum denticulatum* always showed the highest selectivity coefficients for pico- and small nanoplankton, even if other food sizes dominated the community. In cases where food sizes ≥70 μm prevailed (TFC ≥1.3 mm^3 L^{-1}, communities 6 to 9) *D. denticulatum* also established high grazing coefficients for particles ~85 μm. Similarly, *P. avirostris* normally reached highest selectivity coefficients for intermediate food sizes, but expressed high grazing rates on bigger food items ~85–125 μm, when these food sizes were dominant (TFC 1.3–5.5 mm^3 L^{-1}, communities 6 and 7). The selectivity curve of *A. clausi* always matched the peak of the particle-size spectrum; with one exception at very high TFC (8.8 mm^3 L^{-1}, community 9), dominated by food particles >210 μm, not ingestable for *A. clausi*. Community 5 resembles the conditions *in situ* in summer in Blanes Bay.

The EFC calculated for each grazer in every experiment depended on TFC (Figure 3). EFC decreased linearly with increasing TFC for *D. denticulatum* and followed domed curves for *A. clausi* and for *P. avirostris* with maximum values at intermediate and high TFC, respectively (for regression equations see Figure 3). Maximum EFC were 84.2 ± 2.7% SE of the means (based on the three highest values measured) for *D. denticulatum* at TFC <0.1 mm^3 L^{-1}, characterized by food particles <15 μm; 58.8 ± 5.6% for *P. avirostris* (TFC 0.1–0.4 mm^3 L^{-1}, main food size classes 10.25–85 μm); and 57.1 ± 6.1% for *A. clausi* (TFC 0.4–5.5 mm^3 L^{-1}, main food size classes 85–125 μm).

Clearance rates and ingestion rates

Clearance rates of *A. clausi* and of *P. avirostris* showed curvilinear responses with narrow modal ranges to increasing

Table IV: Mean grazing coefficients g (day^{-1}) and selectivity coefficients W′ of Acartia clausi, Penilia avirostris and Doliolum denticulatum for different food sizes (longest linear extension of food organisms)

Food size	Interval		*Acartia clausi*		*Doliolum denticulatum*		*Penilia avirostris*	
class (μm)	mean (μm)	*n*	g (day^{-1})	*W′*	g (day^{-1})	*W′*	g (day^{-1})	*W′*
around 1	1	9	0	0	0.14 ± 0.03	0.51 ± 0.09	0	0
around 2.5	2.5	9	0	0	0.28 ± 0.05	0.99 ± 0.17	0	0
>2.5 to <7.5	5	9	0	0	0.28 ± 0.06	**1.00** ± 0.20	0.05 ± 0.01	0.61 ± 0.18
7.5 to <15	10.25	9	0.12 ± 0.04	0.21 ± 0.07	0.27 ± 0.07	0.94 ± 0.25	0.06 ± 0.01	0.74 ± 0.07
15 to <70	42.5	9	0.17 ± 0.06	0.31 ± 0.11	0.14 ± 0.03	0.49 ± 0.11	0.08 ± 0.02	**1.00** ± 0.19
70 to <100	85	7	0.56 ± 0.14	**1.00** ± 0.25	0.17 ± 0 03	0.59 ± 0.12	0.05 ± 0.01	0.60 ± 0.17
100 to <150	125	6	0.26 ± 0.03	0.46 ± 0.05	0	0	0.02 ± 0.01	0.25 ± 0.08
150 to <200	175	4	0.20 ± 0.04	0.37 ± 0.08	0	0	0	0
200 to <210	205	4	0.10 ± 0.01	0.18 ± 0.02	0	0	0	0
≥210	–	3	0	0	0	0	0	0

Means ± SEM are based on three to nine measurements of *n* (see text for details). All grazing coefficients are significantly different from zero at $P < 0.01$ (99% CI).

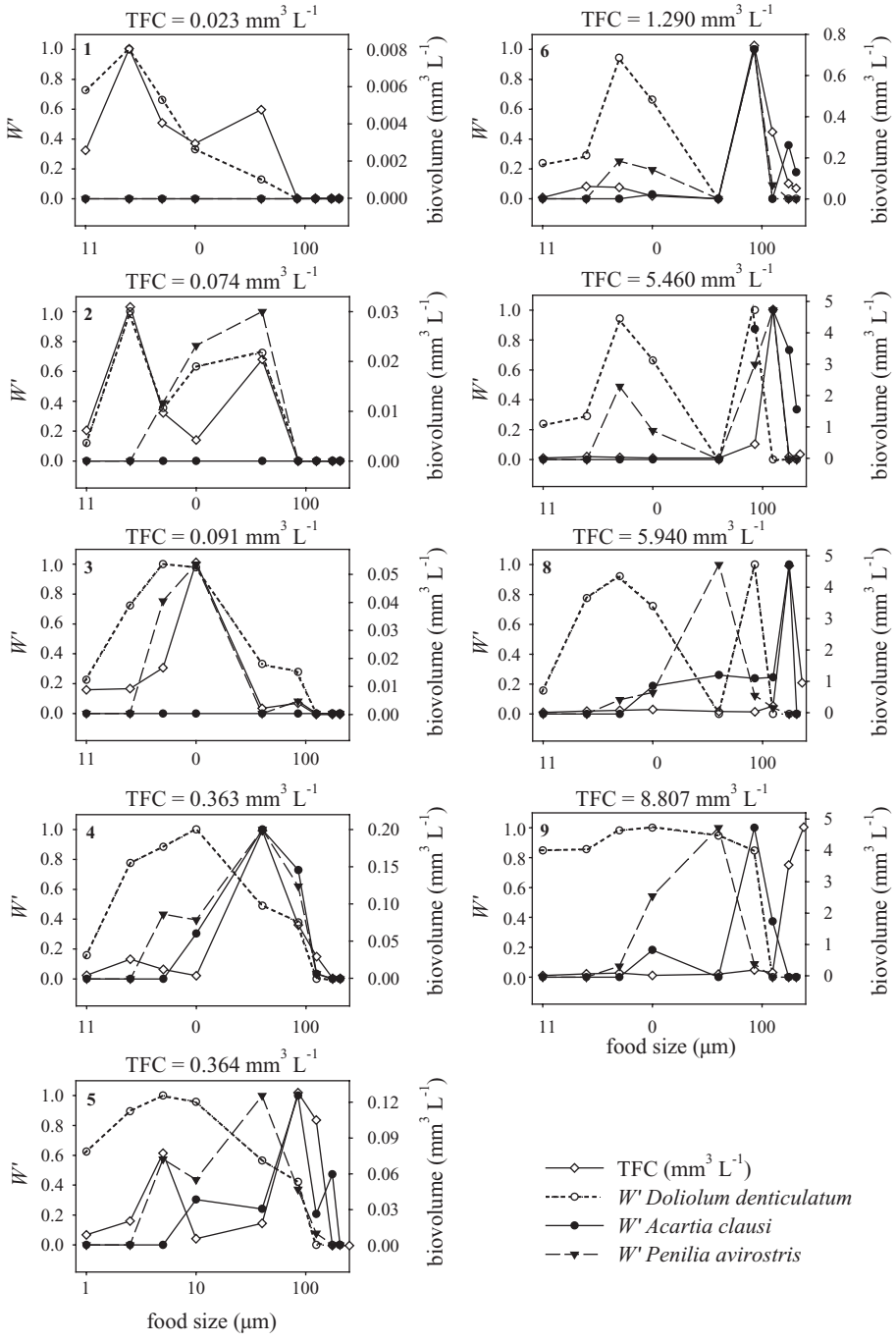

Fig. 2. Selectivity coefficient curves W' of *Acartia clausi*, *Penilia avirostris* and *Doliolum denticulatum* for different food sizes (longest linear extension of food organisms) at different total food concentrations (TFCs) as found in plankton communities (1–9) from Blanes Bay (NW Mediterranean) offered as food in grazing experiments. Community 5 resembles the conditions *in situ* in summer in Blanes Bay. Note logarithmic scale of food size axis.

food concentration [turning points at ~2 and 1 mm^3 L^{-1}, respectively; (Figure 4) using adjustments of the data by eye]. Clearance rates of *D. denticulatum* increased abruptly within a small range of low food concentrations from minimum values measured at 0.02 mm^3 L^{-1} (2.4 mL ind.$^{-1}$ day^{-1}) to values close to maximum rates around 100 mL ind.$^{-1}$ day^{-1} at 0.4 mm^3 L^{-1}. Clearance rates levelled afterwards into a wide plateau, and decreased slightly at ~6 mm^3 L^{-1}. No clearance could be detected for *A. clausi* at food concentrations ≤0.09 mm^3 L^{-1}

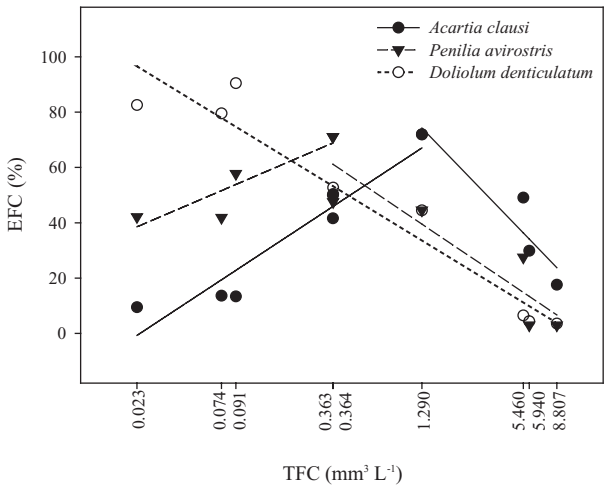

Fig. 3. Effective food concentration (EFC) of *Acartia clausi*, *Penilia avirostris* and *Doliolum denticulatum* as a function of total food concentration (TFC). The respective relationships between EFC and TFC are described by the following linear functions: $EFC_{Ac \text{ for } TFC \leq 1.3} = 62.81 + 16.72$ lnTFC, $r^2 = 0.91$, $P < 0.01$, $F_{(1,4)} = 40.8$; $EFC_{Ac \text{ for } TFC \geq 1.3} = 80.93 - 26.28$ lnTFC, $P < 0.1$, $r^2 = 0.84$, $F_{(1,2)} = 13.1$; $EFC_{Pen \text{ for } TFC \leq 0.4} = 79.89 + 10.88$ lnTFC, $r^2 = 0.78$, $P < 0.1$, $F_{(1,2)} = 7.2$; $EFC_{Pen \text{ for } TFC \geq 0.4} = 43.84 - 17.12$ lnTFC, $r^2 = 0.84$, $P < 0.01$, $F_{(1,4)} = 20.6$; $EFC_{Dol} = 37.53 - 15.54$ lnTFC, $r^2 = 0.94$, $P < 0.0001$, $F_{(1,7)} = 100.0$. Note logarithmic scale of TFC axis.

(equivalent to $\leq$9 μg C L^{-1}), and for *P. avirostris* at food concentrations of 0.02 mm^3 L^{-1} (equivalent to 1.5 μg C L^{-1}) (Figures 2 and 4). Mean clearance rates were 13.9 ± 4.5 mL $ind.^{-1}$ day^{-1} for *A. clausi*, 25.5 ± 5.5 mL $ind.^{-1}$ day^{-1} for *P. avirostris*, and 64.1 ± 11.9 mL $ind.^{-1}$ day^{-1} for *D. denticulatum*. Maximum values were 38.1, 54.9 and 107.3 mL $ind.^{-1}$ day^{-1}, respectively.

Ingestion rates increased linearly with increasing food supply for the two crustacean species until a concentration threshold (around 340 μg C L^{-1}, equivalent to 3.6 mm^3 L^{-1}, for *A. clausi* and around 270 μg C L^{-1}, equivalent to 2.8 mm^3 L^{-1}, for *P. avirostris*) beyond which the relations suggest a plateau, and followed a sigmoidal curve for *D. denticulatum* (Figure 4). Mean weight-specific ingestion rates were 1.3 ± 0.5 μg C μg C_{ind}^{-1} day^{-1} for *A. clausi*, 2.8 ± 1.2 μg C μg C_{ind}^{-1} day^{-1} for *P. avirostris* and 7.7 ± 3.5 μg C μg C_{ind}^{-1} day^{-1} for *D. denticulatum*. Maximum absolute ingestion values measured were 13.9, 15.7 and 67.8 μg C $ind.^{-1}$ day^{-1}, respectively.

Assimilation efficiencies

Assimilation was measured as the percentage of the ingested carbon that was incorporated into the body tissue. Over a broad range of food concentrations *A. clausi* and *P. avirostris* showed assimilation efficiencies of about 40–80% and 30–70%, respectively. Assimilation efficiencies of *D. denticulatum* were lower, with values between 20 and 50%, with one exception at very low food concentration (1.5 μg C L^{-1}, equivalent to 0.02 mm^3 L^{-1}) when assimilation efficiency reached 72% (Figure 5). The range of assimilation efficiencies of crustacean grazers did not differ significantly from each other, and was significantly higher than the range for doliolids (one-way analysis of variance, $P < 0.05$, $F_{(2,32)} = 3.4$ and *post hoc* Tukey-test analyses, $P < 0.05$). Mean assimilation efficiencies differed significantly between all grazers and amounted to 54.8 ± 1.4% for *A. clausi*, to 47.7 ± 1.1% for *P. avirostris* and to 40.5 ± 0.8% for *D. denticulatum*. Assimilation efficiencies of *P. avirostris* ($P \leq 0.1$, $F_{(1,10)} = 3.2$) and of *D. denticulatum* ($P \leq 0.001$, $F_{(1,12)} = 17.7$) decreased significantly with increasing food concentration (linear regressions, for equations see Figure 5). No such significant relationship could be found for *A. clausi*.

Food niche calculations

Doliolids occupied the broadest food niche. Copepods showed the narrowest food niche. The food-niche breadth of cladocerans lay between those of the other grazer groups studied (Figure 6). Differences were not significant (95% CI).

Considering all the experiments, niche overlap was highest among *P. avirostris* and *D. denticulatum* (72.24%) and lowest between *D. denticulatum* and *A. clausi* (39.04%). The selectivity-size spectra of *A. clausi* and *P. avirostris* overlapped to an extent of 62.51% (Figure 1).

DISCUSSION

Copepods, cladocerans and doliolids are the most important mesozooplankton taxa in the NW Mediterranean. Especially the feeding selectivities of marine cladocerans and doliolids are not well known, mainly as a result of the difficulties with culturing these animals. In Spain we had the possibility to conduct grazing experiments with *A. clausi*, *P. avirostris* and *D. denticulatum*, captured a short time before the experiments started. Our results show that the three species differ substantially in their feeding habits, which may help to explain their coexistence in summer in Blanes Bay. Doliolids behaved as passive filter-feeders that ingest food only as a result of anatomical constraints. They encompassed the broadest food niche of all grazers, were the most efficient in filtering pico- and small nanoplankton at low food concentrations and reached the highest clearance and ingestion rates. On the other hand, they attained the lowest assimilation efficiencies and could not adjust their filtration rates to changing food supplies. In contrast, the investigated copepods occupied the narrowest food niche and fed on the biggest food items among all grazers; these occurred in particular at higher enrichment levels. They showed the lowest clearance and ingestion rates, but in return, they reached the highest assimilation efficiencies

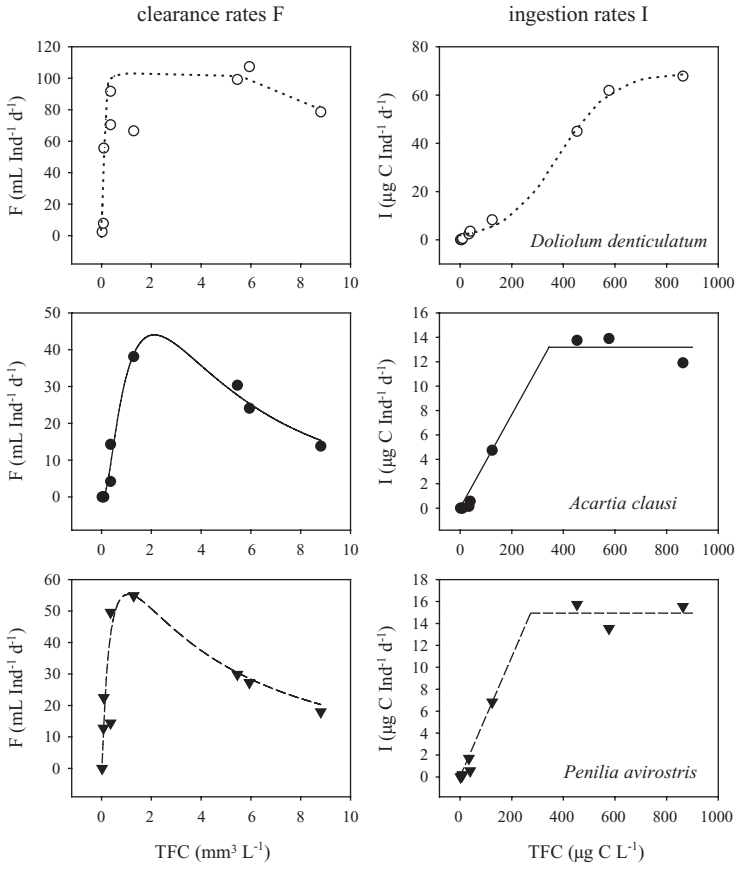

Fig. 4. Clearance rates and ingestion rates of *Acartia clausi*, *Penilia avirostris* and *Doliolum denticulatum* from the NW Mediterranean at different total food concentrations (TFCs) (adjustments of the data performed by eye).

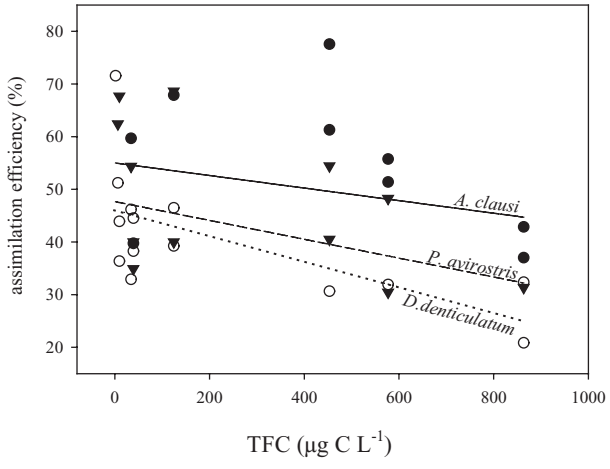

Fig. 5. Assimilation efficiency (A%) of *Acartia clausi* (● *Ac*), *Penilia avirostris* (▼ *Pen*), and *Doliolum denticulatum* (○ *Dol*) as a function of total food concentration (TFC). Assimilation efficiencies of *P. avirostris* and of *D. denticulatum* decreased significantly with increasing food concentration: $A\%_{Pen} = 47.67 - 0.02$ TFC, $P \leq 0.1$, $F_{(1,10)} = 3.2$; $A\%_{Dol} = 42.58 - 0.02$ TFC, $P \leq 0.001$, $F_{(1,12)} = 17.7$. The regression for *A. clausi* ($A\%_{Ac} = 55.01 - 0.12$ TFC) was statistically not significant ($P = 0.366$).

and were able to adjust their clearance rates to changing food concentrations, keeping their ingestion rates stable over a wide range of food densities. Moreover, copepods were the only grazers that probably selected actively beneficial prey. Cladocerans acted as passive filter-feeders like the doliolids, but were more similar to copepods in their functional responses. In general, they seem to be better adapted to intermediate food concentrations and sizes, provided by moderate nutrient levels.

Selectivity-size spectra, selectivity profiles and effective food concentrations

Penilia avirostris covered a food size range of >2.5 to 100 µm. The lower size limit is consistent with the results of Paffenhöfer and Orcutt (Paffenhöfer and Orcutt, 1986) who observed a lower size limit of 2.2 µm. In correspondspondence with Turner *et al.* (Turner *et al.*, 1988), our results indicate no grazing on bacterioplankton. This is in contrast to previous findings (Pavlova, 1959; Sorokin *et al.*, 1970). We did not perform specific bacterial counts in the present experiments, but Katechakis *et al.* (Katechakis *et al.*, 2002) showed that *P. avirostris* influences the bacterioplankton

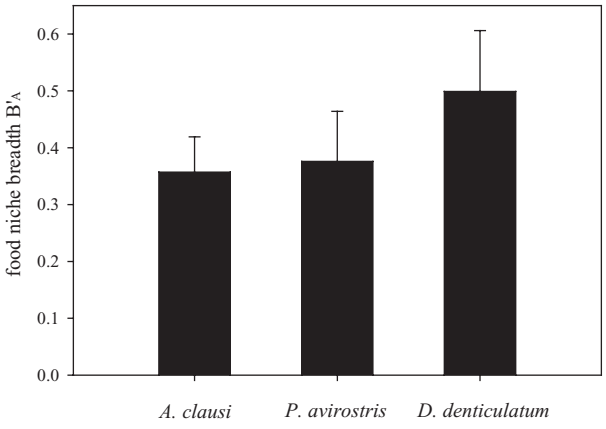

Fig. 6. Food niche breadth of *Acartia clausi*, *Penilia avirostris* and *Doliolum denticulatum* calculated as Hurlbert's standardized niche breadth (B'_A). Error bars represent 95% CI.

only indirectly via a trophic cascade by grazing on nanoflagellates. Other authors (Gore, 1980; Paffenhöfer and Orcutt, 1986; Turner *et al.*, 1988; Kim *et al.*, 1989) reported upper size limits of 15–50 μm. The difference in the upper size limit between our results and those in other studies results from the use of different size scales. Other authors refer to upper size limits as measured by particle width or equivalent spherical diameters (ESD). We based our analyses on the longest linear cell and colony extensions, as ESD may disguise the real dimension of particles that may be handled. In our experiments, grazing coefficients for sizes >37.5 μm result exclusively from feeding on needle-shaped species (*Nitzschia longissima* and *Rhizosolenia* spp.) and on long-chain diatoms (*Skeletonema costatum* and *Thalassiosira* sp.). With valve diameters between 5 and 20 μm these species could be ingested by *P. avirostris* if orientated longitudinally in the filtering current. Therefore, the upper size limit found by us lies within the size spectrum found by other authors, if expressed as particle width.

Doliolum denticulatum filtered the entire food size range from the smallest sizes detected by counts, ~1 μm, to large phytoplankton, ~75 μm, at which grazing coefficients for sizes >35 μm are based on the same longish diatom groups as described for *P. avirostris*. High grazing coefficients of 0.14 day^{-1} (equivalent to 51% of the maximum grazing coefficient, Table IV) for picoplankton indicate that high grazing pressure occurred also on particles <1 μm. Katechakis *et al.* (Katechakis *et al.*, 2002) documented the ingestion of bacteria with sizes ~0.5 μm by *D. denticulatum*. Thus, doliolids were the only mesozooplankton feeding efficiently on particles as small as those consumed by many protozoa [e.g. (Fenchel, 1980)]. This ability is of additional advantage in low-nutrient environments that mainly support the growth of very small algae and do not allow the establishment of large-scale algae [this article (Raven, 1986; Stockner and Antia, 1986; Duarte *et al.*, 2000; Sommer, 2000)]. Besides, relative picoplankton biomass remains more or less constant throughout the year (Harris, 1986; Mura *et al.*, 1996), presenting a safe food source. The ability to take up very small food particles may be an explanation for the great success of tunicates and the gelatinous morphotype in general, especially in ultra-oligotrophic environments (Raymont, 1983); together with decreased gravitational stresses as a result of their watery body tissue (Harbison, 1992), high growth rates among metazoans (Deibel, 1998) and adaptation of their filter apparatus to extremely low food concentrations (Acuña, 2001).

Acartia clausi did not ingest particles <7.5 μm. Feeding on particles >50 μm was based on elongated diatoms with lengths up to 210 μm but valve diameters ≤20 μm. If expressed as particle width, the selectivity-size spectrum found by us is more or less consistent with the results of Nival and Nival (Nival and Nival, 1976) and of Pagano *et al.* (Pagano *et al.*, 2003) who report a size spectrum from 3 μm to at least 50 and 36 μm ESD, respectively. If expressed as the longest linear dimension, it corresponds with the findings of Hodgkin and Rippingale (Hodgkin and Rippingale, 1971) who showed that *A. clausi* may collect particles up to ~250 μm. In contrast to cladocerans and doliolids, copepods did not feed on components of the microbial food web in our experiments. They ingested even larger ciliates at only a very low rate. Gismervik and Andersen (Gismervik and Andersen, 1997) showed that *A. clausi* may switch between algal and ciliate food depending on their respective abundance. Therefore, our findings may also result from low ciliate abundances (<<1 to 4.2% of total biovolume). It is known that *A. clausi* can affect the microbial food web indirectly via a trophic cascade (Katechakis *et al.*, 2002).

Within the size-limits of ingestion the selectivity profile of *A. clausi* always matched the peak of available food particles (Figure 2). This peak tracking behaviour can be interpreted as an active selection of large diatoms ≥70 μm, as these were the dominant food taxa corresponding to the peaks. Such a preference is in agreement with other studies (Donaghay and Small, 1979; Guisande *et al.*, 2002) and is known also from other marine copepods [reviewed in (Kleppel, 1993)]. Nevertheless, grazing always occurred on less abundant, smaller food organisms. Hence, taking into account the investigations of various other authors, our results indicate that *A. clausi* might supplement a passive-mechanical filtering mode with a raptorial mode (particle detection and capture), depending on food size (Donaghay and Small, 1979; Pagano *et al.*, 2003), food quality (Donaghay and Small, 1979; Ayukai, 1987; Wiadnyana and Rassoulzadegan, 1989; Guisande *et al.*, 2002), and food quantity (Moraitou-Apolostopoulou and

Verriopoulos, 1976; Gismervik and Andersen, 1997). This ability seems to be lacking in *D. denticulatum* and *P. avirostris*.

In contrast to copepods, doliolids and cladocerans showed relatively invariant grazing patterns. This argues in favour of passive-mechanical filtering modes, consistent with the considerations in the literature [doliolids reviewed in (Bone, 1998); *P. avirostris* in (Pavlova, 1959; Paffenhöfer and Orcutt, 1986; Lipej *et al.*, 1997)]. Nevertheless, in some experiments doliolids and cladocerans expressed grazing peaks for dominant particles bigger than their normally preferred food sizes. But these additional peaks, separated from the other peaks by zero-grazing values, are based exclusively on the ingestion of needle-shaped diatoms, whose valve diameters are within the normally preferred food-size spectra. Therefore, we do not rate these ingestions as intended captures of beneficial prey, but as accidental ingestions, depending on the orientation of the algae in the filtering currents.

The EFC of all grazers depended on TFC and on food size composition. Both factors depended again on the nutrient conditions in which the food communities grew (Table I, Figure 2). The results suggest that ultra-oligotrophic to oligotrophic conditions, providing low TFC and small food sizes, are advantageous for *D. denticulatum*, while *P. avirostris* and *A. clausi* profit by oligotrophic to mesotrophic and mesotrophic to eutrophic conditions, respectively. Nevertheless, at levels similar to the normal nutrient loading rate at Blanes Bay all grazers met similar EFC of around 50% TFC (community 5, TFC = 0.364 $mm^3\ L^{-1}$). This might be one of the preconditions necessary to enable the coexistence of copepods, cladocerans and doliolids in summer in Blanes Bay. We will discuss this point in more detail together with the food-niche calculations.

Clearance rates and ingestion rates

Acartia clausi and *P. avirostris* reached similar clearance and ingestion rates and showed equivalent functional responses. *Doliolum denticulatum* generally attained higher rates at similar food densities and differed in its functional response from crustacean grazers. This speaks for a different metabolic activity of crustacean and gelatinous mesozooplankton and for an adaptation to different pelagic environments.

Clearance and ingestion rates of *A. clausi* and of *P. avirostris* are within the range of rates found in the literature (Table V). No clearance or ingestion rates have been published for *D. denticulatum* so far. Therefore, we listed data published for other doliolid species. In general, we tried to compare with animals similar in size or biomass to the individuals used in our experiments. Nevertheless, a comparison was not always possible because of the use of different rate units. If possible, we converted units for clearance rates to mL $ind.^{-1}\ day^{-1}$ and ingestion rates to μg C $ind.^{-1}\ day^{-1}$. Our results for ingestion rates include a degree of uncertainty as conversion of phytoplankton biovolume to carbon biomass depends very much on the conversion factor chosen. We decided to follow the estimations of Nalewajko (Nalewajko, 1966), which treat all phytoplankton species equally. Other computations emphasize small taxa [e.g. (Strathmann, 1967)] or big algal sizes [e.g. (Rocha and Duncan, 1985)] and may lead to deviating results. Moreover, rate measurements are always influenced by a variety of parameters, such as temperature, type of food source, food density, life history of animals, and choice of experimental method.

The relationship between clearance rate and TFC followed a bell-shaped curve with narrow modal ranges for both crustacean species. For *A. clausi* this type of model has been observed by other authors (Gismervik and Andersen, 1997; Pagano *et al.*, 2003). For *P. avirostris* a decrease of clearance rates with increasing food concentration has been documented (Pavlova, 1959; Paffenhöfer and Orcutt, 1986; Wong *et al.*, 1992), but not an initial increase that indicates a switching from non-feeding to feeding activities (Marten, 1973). Non-feeding activities suggest that TFC or EFC, or both, are too low to support basic metabolism. Our results indicate that this was the instance when TFC was $<0.1\ mm^3\ L^{-1}$ for *A. clausi* and at TFC $\leq 0.02\ mm^3\ L^{-1}$ for *P. avirostris*. Paffenhöfer and Orcutt (Paffenhöfer and Orcutt, 1986) observed feeding activities of *P. avirostris* also at lower food concentrations ($0.01\ mm^3\ L^{-1}$), but reproduction did not occur at these levels. No comparable studies are available for *A. clausi*. *Doliolum denticulatum* also fed on the lowest food concentrations offered.

Acartia clausi and *P. avirostris* decreased their filtration efforts at higher food concentrations. Nevertheless, their ingestion rates remained stable. This behaviour points to an optimal adjustment of energy expenses. According to Paffenhöfer (Paffenhöfer, 1988), such an ability corresponds to species adapted to varying trophic conditions. Indeed, *A. clausi* (Raymont, 1983) and *P. avirostris* (Paffenhöfer and Orcutt, 1986) occur most commonly in near- and in-shore environments that are often subject to fluctuating particulate densities. *Doliolum denticulatum* did not show this kind of behavioural flexibility. Doliolids increased their filtration rates from minimum to maximum clearance almost without transition, and kept rates constantly high, despite increasing food supplies. Hence, ingestion rates followed a sigmoidal curve. According to Holling (Holling, 1959), an S-shaped functional response has the potential to regulate prey density. This response is also termed a switching response, following the definition of Murdoch (Murdoch, 1969):

Table V: Clearance rates (mL ind.$^{-1}$ day^{-1}) and ingestion rates (μg C ind.$^{-1}$ day^{-1}) of Acartia clausi, Penilia avirostris and three doliolid species

	Clearance rate	Mean	Ingestion rate	Mean	Author(s)
Acartia clausi	3.5–24				Ayukai (1987)
	6.6–74				Broglio *et al.* (2001)
			*		Donaghay and Small (1979)
	2.0–20		0.2–5.7[a]		Gismervik and Andersen (1997)
			*		Guisande *et al.* (2002)
	*		1.3–4.9[b]	3.3	Pagano *et al.* (2003)
	3.5–20[c]				Tiselius (1998)
	3.1–22		*		Turner and Granéli (1992)
	34–630		*		Wiadnyana and Rassoulzadegan (1989)
	0–38	**14**	**0–14**	**5.0**	**this study**
Penilia avirostris	4.8–26		*		Paffenhöfer and Orcutt (1986)
	41–252	101	*		Pavlova (1959)
	18–56		*		Turner *et al.* (1988)
	4.8–30	21	*		Turner *et al.* (1998)
	0.1–20	2.2	*		Wong *et al.* (1992)
	0–55	**26**	**0–16**	**6.0**	**this study**
Dolietta gegenbauri	0.5–264	106			Crocker *et al.* (1991)
	10–355[d]	46[e]	*		Deibel (1982)
	24[f]				based on data from Deibel (1982) and on equations given in Madin and Deibel (1998)
	20–175[g]		3–8[g]		Gibson and Paffenhöfer (2000)
	24–233	139[h]			Tebeau and Madin (1994)
Doliolum nationalis	60–140				Deibel and Paffenhöfer (1988)
Doliolum denticulatum	**2.4–107**	**64**	**0.01–68**	**21**	**this study**

*Rates measured by author(s), but conversion of units not possible.
[a]Converted with an estimated biomass of 750 pg C cell^{-1} *T. weissflogii* (their figure 2).
[b]Converted with an estimated biomass of 3.8 μg C ind.$^{-1}$ (their table 3 and figure 3).
[c]Converted with an estimated dry weight of 11.6 μg C ind.$^{-1}$ (his table 1 and figure 5).
[d]Including phorozooids, [e]gonozooids with a biomass of 2.7 μg C ind.$^{-1}$ (his figure 1).
[f]Gonozooids 1.5 mm long.
[g]Gonozooids with a biomass of 5 μg C ind.$^{-1}$ (their figures 2, 3, 5, 6 and 8).
[h]Gonozooids.

'As a prey becomes relatively more abundant, switching occurs if the relative amount which that species forms of the predator's diet increases disproportionately in comparison with the expected amount.' As shown above, *D. denticulatum* behaved like a passive filter-feeder. Therefore, 'switching' might be interpreted as an abrupt change from low to high feeding activity, as suggested by our data. The slight decrease of clearance rates at very high food concentrations may be the result of the filtration apparatus of *D. denticulatum* becoming blocked, as observed for other tunicates (Deibel, 1985; Harbison *et al.*, 1986).

Assimilation efficiencies

Both crustacean grazers reached significantly higher assimilation efficiencies than doliolids. A reason could be that their body tissues are more similar to the food guild's biochemical composition. Assimilation efficiencies depend essentially on food quality. The more similar the biochemical composition of the food to the body tissue of the consumer, the higher its assimilation efficiency (Valiela, 1991). Mean efficiencies of copepods were significantly higher than those of cladocerans. *Acartia clausi* may have profited from its presumed ability

to capture actively beneficial prey. With this, the high abundance of diatoms may have been advantageous [e.g. (Guisande *et al.*, 2002)]. Considering all experiments, the assimilation efficiencies of *A. clausi* showed no clear dependency on food density. This is consistent with the results of Pechen-Finenko (Pechen-Finenko, 1977). Nevertheless, *A. clausi* attained maximum efficiencies at food concentrations close to the concentration threshold where maximum ingestion rates were reached (Figures 4 and 5). At higher food densities assimilation efficiency decreased continuously, as occurs in other marine copepods (Conover, 1978). Similarly, assimilation efficiencies of *P. avirostris* and of *D. denticulatum* decreased significantly with increasing food concentration. These observations might be understood as a result of the digestibility of food and its retention time in the gut, related to the superfluous feeding theory proposed by Beklemishev (Beklemishev, 1962). The theory suggests that at high food concentrations retention time in the gut might be too short to support effective digestion of food. At lower concentrations, the retention time will be longer and the digestion of the food may be more complete, leading to increased assimilation efficiencies. Moreover, digestion times seem to increase with prey size [e.g. (Martinussen and Bamstest, 1999; Suchmann and Sullivan, 2000)]. In our experiments increasing food concentrations occurred with increasing food dimensions (Table I, Figure 2). The efficiency loss was strongest for doliolids, maybe because they are not able to reduce filtration, and thus ingestion rates, with increasing food concentration.

The assimilation efficiencies we measured for *A. clausi* are within the range documented for marine copepods [e.g. (Gaudy, 1974; Pechen-Finenko, 1977)]. However, assimilation efficiencies computed from short-term experiments like ours do not account for long-term effects, such as losses of ingested material through moulting and mortality. Therefore, it is likely that assimilation efficiencies on a population level are lower than those calculated for individuals. Moreover, assimilation efficiencies of individuals may vary depending on the life-cycle phase of the organism investigated (Jones *et al.*, 2002).

Assimilation efficiencies of marine cladocerans and doliolids are poorly investigated. However, our results are consistent with findings for freshwater cladocerans (Lair, 1991; Urabe and Watanabe, 1991) and for predatory gelatinous zooplankton (Kremer and Reeve, 1989; Reeve *et al.*, 1989; Stibor and Tokle, 2003).

Food-niche calculations

Food-niche breadth calculations sustain that in feeding *A. clausi* is more specialized than *P. avirostris* and *D. denticulatum*. The food-niche breadth of doliolids might even have been underestimated in our experiments as we did not record sizes <1 μm. Nevertheless, a narrow food niche might be compensated by high abundances of preferred prey (high EFC) at food concentrations enabling high ingestion rates (suitable TFC) and optimum assimilation efficiencies. Thus, especially those organisms which can actively detect and capture beneficial prey may rival a competitor with a broader selectivity-size spectrum.

Food-niche overlap was highest between the filter feeders. However, although all grazers competed for food sizes between 7.5 and 100 μm, none of the grazing spectra overlapped any of the others completely. *Acartia clausi*, *P. avirostris* and *D. denticulatum* reached their highest grazing coefficients for separated food-size classes (Figure 1, Table IV). Different niche allocation may be one explanation for the coexistence of copepods, cladocerans and doliolids in Blanes Bay at certain times; besides the influence of hydrographic (Sabatès and Masó, 1990; Masó and Tintoré, 1991) and seasonal (Andreu and Duarte, 1996) dynamics. Nevertheless, niche overlap measurements based only on single niche dimensions, such as food size, do not describe overall niche overlap in a multidimensional niche space (Abrams, 1980; Holt, 1987). Therefore, our calculations may only reflect tendencies in the relationship between niche overlap and competition. Yet, because of the fundamental importance that food size has for the feeding relationships in pelagic communities [e.g. (Sommer and Stibor, 2002)], we consider our results to be an appropriate measurement within this context. Our results indicate that none of the mesozooplankton groups studied here should be out-competed on a food-resource basis, if the whole size spectrum from pico- to microphytoplankton is available, and effective and TFCs are sufficiently high for every grazer to support basic metabolism. Our data suggest that this is the case at food concentrations ~0.4 to 1.3 $mm^3\ L^{-1}$, as is most commonly found in Blanes Bay.

ACKNOWLEDGEMENTS

This article is a contribution to the MAST III-project COMWEB founded by the EU (MAST3-CT96-0052). We thank C. M. Duarte and the Centro de Estudios Avanzados de Blanes (CEAB, Blanes, Catalunya, Spain) for the supply of laboratory space for our experiments. We also thank I. Jenkinson and two anonymous reviewers for their helpful comments on the manuscript.

REFERENCES

Abrams, P. (1980) Some comments on measuring niche overlap. *Ecology*, **61**, 44–49.

Acuña, J. L. (2001) Pelagic tunicates: why gelatinous? *Am. Nat.*, **158**, 100–107.

Alldredge, A. L. and Madin, L. P. (1982) Pelagic tunicates: unique herbivores in the marine plankton. *Bioscience*, **32**, 655–663.

Andreu, P. and Duarte, C. M. (1996) Zooplankton seasonality in Blanes Bay (northwest Mediterranean). *Publ. Espec. Inst. Esp. Oceanogr.*, **22**, 47–54.

Ayukai, T. (1987) Discriminate feeding of the calanoid copepod *Acartia clausi* in mixtures of phytoplankton and inert particles. *Mar. Biol.*, **94**, 579–587.

Beklemishev, C. W. (1962) Superfluous feeding of marine herbivorous zooplankton. *Rapp. P-v. Reun. Cons. Perm. Int. Expl. Mer.*, **153**, 108–113.

Bone, Q. (ed.) (1998) *The Biology of Pelagic Tunicates*. Oxford University Press, Oxford.

Bøsheim, K. Y. and Bratbak, G. (1987) Cell volume to carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. *Mar. Ecol. Progr. Ser.*, **8**, 211–223.

Broglio, E., Johansson, M. and Jonsson, P. R. (2001) Trophic interaction between copepods and ciliates: effects of prey swimming behavior on predation risk. *Mar. Ecol. Progr. Ser.*, **220**, 179–186.

Conover, R. J. (1978) Transformation of organic matter. In Kinne, O. (ed.), *Marine Ecology*, Vol. 4. Wiley, New York, pp. 221–499.

Crocker, K. M., Alldredge, A. L. and Steinberg, D. K. (1991) Feeding rates of the doliolid, *Dolioletta gegenbauri*, on diatoms and bacteria. *J. Plankton Res.*, **13**, 77–82.

DeBiase, A. E. R., Sanders, R. W. and Porter, K. G. (1990) Relative nutritional value of ciliate protozoa and algae as food for *Daphnia*. *Microb. Ecol.*, **19**, 99–210.

Deibel, D. (1982) Laboratory-measured grazing and ingestion rates of the salp, *Thalia democratica* Forskal, and the doliolid, *Dolioletta gegenbauri* Uljanin (Tunicata, Thaliacea). *J. Plankton Res.*, **4**, 189–202.

Deibel, D. (1985) Blooms of the pelagic tunicate, *Dolietta gegenbauri*: are they associated with Gulf Stream frontal eddies? *J. Mar. Res.*, **43**, 211–236.

Deibel, D. (1998) The abundance, distribution and ecological impact of doliolids. In Bone, Q. (ed.), *The Biology of Pelagic Tunicates*. Oxford University Press, Oxford, pp. 171–186.

Deibel, D. and Paffenhöfer, G. A. (1988) Cinematographic analysis of the feeding mechanism of the pelagic tunicate *Doliolum nationalis*. *Bull. Mar. Sci.*, **43**, 404–412.

Donaghay, P. L. and Small, L. F. (1979) Food selection capabilities of the estuarine copepod *Acartia clausi*. *Mar. Biol.*, **52**, 137–146.

Duarte, C. M., Agustí, S. and Agawin, N. S. R. (2000) Response of a Mediterranean phytoplankton community to increased nutrient inputs: a mesocosm experiment. *Mar. Ecol. Progr. Ser.*, **195**, 61–70.

Fenchel, T. (1980) Suspension feeding in ciliated protozoa: functional response and particle size selection. *Microb. Ecol.*, **6**, 1–11.

Frost, B. W. (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnol. Oceanogr.*, **17**, 805–815.

Gaudy, R. (1974) Feeding four species of pelagic copepods under experimental conditions. *Mar. Biol.*, **25**, 125–141.

Gibson, D. M. and Paffenhöfer, G. A. (2000) Feeding and growth rates of the doliolid, *Dolioletta gegenbauri* Uljanin (Tunicata Thaliacea). *J. plankton Res.*, **22**, 1485–1500.

Gismervik, I. and Andersen, T. (1997) Prey switching by *Acartia clausi*: experimental evidence and implications of intraguild predation assessed by a model. *Mar. Ecol. Progr. Ser.*, **157**, 247–259.

Gore, M. A. (1980) Feeding experiments on *Penilia avirostris* Dana (Cladocera: Crustacea). *J. Exp. Mar. Biol. Ecol.*, **44**, 253–260.

Guisande, C., Maneiro, I., Riveiro, I., Barreiro, A. and Pazos, Y. (2002) Estimation of copepod trophic niche in the field using amino acids and marker pigments. *Mar. Ecol. Progr. Ser.*, **239**, 147–156.

Harbison, G. R. (1992) The gelatinous inhabitants of the ocean interior. *Oceanus*, **35**, 18–23.

Harbison, G. R., McAlister, V. L. and Gilmer, R. W. (1986) The response of the salp, *Pegea confoederata*, to high levels of particulate material: starvation in the midst of plenty. *Limnol. Oceanogr.*, **31**, 371–382.

Harris, G. P. (1986). *Phytoplankton Ecology*. Chapman Hall, London, New York.

Hillebrand, H., Dürselen, C. D., Kirschtel, D., Pollingher, U. and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.

Hodgkin, E. P. and Rippingale, R. J. (1971) Interspecies conflicts in estuarine copepods. *Limnol. Oceanogr.*, **16**, 573–576.

Holling, C. S. (1959) The components of predation as revealed by a study of small mammal predation of European pine sawfly. *Can. Entomol.*, **91**, 293–332.

Holt, R. D. (1987) On the relation between niche overlap and competition: the effect of incommensurable niche dimensions. *Oikos*, **48**, 110–114.

Horn, H. S. (1966) Measurement of overlap in comparative ecological studies. *Am. Nat.*, **100**, 419–423.

Hurlbert, S. H. (1978) The measurement of niche overlap and some relatives. *Ecology*, **59**, 67–77.

Ikeda, T. (1985) Metabolic rates of epipelagic marine zooplankton as a function of body mass and temperature. *Mar. Biol.*, **85**, 1–11.

Jones, R. H., Flynn, K. J. and Anderson, T. R. (2002) Effect of food quality on carbon and nitrogen growth efficiency in the copepod *Acartia tonsa*. *Mar. Ecol. Progr. Ser.*, **235**, 147–156.

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

Kim, S. W., Onbé, T. and Yoon, Y. H. (1989) Feeding habits of marine cladocerans in the Inland Sea of Japan. *Mar. Biol.* **100**, 313–318.

Kleppel, G. S. (1993) On the diets of calanoid copepods. *Mar. Ecol. Progr. Ser.*, **99**, 183–195.

Krebs, C. J. (1999) *Ecological Methodology*, Harper Collins Publishers, New York.

Kremer, P. and Reeve, M. R. (1989) Growths dynamics of a ctenophore (*Mnemiopsis*) in relation to variable food supply. II. Carbon budgets and growth model. *J. Plankton Res.*, **11**, 553–574.

Lair, N. (1991) Grazing and assimilation rates of natural populations of planktonic cladocerans in an eutrophic lake. *Hydrobiologia*, **215**, 51–62.

Lipej, L., Mozetic, P., Turk, V. and Malej, A. (1997) The trophic role of the marine cladoceran *Penilia avirostris* in the Gulf of Trieste. *Hydrobiologia*, **360**, 197–203.

Lund, J. W. G., Kipling, C. and LeCren, E. D. (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*, **11**, 147–170.

Madin, L. P. and Deibel, D. (1998) Feeding and energetics of Thaliacea. In Bone, Q. (ed.), *The Biology of Pelagic Tunicates*. Oxford University Press, Oxford, pp. 81–103.

Marten, G. G. (1973) An optimization equation for predation. *Ecology*, **54**, 92–101.

Martinussen, M. B. and Bamstest, U. (1999) Nutritional ecology of gelatinous planktonic predators. Digestion rates in relation to type and amount of prey. *J. Mar. Biol. Ecol.*, **232**, 61–84.

Masó, M. and Tintoré, J. (1991) Variability of the northeast Spanish coast. *J. Mar. Systems*, **1**, 441–450.

Moraitou-Apolostopoulou, M. and Verriopoulos, G. (1976) Laboratory studies on the feeding of *Acartia clausi* (Copepoda, Calanoida). *Bolletino di Pesca Piscicoltura e Idrobiologia*, **31**, 73–86.

Mura, M. P., Agusti, S., Cebrian, J. and Satta, M. P. (1996) Seasonal variability of phytoplankton biomass and community composition in Blanes Bay (1992–1994). *Publ. Espec. Inst. Esp. Oceanogr.*, **22**, 23–29.

Murdoch, W. W. (1969) Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecol. Monogr.*, **39**, 335–354.

Nalewajko, C. (1966) Dry weight, ash and volume data for some freshwater planktonic algae. *J. Fish. Res. Can.*, **23**, 1285–1287.

Nival, P. and Nival, S. (1976) Particle retention efficiencies of an herbivorous copepod, *Acartia clausi* (adult and copepodite stages): effects on grazing. *Limnol. Oceanogr.*, **21**, 25–38.

Paffenhöfer, G. A. (1988) Feeding rates and behaviour of zooplankton. *Bull. Mar. Sci.*, **43**, 430–445.

Paffenhöfer, G. A. and Orcutt, J. D. (1986) Feeding, growth and food conversion of the marine cladoceran *Penilia avirostris*. *J. Plankton Res.*, **8**, 741–754.

Paffenhöfer, G. A., Stewart, T. B., Youngblouth, M. J. and Bailey, T. G. (1991) High resolution vertical profiles of pelagic tunicates. *J. Plankton Res.*, **13**, 971–981.

Pagano, M., Kouassi, E., Saint, J. L., Arfi, R. and Bouvy, M. (2003) Feeding of *Acartia clausi* and *Pseudodiaptomus hessei* (Copepoda: Calanoida) on natural particles in a tropical lagoon (Ebrie, Cote d'Ivoire). *Estuarine Coastal Shelf Sci.*, **56**, 433–445.

Pavlova, E. V. (1959) On grazing by *Penilia avirostris* Dana. *Trans. Sevastopol. Biol. Stn. Akad. Nauk. Ukr. SSR*, **11**, 63–71. (English translation by Translation Bureau, *Fish. Res. Bd.*, Canada, **967**).

Pechen-Finenko, G. A. (1977) Efficiency of food assimilation by planktonic crustaceans in different trophic conditions. *Zool. Zh.*, **56**, 1459–1466.

Raven, J. A. (1986) Physiological consequences of extremely small size for autotrophic organisms in the sea. *Can. Bull. Fish. Aqu. Sci.*, **214**, 1–70.

Raymont, J. E. G. (1983) *Plankton and Productivity in the Oceans*, 2nd edn, Vol. 2: *Zooplankton*. Pergamon Press, Oxford.

Reeve, M. R., Syms, M. A. and Kremer, P. (1989) Growth dynamics of a ctenophore (*Mnemiopsis*) in relation to variable food supply. I. Carbon biomass, feeding, egg production, growth and assimilation efficiency. *J. Plankton Res.*, **11**, 535–552.

Rocha, O. and Duncan, A. (1985) The relationship between cell carbon and cell volume in freshwater algal species used in zooplankton studies. *J. Plankton Res.*, **7**, 279–294.

Sabatès, A. and Masó, M. (1990) Effects of a shelf slope front on the spatial distribution of mesopelagic fish larvae in the Western Mediterranean. *Deep Sea Res.*, **37**, 1085–1098.

Schneider, G. (1992) A comparison of carbon-specific respiration rates in gelatinous and non-gelatinous zooplankton: a search for general rules in zooplankton metabolism. *Helgoländer Meeresuntersuchungen*, **46**, 377–388.

Sommer, U. (2000) Scarcity of medium-sized phytoplankton in the northern Red Sea explained by strong bottom-up and weak top-down control. *Mar. Ecol. Progr. Ser.*, **197**, 19–25.

Sommer, U. and Stibor, H. (2002) Copepoda – cladocera – tunicata: the role of three major mesozooplankton groups in pelagic food webs. *Ecol. Res.*, **17**, 161–174.

Sorokin, Y. I., Petipa, T. S. and Pavlova, E. V. (1970) Quantitative estimate of marine bacterioplankton as a source of food. *Oceanology*, **10**, 253–260.

Stibor, H. and Tokle, N. (2003) Feeding and asexual reproduction of the jellyfish *Sarsia gemmifera* in response to resource enrichment. *Oecologia*, **135**, 202–208.

Stockner, J. G. and Antia, N. J. (1986) Algal picoplankton from marine and freshwater ecosystems: a multidisciplinary perspective. *Can. J. Fish. Aquat. Sci.*, **214**, 2472–2503.

Strathmann, R. H. (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. *Limnol. Oceanogr.*, **12**, 411–418.

Suchmann, C. L. and Sullivan, K. (2000) Effect of prey size on vulnerability of copepods to predation by the scyphomedusae *Aurelia aurita* and *Cyanea* sp. *J. Plankton Res.*, **22**, 2289–2306.

Tebeau, C. M. and Madin, L. P. (1994) Grazing rates for three life history stages of the doliolid *Dolietta gegenbauri* Uljanin (Tunicata, Thalicea). *J. Plankton Res.*, **16**, 1075–1081.

Tiselius, P. (1998) Short term feeding response to starvation in three species of small calanoid copepods. *Mar. Ecol. Prog. Ser.*, **168**, 119–126.

Turner, J. T. and Granéli, E. (1992) Zooplankton feeding ecology: grazing during enclosure studies of phytoplankton blooms from the west coast of Sweden. *J. Exp. Mar. Biol. Ecol.*, **157**, 19–31.

Turner, J. T., Hopcroft, R. R., Lincoln, J. A., Huestis, C. S., Tester, P. A. and Roff, J. C. (1998) Zooplankton feeding ecology: grazing by marine copepods and cladocerans upon phytoplankton and cyanobacteria from Kingston Harbour, Jamaica. *Mar. Ecol.*, **19**, 195–208.

Turner, J. T., Tester, P. A. and Ferguson, R. L. (1988) The marine cladoceran *Penilia avirostris* and the 'microbial loop' of pelagic food webs. *Limnol. Oceanogr.*, **33**, 245–255.

Urabe, J. and Watanabe, Y. (1991) Effect of food concentration and the assimilation and production efficiencies of *Daphnia galeata* G. O. Sars (Crustacea, Cladocera). *Funct. Ecol.*, **5**, 635–641.

Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Verein Theor. Angew. Limnol.*, **9**, 1–38.

Valiela, I. (1991) Ecology of water columns. In Barnes, R. S. and Mann, K. H. (eds.), *Fundamentals of Aquatic Ecology*. Blackwell Scientific Ltd, Oxford, pp. 29–56.

Vanderploeg, H. A. and Scavia, D. (1979) Calculation and use of selectivity coefficients of feeding: zooplankton grazing. *Ecol. Modeling*, **7**, 135–149.

Vanderploeg, H. A., Scavia, D. and Liebig, J. R. (1984) Feeding rate of *Diaptomus sicilis* and its realtion to selectivity and effective food concentration in algal mixtures in Lake Michigan. *J. Plankton Res.*, **6**, 919–941.

Wiadnyana, N. N. and Rassoulzadegan, F. (1989) Selective feeding of *Acartia clausi* and *Centropages typicus* on microzooplankton. *Mar. Ecol. Progr. Ser.*, **53**, 37–45.

Wong, C. K., Chan, A. L. C. and Tang, K. W. (1992) Natural ingestion rates and grazing impact of the marine cladoceran *Penilia avirostris* Dana in Tolo Harbour, Hong Kong. *J. Plankton Res.*, **14**, 1757–1765.

PAPER A2

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)

Katechakis A, Stibor H, Sommer U, Hansen T (2002)
***Marine Ecology Progress Series* 234:55–69**

Vol. 234: 55–69, 2002 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published June 3

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)

Alexis Katechakis[1,*], Herwig Stibor[1], Ulrich Sommer[2], Thomas Hansen[2]

[1]Zoologisches Institut, Ludwig-Maximilians-Universität, Abteilung Aquatische Ökologie, Karlstraße 23-25, 80333 München, Germany

[2]Institut für Meereskunde, Christian-Albrechts-Universität, Abteilung Experimentelle Ökologie, Düsternbrooker Weg 20, 24105 Kiel, Germany

ABSTRACT: We report how different zooplankton groups (doliolids, cladocerans and copepods) are able to influence the coastal pelagic food web, including the microbial food web, in waters of the NW Mediterranean. We studied the effect of grazing and of grazing-induced nutrient recycling mediated by different types of zooplankton grazing on a natural phytoplankton community. Experiments were conducted in semicontinuous 2-stage chemostats. The 1st stage vessels contained seawater from Blanes Bay, Spain (NW Mediterranean) including its natural phytoplankton community; the 2nd stage vessels contained the same seawater and copepods, cladocerans or doliolids. At daily intervals we transferred part of the medium from the 2nd to the 1st stage flasks, which contained ungrazed algae and excreted nutrients. In this way, the zooplankton could influence phytoplankton dynamics both by selective grazing and by differential excretion of limiting nutrients. In the 2nd stage flasks grazing changed the algal community composition. Doliolids and cladocerans promoted the growth of large algae and copepods shifted the size spectrum towards small sizes. This effect was transferred to the 1st stage flasks. Doliolids, cladocerans and copepods also affected the microbial food web in different ways. Size-selective grazing led to differences in the nanoplankton concentrations. These in turn affected bacterial concentrations in a trophic cascade. The potential to modify a given algal population increased with increasing selectivity of the grazer.

KEY WORDS: Doliolids · Cladocerans · Copepods · Grazing · Marine pelagic food web · Microbial food web · Trophic cascade

INTRODUCTION

The grazing behaviour of herbivorous mesozooplankton is one of the critical factors structuring pelagic food webs. Herbivores distribute the organic matter synthetized by autotrophs to higher trophic levels. In spite of some recent controversy (Miralto et al. 1999, Tang & Dam 2001), the energy flow from diatoms via crustaceans to fishes is considered particularly efficient (Cushing 1975, Officer & Ryther 1980, Iverson 1990, Sommer et al. 2002). In contrast, gelatinous zooplankton are considered a poor food base for commercial fish stocks (Verity & Smetacek 1996), due to their high volume to plasma ratio and their low protein content (Cushing 1975).

*E-mail: katechakis@zi.biologie.uni-muenchen.de

There are many studies concerning grazing by marine copepods and their influence on the marine pelagic food web structure (e.g. Kiørboe 1998). Gelatinous mesozooplankton and cladocerans are poorly investigated in this context, although seasonally they may dominate zooplankton communities at times (e.g. Alldredge & Madin 1982, Deibel 1982a,b, 1998, Crocker et al. 1991, Paffenhöfer et al. 1991, Andreu & Duarte 1996).

Besides exerting direct grazing pressure, zooplankton may also influence the phytoplankton community indirectly (Gismervik et al. 1996 review, Andersen 1997). Whilst feeding on algae, herbivores release nutrients through excretion and sloppy feeding. The regeneration of dissolved nutrients may influence the gross growth rate of the algal community. A changing nutrient-stoichiometry can alter its composition (e.g. Officer & Ryther 1980, Tilman 1982, Sommer 1983, 1994a, 1996, 1998a, Tilman et al. 1986, Hessen & Andersen 1992, Escaravage et al. 1996, Schöllhorn & Granéli 1996). This might feed back on the competition within the herbivorous zooplankton and affect the energy transfer in the pelagic food web (e.g. Sommer 1998b).

To study the effects of grazing and grazing-induced nutrient regeneration, we conducted experiments with mesozooplankton from Blanes Bay (Catalan Sea, NW Mediterranean) feeding on a natural phytoplankton assemblage. Three zooplankton groups dominated in Blanes Bay in summer: copepods, cladocerans and doliolids. Short-term grazing experiments (several hours) with these zooplankton groups showed that they differ in their size preference for algae; therefore, they can influence the competition between different-sized algal groups (Katechakis 1999). This makes them well-suited for longer experiments (several weeks) to investigate how copepods, cladocerans and doliolids influence the algal community over several phytoplankton generations.

MATERIALS AND METHODS

Experimental setup. Experiments were performed in semicontinuous 2-stage chemostats, consisting of 600 ml tissue culture flasks. The 1st stage flasks were filled with the natural phytoplankton community occurring in summer in Blanes Bay (Catalan Sea, NW Mediterranean, 42° 18' 26" N, 3° 18' 11" E); water was filtered through a plankton net with a mesh size of 100 µm to exclude mesozooplankton. To the 2nd stage flasks (reaction chambers) we added 20 copepods (*Acartia* sp.), 20 cladocerans (*Penilia avirostris*) or 15 doliolids (solitary gonozooids of *Doliolum denticulatum*), at higher densities than those in summer in Blanes Bay (natural densities: 500 to 780 copepods m^{-3}, 750 to 1250 cladocerans m^{-3}, 90 doliolids m^{-3}: Andreu & Duarte 1996). We were careful to incubate similar biovolumes of grazers in the various flasks. We estimated biovolumes from size measurements. All treatments were replicated 3 times, including controls without grazers. The replicates were placed randomly in a water bath at a temperature between 21 and 23°C. The *in situ* surface temperature in Blanes Bay was 25 to 26°C (measured with a WTW LF 20 temperature sensor). The 1st stage flasks were ventilated with air pumps and illuminated with 6 fluorescent tubes (3× Osram light code 77, 3× Osram light code 21-840, 36 W each). The reaction chambers remained dark and were not ventilated—preliminary experiments had shown that bubbling affected especially cladocerans and doliolids adversely. We took 150 ml from the 1st and 2nd stage flasks daily (dilution rate, $D = 0.25\ d^{-1}$). The 150 ml from the 1st stage flasks were transferred to the 2nd stage flasks. Of the 150 ml taken from the 2nd stage flasks, 75 ml were returned to the 1st stage flasks, together with uneaten algae and recycled nutrients but without transferring mesozooplankton grazers; 75 ml were used for sampling or discarded. Sampling was done 4 times during the experiment: at the beginning, after 6 d, after 12 d and at the end. Sampling of 1st stage flasks after 6 d and after 12 d resulted in dilution rates higher than $0.25\ d^{-1}$; however we estimated this to be no problem taking into account the duration of the experiment. Sampling at the beginning and at the end did not influence the dilution rate. The 75 ml deficits in 1st stage flasks were made up with fresh medium (Fig. 1) consisting of sterile-filtered seawater (0.2 µm cellulose-acetate filters) enriched with nutrients (N, 21 µM: 50% $NaNO_3$ and 50% NH_4Cl; P, 1 µM: $Na_2HPO_4 \cdot 2H_2O$; Si, 7 µM: $Na_2O_3Si \cdot 5H_2O$), which is similar to the *in situ* supply from natural terrestial and human sources during summer in Blanes Bay (Y. Olsen unpubl. data). During the experimental period of 17 d we visually controlled whether grazers were intact several times a day by observing their swimming behaviour in the flasks. Injured individuals were replaced if necessary. Animals were not reproducing during the experiment.

Sample preparation and analysis. The recirculating design permitted the zooplankton to influence the phytoplankton community in 2 ways—directly through grazing impact and indirectly through excretion of limiting nutrients. To determine grazer-induced changes in abundance, species composition, biovolume and the biomass of the nano- and microplankton, we preserved samples with Lugol's iodine (5 g I_2 + 10 g KI ad 100 ml aq. dest.). We counted the samples using an inverse microscope (Leica DMIL; Utermöhl 1958). If present, we counted at least 400 cells of each species to

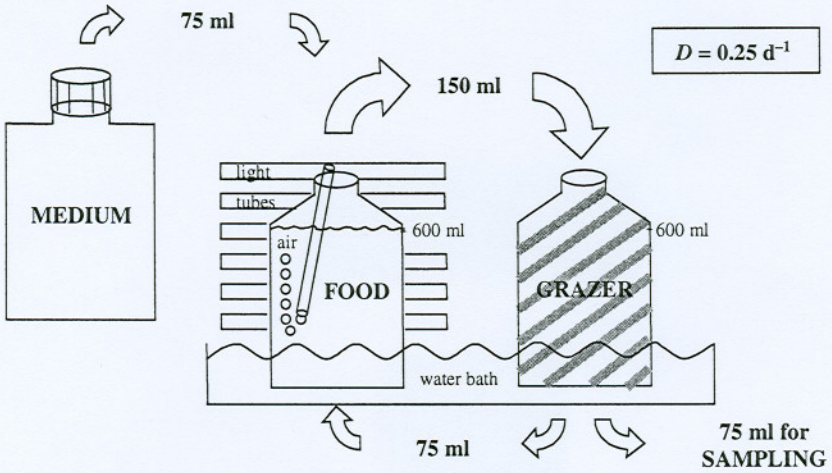

Fig. 1. Scheme illustrating the experimental setup (for details see 'Materials and methods'). *D*: dilution rate

achieve an error of <10% (Lund et al. 1958). Biovolumes were calculated using the equations of Hillebrand et al. (1999); for this purpose we measured the linear dimensions of 20 specimens of each species. Carbon contents were estimated after Strickland & Parsons (1972).

Booth et al. (1982) and Reid (1983) criticized the Utermöhl method, suggesting it underestimates pico- and small nanoplankton abundances drastically. Therefore we determined the abundances of bacteria, naked flagellates <5 µm and dinoflagellates <10 µm by staining with DAPI (4,6-diamidino-2-phenylindol) (Porter & Feig 1980). We fixed samples in formalin (final concentration: 2%) and stained them with a final concentration of 1.76 µg DAPI ml^{-1} for bacteria and 2.45 µg DAPI ml^{-1} for flagellates. After 10 min, the samples were filtered onto black 0.2 µm polycarbonate filters (Millipore) and 0.8 µm filters (Nuclepore), respectively. Filters were rinsed with 5 ml washing solution (sterile filtered tap water, 2% formaldehyde). Counts were done using an epifluorescence microscope (Leitz DMRB) equipped with a blue light and an UV-light filter set. For bacteria, at least 400 cells of each morphotype were enumerated if present. Bacteria attached to particles were counted as 'particle-bound bacteria cells', independent of particle size and abundance measurements. Naked flagellates and dinoflagellates were divided into 3 size classes: 2.5 to 5 µm, >5 to 7.5 µm and >7.5 to 10 µm, of which at least 400, 200 or 100 cells, respectively, were counted. To calculate biovolumes we measured the linear dimensions of 50 specimen of each morphotype (Fuhrmann & Azam 1980, Bjørnsen 1986). Flagellate biovolumes were calculated on the base of the respective interval means of every size class (3.75, 6.3, 8.8 µm). The carbon content of bacteria was calculated by multiplying cell numbers with 23.3 fg C $cell^{-1}$ (Simon & Azam 1989). Flagellate biomass was estimated with 0.22 pg C μm^{-3} according to Bøsheim & Bratbak (1987), that of ciliates with 0.15 pg C μm^{-3} (DeBiase et al. 1990). Moreover, under blue light stimulation, the differentiation of autotrophic cells (chlorophyll *a*: red autofluorescence) and heterotrophic cells (green coloration) was possible, as well as the detection of cyanobacteria (chl *a* + accessory phycobilines: yellow-orange coloration). DAPI-countings were done for 1st stage flasks.

Dissolved inorganic nutrients were analyzed with a continuous flow analyser using the methods of Grasshoff et al. (1983) for silicate, nitrate, ammonium and phosphate. For the determination of particulate carbon and nitrogen we filtered samples onto precombusted Whatman GF/C filters and measured them with a Fisons CN-analyser (NA 1500N).

Similarities between the resulting communities at the end of the experiment in the 1st stage flasks were expressed as Euclidean distances (Eq. 1), based on the following groups: cyanobacteria, naked flagellates, ciliates, dinoflagellates, diatoms and amoeba.

$$\Delta_{jk} = \sqrt{\sum_{i=1}^{n} (X_{ij} - X_{ik})^2} \quad (1)$$

where Δ_{jk} = Euclidean distance between Chemostats j and k; X_{ij} = proportion of Group i of total biovolume in Chemostat j (X_{ik} analog) and n = total number of groups. Δ_{jk} increases with increasing n. To compensate for this we calculated the average distance d_{jk} (Eq. 2):

$$d_{jk} = \sqrt{\frac{\Delta_{jk}^2}{n}} \quad (2)$$

Both Δ_{jk} and d_{jk} vary from 0 to $+\infty$; the larger the distance, the less similar are the 2 communities.

Data analysis. For statistical analysis, SigmaStat 2.0 and SPSS 10.0.5 software was used.

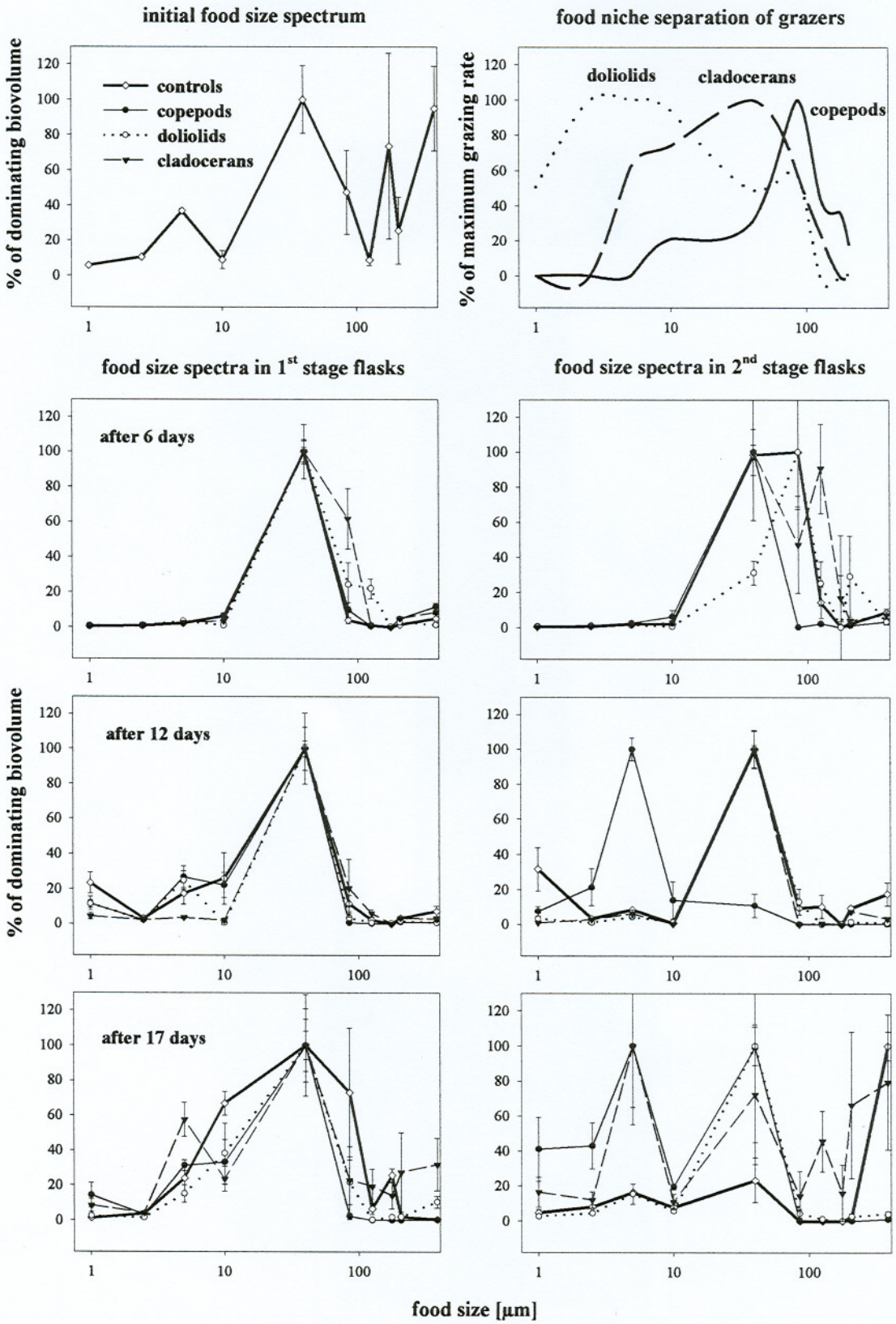

Fig. 2. Changes in the size composition of the summer plankton community of Blanes Bay (NW Mediterranean) under prolonged grazing pressure by doliolids, cladocerans or copepods in semicontinuous 2-stage chemostats (1st stage: food without grazer, 2nd stage: reaction chamber). Top left: initial seawater; top right: grazing spectra of doliolids, cladocerans and copepods as evaluated in grazing experiments (Katechakis 1999). Data points are means of triplicates; error bars represent ±SE of the means. Note logarithmic scale of particle-size axes

RESULTS

Changes in composition of food guilds

Size composition

Fig. 2 shows how the size composition within the food changed under the persisting grazing pressure of copepods, cladocerans or doliolids. We subdivided the plankton community into 9 size classes with mean intervals of 1, 2.5, 5, 10, 40, 85, 125, 175 and 205 µm. Colonial species were assigned to classes according to the biggest linear dimension of colonies. For the following comparisons of size classes we set the biovolume of the most abundant size class at 100% and refer to it as the 'dominating biovolume'.

Initial seawater

The size spectrum of food types initially presented to grazers was dominated by organisms between 15 and 70 µm (for comparitive purposes we set the biovolume at 100 ± 19.2% SE of the means: Fig. 2). Microplankton from 150 to 200 µm and >210 µm made up 73.7 ± 52.7% of the dominating biovolume and 95.0 ± 24.0% of the dominating biovolume, respectively. Among small size classes only individuals around 5 µm were important (36.6 ± 1.4%).

Second stage flasks (reaction chambers)

In 2nd stage flasks copepods, cladocerans and doliolids caused size compositions according to their respective grazing spectra, as evaluated in grazing experiments (Katechakis 1999 and present Fig. 2: right), with the following exceptions: After 17 d, chemostats with cladocerans showed high biovolumes of intermediate food sizes between 15 and 70 µm (72.4 ± 40.0% of the dominating biovolume) compared to controls, and in chemostats with doliolids large food items >100 µm were efficiently reduced. Controls were dominated by intermediate food sizes after 6 d and 12 d. Lastly, organisms >210 µm prevailed in control flasks. Differences among treatments were tested for significance using 2-way ANOVAs with the factor grazer type as a fixed factor and food size as a random factor. For percentages of dominating biovolume original data were arcsine-transformed. The interaction between different grazers and phytoplankton size composition was significant ($p \leq 0.001$, $F_{9,24} = 4.904$).

First stage flasks

Shifts in food size composition were transferred to 1st stage flasks ($p \leq 0.05$, $F_{9,24} = 2.443$) through recurrent inoculation with small amounts of material from the 2nd stage flasks.

Taxonomic composition

Initial seawater: The initial community was dominated by diatoms, ciliates and organisms <5 µm. Dinophyceae and naked flagellates were of little importance. Abundances of amoebae lay below the detection limit initially but became detectable later. The most important species were *Rhizosolenia fragilissima* and *Skeletonema costatum*. Together they accounted for more than 55% of the total food biovolume (for details see Table 2). The whole taxonomic spectrum is listed in Table 1.

Second stage flasks (reaction chambers): By Day 6, the taxonomical composition of the various chemostats differed little. Diatoms extended their dominance in all treatments. Compared to the initial community, pico- and nanoplankton showed substantial decreases in all flasks. Ciliates decreased in the copepod and doliolid treatments (Tables 2 & 3). After 12 d the communities had changed radically. In all flasks with grazers, non-siliceous species had become predominant: naked flagellates in chemostats with copepods, dinoflagellates (mainly *Peridinium* sp. accompanied by *Prorocentrum micans*) in those with doliolids or cladocerans. After 17 d, communities with different treatments differed greatly from each other. By Day 12 of the experiment, the prevailing naked flagellates had declined in the copepod chambers, while pico- and nanoplankton <5 µm and *Peridinium* sp. increased. *Peridinium* sp. was also the outstanding taxon under the influence of doliolids. In both chemostats with cladocerans and controls, diatoms gained importance, whereas dinophyceaes declined slightly. For details see Table 3.

First stage flasks: Here the central characteristic was the rise in dinophyceaens at the expense of diatoms. The change was expressed by the shift from *Rhizosolenia* spp. and *Skeletonema costatum* to *Peridinium* sp. and *Prorocentrum micans* as the most important species. Except for cladoceran treatments this was valid for all chemostats, although most evident in copepod systems.

Similarity of communities: The most dissimilar communities resulted from the influence of selective grazers (copepods) on the one hand and unselective filter-feeders (cladocerans or doliolids) on the other hand (Table 4). The latter were more similar to each other. The most similar communities were chemostats with doliolids and those serving as controls.

Changes in composition of microbial food web (1st stage flasks)

Bacteria and cyanobacteria

Solitary bacteria abundances: Solitary bacteria (diameter 0.3 µm, biovolume 0.014 μm^3) increased in all

Table 1. Taxonomic list of all plankton food in chemostat experiments. Biovolumes were calculated using the equations of Hillebrand et al. (1999). Carbon contents were estimated after Strickland & Parsons (1972) for phytoplankton, after Bøsheim & Bratbak (1987) for flagellates and after DeBiase et al. (1990) for ciliates. ANF: autotrophic nanoflagellates; HNF: heterotrophic nanoflagellates

Taxon	Geometrical shape	Cell dimension (µm) biggest extension	Biovolume (μm^3 $cell^{-1}$)	Biomass (pg C $cell^{-1}$)
Picoplankton				
1 µm	Sphere	1	0.52	0.07
Nanoplankton				
2.5 µm	Sphere	2.5	8.2	1.2
5 µm	Sphere	5	65	9.2
Cyanobacteria				
Coccal	Sphere	0.5	0.07	0.01
Filamentous	Cylinder	7.0–140	0.88–17.6	1.4–28
Bacillariophyceae				
Centrales				
Biddulphia sp.	Elliptic prism	15	442	39
Coscinodiscus sp.	Cylinder	12.5–40	920–12566	81–1100
Chaetoceros sp. A	Elliptic prism	5	79	6.9
Chaetoceros sp. B	Elliptic prism	20	707	62
Leptocylindrus sp.	Cylinder	45	884	77
Rhizosolenia deliculata	Cylinder	28	2160	189
Rhizosolenia fragilissima	Cylinder	18–75	344–5890	30–515
Rhizosolenia stolterfothii	Cylinder	38–200	1657–62832	145–5498
Rhizosolenia sp. A	Cylinder	70	3093	271
Rhizosolenia sp. B	Cylinder	100–500	1964–9817	172–859
Skeletonema costatum	Cylinder + 2 halfspheres	7.5–25	94–1104	8–97
Thalassiosira sp.	Cylinder	20	3534	309
Pennales				
Licmophora sp.	Gomphonemoid	75	10000	875
Navicula sp.	Elliptic prism	15	147	13
Nitzschia closterium	Prism on parallelogram	30	94	8.2
Nitzschia longissima	Prism on parallelogram	75	125	11
Nitzschia sp. A	Prism on parallelogram	17.5–30	47–156	4.1–14
Nitzschia sp. B	Prism on parallelogram	70	125	11
Thalassionema nitzschioides	Box	40	785	69
Dinophyceae				
Dinophysiales				
Dinophysis sp.	Ellipsoid	50	10472	1466
Peridiniales				
Ceratium tripos	3 cones + cylinder	50	25000	3500
Gymnodinium sp.	Ellipsoid	10	654	92
Peridinium sp.	Ellipsoid	15–30	1767–9425	247–1319
Prorocentrales				
Prorocentrum micans	Cone + halfsphere	30–50	2209–6283	309–880
Prymnesiophyceae				
Coccolithus sp.	Sphere	7.5	221	31
Phaeocystis pouchetii	Sphere	7.5	221	73
Other flagellates				
ANF spp.	Sphere	2.5–10	8.2–523	1.8–73
HNF spp.	Sphere	2.5–10	8.2–523	1.8–73
Ciliata				
Ciliate sp.	Ellipsoid	25	29452	4123
Amoeba				
Amoeba sp.	Irregular	7.5–15	331–2651	46–371

Table 2. Taxonomic composition (% of total food guild biovolume and SE of means in chemostats) of the food presented to grazers in the initial seawater at the beginning of the experiment

Functional group	%
Pico/nanoplankton <5 µm	15.26 ± 0.87
Diatoms	
Total	57.21 ± 6.70
Skeletonema costatum	20.66 ± 0.38
Rhizosolenia fragilissima	35.21 ± 6.00
Dinophyceae	2.52 ± 1.39
Naked flagellates	2.51 ± 2.54
Ciliates	22.49 ± 5.25
Amoeba	0

treatments. At the end of the experiment, controls showed lower values than chemostats with grazers. Biovolumes were highest in cladoceran-influenced systems followed by those systems affected by doliolids or copepods (Fig. 3). The cell numbers in the 1st stage chemostats differed significantly from each other (1-way ANOVA, $p \leq 0.001$, $F_{4,14} = 16.64$). Post hoc Tukey-test analyses showed a significant difference between the cladoceran and all the other treatments.

Particle-bound bacteria: We could not find any of these in natural seawater; 17 d later, in the chambers with doliolids most bacteria were attached to particles (2.81×10^5 cells ml^{-1}), while the remaining chambers had densities of 55 200 cells ml^{-1} (cladocerans), 51 886 cells ml^{-1} (controls), and 8529 cells ml^{-1} (copepods). Differences among treatments were significant (1-way ANOVA, $p \leq 0.001$, $F_{4,14} = 15.132$). Post hoc Tukey-test analyses showed that the doliolid treatments formed a separate group.

Coccal cyanobacteria abundances: Coccal cyanobacteria (diameter 0.5 µm, biovolume 0.065 µm^3) were below the detection limit in the initial samples and did not occur in chemostats with doliolids. They reached highest abundances (5.66×10^5 cells ml^{-1}) in controls, followed by the treatments with cladocerans and with copepods, in that order (Fig. 3). Chemostats differed significantly from each other (1-way ANOVA, $p \leq 0.05$, $F_{4,14} = 4.109$). Post hoc Tukey-test analyses showed that systems with doliolids and the initial sample represented separate groups.

Filamentous cyanobacteria: These could not be found at the beginning of the experiment but occurred in all treatments at the end. Filamentous cyanobacteria had a diameter of 0.4 µm and covered lengths from 7 to 140 µm in all chemostats. The mean sizes of filamentous cyanobacteria were larger in treatments with doliolids as grazers (length 38.2 ± 1.6 µm SE, biovolume 4.8 ± 0.2 µm^3 SE) than in other treatments (copepods: 29.1 ± 1.8 µm and 3.7 ± 0.2 µm^3, cladocerans: 25.9 ± 1.1 µm and 3.3 ± 0.1 µm^3) and in controls (29.1 ± 0.6 µm and 3.7 ± 0.1 µm^3). Doliolid chambers also contained the most filamentous cyanobacteria (1.48×10^5 µm^3 ml^{-1}), followed by those with cladocerans or copepods and controls. Differences between treatments were significant (1-way ANOVA, $p \leq 0.05$, $F_{(4;14)} = 3.495$). Post hoc Tukey-test analyses showed that doliolid treatments formed a separate group.

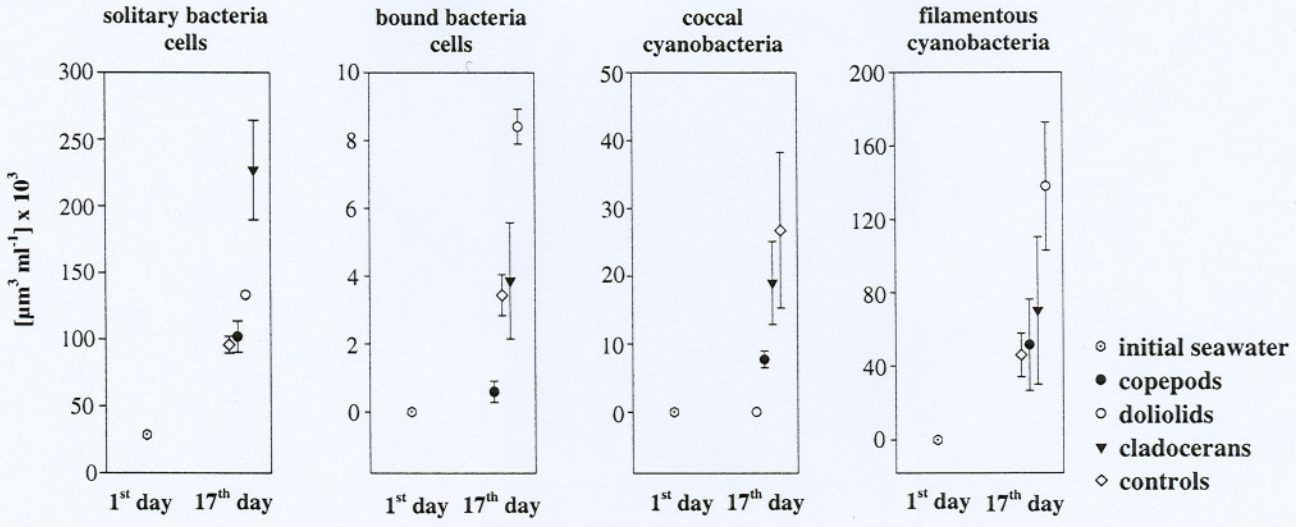

Fig. 3. Changes in the composition of the bacterial community of Blanes Bay (NW Mediterranean) under prolonged grazing pressure by doliolids, cladocerans or copepods (1st stage flasks). Data points are means of triplicates; error bars represent ±SE of the means

Flagellates

We found a significant negative correlation between bacterial abundance (bacteria + coccal cyanobacteria) and the appearance of heterotrophic nanoflagellates (HNF) from 5.1 to 10 µm size (Fig. 4). At the end of the experiment, chemostats with cladocerans as grazers showed the lowest HNF biovolumes (3.68×10^5 µm^3

Table 3. Changes in taxonomic composition (% of total food guild biovolume and SE of the means in chemostats) of the summer plankton community in Blanes Bay (NW Mediterranean) under prolonged grazing pressure by doliolids, cladocerans or copepods in semicontinuous 2-stage chemostats (1st stage: food without grazers, 2nd stage: reaction chamber)

Functional group	1st stage after 6 d	1st stage after 12 d	1st stage after 17 d	2nd stage after 6 d	2nd stage after 12 d	2nd stage after 17 d
Copepods						
Pico/nanoplankton <5 µm	2.19 ± 0.27	9.23 ± 1.59	5.60 ± 1.20	3.55 ± 0.94	16.91 ± 5.62	60.51 ± 18.54
Diatoms total	59.33 ± 3.29	5.89 ± 1.36	3.40 ± 1.60	88.39 ± 2.97	7.84 ± 4.28	3.98 ± 1.07
Skeletonema costatum	3.75 ± 1.99			33.09 ± 3.33		
Rhizosolenia fragilissima	53.52 ± 4.79			46.01 ± 4.42		
Dinophyceae total	15.13 ± 3.48	63.40 ± 7.63	52.33 ± 11.22			28.92 ± 18.68
Peridinium sp.		54.13 ± 2.25	50.14 ± 10.70			0.75 ± 0.61
Prorocentrum micans		2.90 ± 0.20	1.64 ± 1.06			
Naked flagellates	4.44 ± 1.34	21.38 ± 7.28	31.35 ± 7.52	5.18 ± 3.19	73.00 ± 10.46	6.47 ± 0.50
Ciliates	18.91 ± 4.38	0	0	1.68 ± 0.73	0	0
Amoeba	0	0.10 ± 0.09	0.66 ± 0.43	0	0	0
Doliolids						
Pico/nanoplankton <5 µm	2.79 ± 0.65	9.43 ± 1.86	3.63 ± 0.38	1.61 ± 0.54	5.57 ± 1.52	5.02 ± 1.06
Diatoms total	90.77 ± 1.70	18.22 ± 1.51	26.97 ± 11.86	86.59 ± 0.79	13.49 ± 3.22	12.89 ± 2.07
Skeletonema costatum	11.03 ± 5.40		18.45 ± 10.49	56.90 ± 11.26		
Rhizosolenia fragilissima	35.59 ± 2.67		7.04 ± 1.06	27.80 ± 12.34		
Dinophyceae total	3.93 ± 1.39	54.66 ± 3.26	43.63 ± 0.24	7.50 ± 2.37	64.20 ± 7.99	66.20 ± 6.79
Peridinium sp.		47.19 ± 4.57	38.31 ± 1.95		61.08 ± 8.78	59.86 ± 9.35
Prorocentrum micans		7.60 ± 3.92	6.84 ± 2.48		2.72 ± 0.91	6.34 ± 2.68
Naked flagellates	0.48 ± 0.16	16.84 ± 3.60	25.73 ± 12.49	0.33 ± 0.15	0.58 ± 0.38	14.42 ± 4.53
Ciliates	2.03 ± 1.66	0	0	3.97 ± 3.24	0	0
Amoeba	0	0.85 ± 0.70	0.03 ± 0.02	0	16.17 ± 7.31	1.49 ± 0.86
Cladocerans						
Pico/nanoplankton <5 µm	2.26 ± 0.36	6.26 ± 2.12	4.78 ± 0.39	0.98 ± 0.20	4.66 ± 0.09	14.83 ± 2.33
Diatoms total	60.58 ± 3.52	26.69 ± 10.76	41.15 ± 5.26	66.52 ± 6.07	15.66 ± 7.78	54.56 ± 5.05
Skeletonema costatum	6.20 ± 2.13		8.73 ± 6.67	56.90 ± 11.26		8.78 ± 3.15
Rhizosolenia fragilissima	51.60 ± 1.23		18.68 ± 8.86	27.80 ± 12.34		34.27 ± 2.73
Dinophyceae						
Total	19.23 ± 6.26	65.00 ± 12.31	28.11 ± 7.28	6.44 ± 1.61	54.65 ± 21.56	11.56 ± 7.93
Peridinium sp.		63.24 ± 11.85			54.23 ± 21.55	
Prorocentrum micans		1.83 ± 0.47			0.33 ± 0.05	
Naked flagellates	1.73 ± 0.40	1.83 ± 0.71	22.89 ± 4.01	0.63 ± 0.25	0.18 ± 0.10	16.78 ± 10.03
Ciliates	16.21 ± 4.65	0	0	25.43 ± 6.32	22.13 ± 12.78	0
Amoeba	0	0.33 ± 0.08	3.07 ± 2.44	0	2.74 ± 1.01	2.27 ± 1.00
Controls						
Pico/nanoplankton <5 µm	2.57 ± 0.08	16.48 ± 2.30	6.16 ± 0.40	1.25 ± 0.21	20.61 ± 7.12	15.80 ± 0.77
Diatoms total	64.89 ± 5.45	19.22 ± 2.70	22.33 ± 4.84	67.58 ± 10.54	44.10 ± 3.29	58.16 ± 3.35
Skeletonema costatum	16.90 ± 10.93			30.23 ± 10.09	14.88 ± 7.12	0.31 ± 0.02
Rhizosolenia fragilissima	42.49 ± 16.00			35.07 ± 1.50	27.18 ± 6.86	55.63 ± 3.66
Dinophyceae total	17.25 ± 1.45	43.47 ± 5.36	43.99 ± 5.32	3.90 ± 1.07	31.59 ± 6.93	21.97 ± 1.34
Peridinium sp.		30.89 ± 4.64	26.83 ± 2.81		21.36 ± 8.30	
Prorocentrum micans		12.77 ± 1.45	13.38 ± 0.24		9.77 ± 0.73	
Naked flagellates	4.60 ± 1.43	18.34 ± 7.61	27.53 ± 0.08	0.90 ± 0.22	1.62 ± 1.24	4.08 ± 1.24
Ciliates	10.69 ± 4.44	0.30 ± 0.24	0	26.36 ± 9.12	0	0
Amoeba	0	2.19 ± 1.13	0	0	2.07 ± 1.26	0

Table 4. Similarity between plankton communities at the end of the experiment expressed as average distances, d_{jk} (see 'Materials and methods': Eq. 2)

1st stage	Copepods	Doliolids	Cladocerans	Controls
Copepods	–	11.09	18.91	8.95
Doliolids		–	8.76	2.28
Cladocerans			–	10.32
Controls				–

ml^{-1}) and highest bacterial biovolumes (2.50×10^5 μm^3 ml^{-1}). In contrast, treatments with copepods showed the highest HNF biovolumes (1.05×10^6 μm^3 ml^{-1}) and lowest bacterial biovolumes (1.10×10^5 μm^3 ml^{-1}), with even fewer bacteria than the controls (1.26×10^5 μm^3 ml^{-1}). Under the influence of doliolids HNF had lower biovolumes (6.69×10^5 μm^3 ml^{-1}) and bacteria higher biovolumes (1.42×10^5 μm^3 ml^{-1}) than treatments with copepods and controls.

The ratio of autotrophic to heterotrophic flagellates (5.1 to 10 µm size) increased during the experimental term compared to the initial seawater (21% autotrophs). The increase was significant for all treatments (1-way ANOVA, $p \leq 0.05$, $F_{4,14} = 6.866$). Chemostats with doliolids had the highest proportion of autotrophs (63 ± 4% SE of the means, cladocerans: 43 ± 12%, copepods: 31 ± 4%, controls: 27 ± 5%). Post hoc Tukey-test analyses showed that systems with doliolids formed a separate group (Fig. 5).

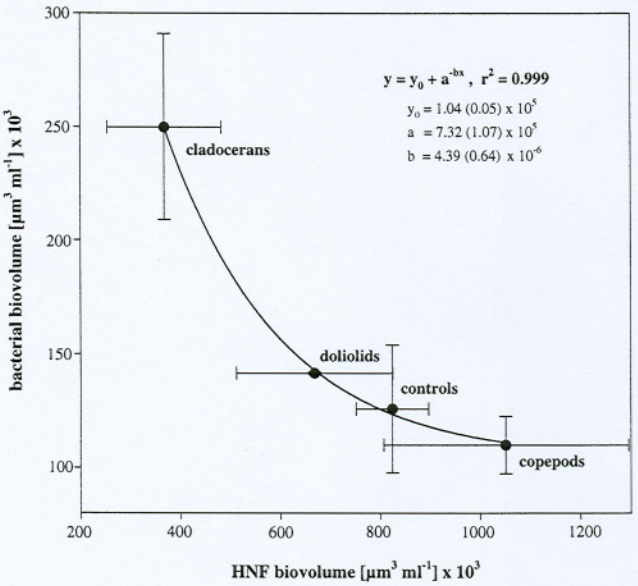

Fig. 4. Nonlinear regression analysis of heterotrophic nanoflagellate (HNF) biovolume (5.1 to 10 µm size) on bacterial biovolume (bacteria + coccal cyanobacteria). Values in parentheses are SE, $p < 0.05$. Data points are means of triplicates; error bars represent ±SE of the means

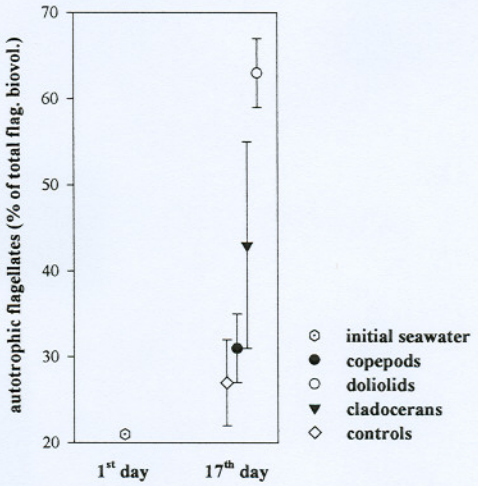

Fig. 5. Changes in the density of autotrophic flagellates as percentage total flagellate biovolume (5.1 to 10 µm size). Data points are means of triplicates; error bars represent ±SE of the means

Changes in nutrient regime

C:N ratios

The C:N ratios of copepods, cladocerans and doliolids did not differ significantly from each other (1-way ANOVA, $p = 0.576$, $F_{2,7} = 0.168$). At the end of the experiment, the particulate C:N ratios in 1st stage flasks approached the Redfield ratio (106:16, Redfield 1958; cf. Copin-Montegut & Copin-Montegut 1983) best in controls (117:16) and deviated most from it in treatments with cladocerans (141:16). Differences were not significant (1-way ANOVA, $p = 0.681$, $F_{4,13} = 0.586$). For results see Table 5.

Dissolved nutrients

At the end of the experiment silicate, nitrate, ammonium and phosphate concentrations were higher in chemostats influenced by grazers than in controls (Fig. 6). In 1st stage flasks differences were only significant for silicate (1-way ANOVA $p \leq 0.05$, $F_{3,9} = 15.176$) between copepod and doliolid treatments, between doliolid treatments and controls, and between controls and cladoceran treatments (post hoc Tukey-test $p < 0.05$). In 2nd stage flasks differences were only significant for ammonium (1-way ANOVA, $p \leq 0.001$, $F_{3,9} = 45.636$). Copepod treatments showed higher values than all other treatments and the ammonium concentrations in the cladoceran treatments were higher than in controls. These differences were significant (post hoc Tukey-test, $p < 0.05$).

Table 5. C:N ratio of grazers and of the summer plankton community in Blanes Bay (NW Mediterranean) before (initial seawater) and after prolonged grazing pressure by doliolids, cladocerans or copepods. Values in parentheses are SE of the means

Sample	C:N (SE)
Food guild	
Initial seawater	9.27 (0.36)
Copepod-chemostats	8.64 (0.40)
Doliolid-chemostats	8.72 (1.51)
Cladoceran-chemostats	8.79 (0.17)
Controls	7.32 (1.10)
Grazer	
Copepods	4.57 (0.25)
Doliolids	4.50 (0.05)
Cladocerans	4.84 (0.23)
Redfield ratio	6.63

At the end of the experiment, Si:N stoichiometry was more or less in accordance with the Redfield ratio (1:1 in all 1st stage flasks and in 2nd stage control flasks), but lower in chambers with grazers (Fig. 7). The N:P ratio was much lower than the Redfield ratio (16:1) in all 1st stage flasks. In 2nd stage flasks grazer exudates effected higher N:P values. Systems influenced by copepods corresponded best with the Redfield ratio. Chemostats with doliolids or cladocerans caused lower N:P ratios, although they were higher than those found in controls.

DISCUSSION

We used semicontinuous, re-circulating, 2-stage chemostats to study how doliolids, cladocerans and copepods can influence the phytoplankton community structure in Blanes Bay (Catalan Sea, NW Mediterranean). Semi-continuous chemostats permit a good approximation to the results gained from continuous designs (Sommer 1985), and have been proved to be effective tools in testing for direct and indirect effects of herbivore grazing on algae (e.g. Sommer 1988, 1998b).

Changes in composition of food guilds

Phytoplankton between 15 and 70 µm dominated both stages of all chemostats influenced by herbivores. This is surprising, because food size spectra of all grazer types span this size class (Katechakis 1999). Possible reasons could be unpalatability of organisms, toxicity, higher competitive abilities than other phytoplankton, or allelopathic effects. We will discuss these alternatives below.

Experiments were conducted in late August when copepods, cladocerans and doliolids are present in high abundances in Blanes Bay (500 to 780, 750 to 1250 and 90 ind. m^{-3}, respectively: Andreu & Duarte 1996), and the clear-water stadium has almost been reached (Mura et al. 1996, Satta et al. 1996). Hence, the natural (initial) community may already have been adapted to high grazing pressure. On the other hand, mostly Bacillariophyceae, mainly *Skeletonema costatum* and *Rhizosolenia fragilissima*, comprised the size class between 15 and 70 µm until Day 6 (Table 2) and both species are considered food suitable for copepods (e.g. Paffenhöfer & Knowles 1978, Ryther & Sanders 1980), cladocerans and doliolids (Katechakis 1999).

By Day 12, Dinophyceae dominated the same size class, principally *Peridinium* sp. and *Prorocentrum micans*. Both taxa are classified as potentially toxic. This possibly affected grazers adversely and benefited dinoflagellates compared to other plankton. Toxicity can be a potent instrument against grazers, particularly against selective feeders such as copepods (Granéli 1990). As we did not test for toxicity we do not know if species were really toxic. However, we did not observe any obvious detrimental effect on the grazers.

Another explanation for the observed dominance of dinoflagellates could have been allelopathic effects on other phytoplankton species. To test for allelopathy was not a topic of this work. Also, we could find no reports about possibly allelopathic effects of *Peridinium* or *Prorocentrum* species in the literature.

Organisms of intermediate size may also profit, by being (1) too large to be fed on by protozoans, but (2) small enough to be better competitors than larger algae for nutrients.

Only in 2nd stage flasks of the control treatments did large phytoplankton (>210 µm) dominate at the end of the experiment (Fig. 2). These were exclusively diatoms of the genus *Rhizosolenia* and sporadically *Nitzschia* spp. colonies (Table 1). They may have developed because of the high Si:N ratios in 2nd stage flasks in the control treatments (Fig. 7). A similar rise in large (and, due to their size, inedible) algae was observed during mesocosm experiments with high nutrient supplies in Blanes Bay (Y. Olsen unpubl. data). Differences between the 1st stage and 2nd stage control flasks may have resulted from higher Si concentrations in the 2nd stage flasks (Fig. 6). In addition it is conceivable that Bacillariophyceae benefited from dark incubation in the 2nd stage flasks, which contained high cell quotas of nitrogen and phosphorus. However, measurements cell quotas for different marine phytoplankton (Y. Olsen unpubl. data) provide no evidence for this assumption.

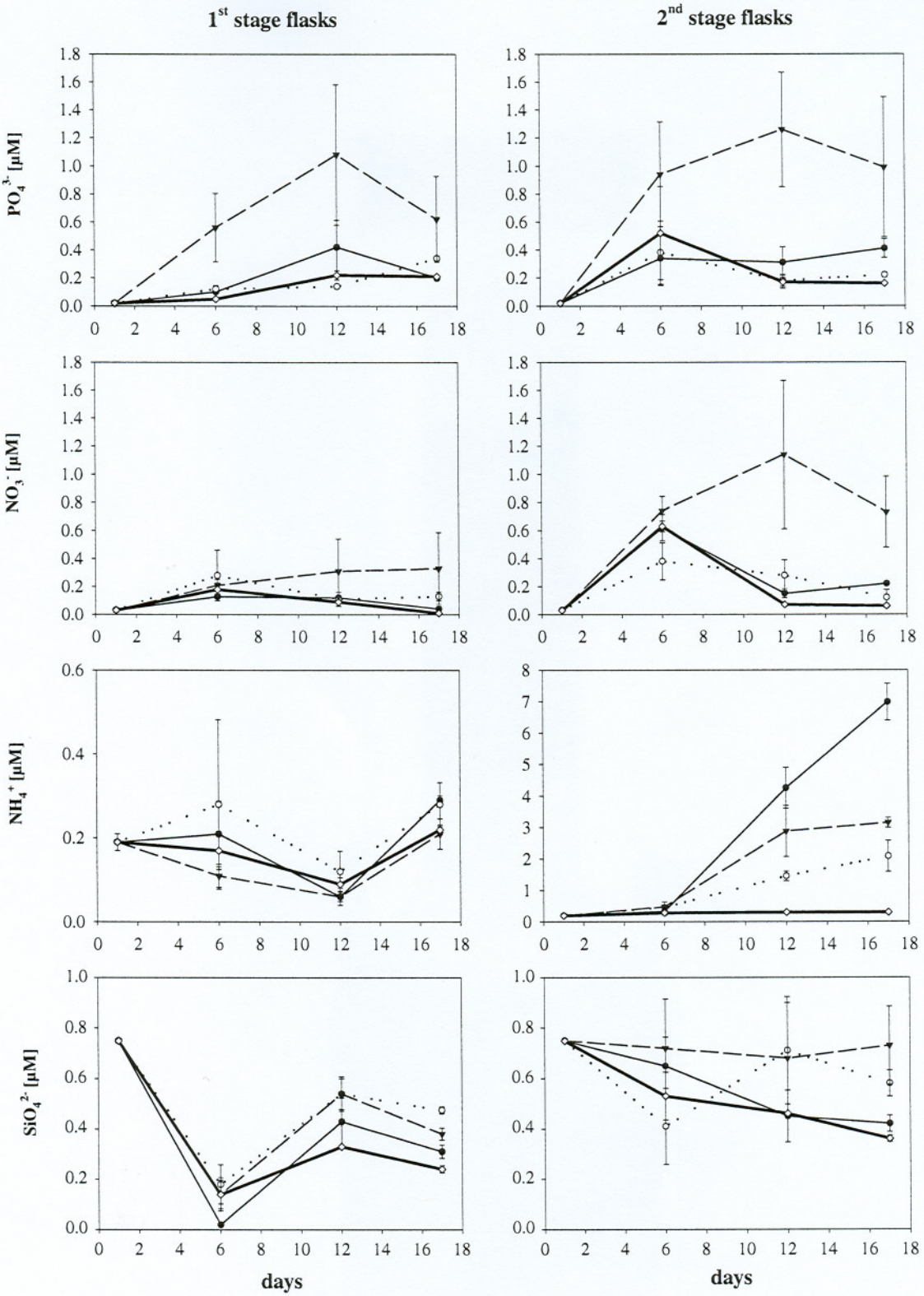

Fig. 6. Changes in the dissolved nutrient concentrations in 1st stage (left) and 2nd stage (right) flasks during the course of the experiment. Data points are means of triplicates; error bars represent ±SE of the means

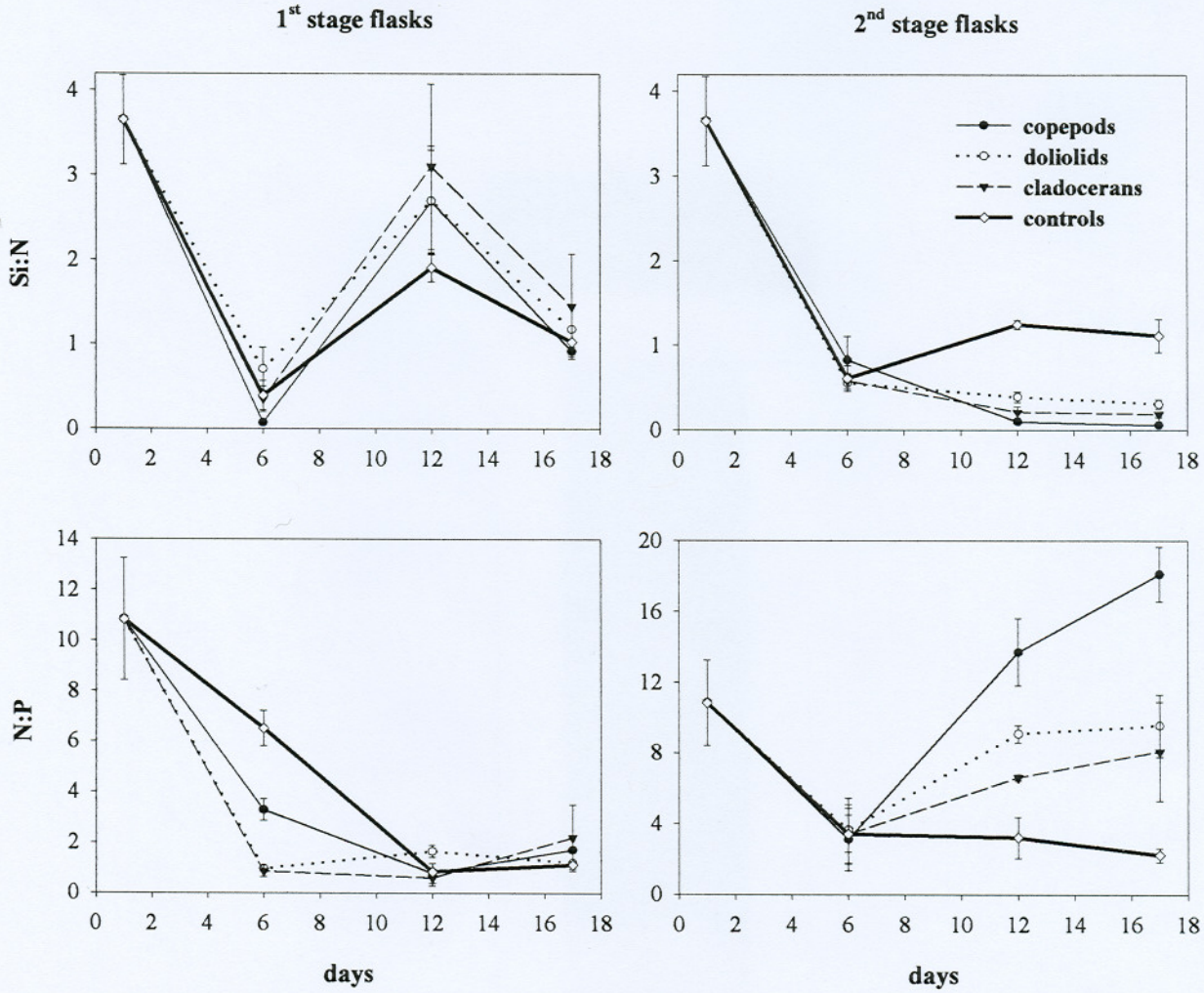

Fig. 7. Changes in the Si:N and N:P stoichometry in 1st stage (left) and 2nd stage (right) flasks during the course of the experiment. Data points are means of triplicates; error bars represent ±SE of the means

Various studies have suggested that competition between diatoms and flagellates is determined by the stoichiometry of dissolved nutrients. If Si is not limiting, diatoms usually dominate over non-siliceous species (e.g. Officer & Ryther 1980, Tilman et al. 1986, Cadée & Hegeman 1991, Sommer 1994a,b,c, 1998a,b). In the present study, grazers have influenced the nutrient regime in reaction chambers through their excretions; by increasing the dissolved nutrient concentrations, they changed the stoichiometry in relation to controls. This effect was weaker in 1st stage than in 2nd stage flasks. The stoichiometry of 1st stage flasks deviated little from that in controls at the end of the experiment (Fig. 7). It seems that the food organisms immediately assimilated the added dissolved nutrients indicating that their growth was nutrient-limited. The C:N data (Table 5) support this assumption. Biomass stoichiometry is an indicator of nutrient status (Droop 1974, 1975, Healey 1978, Healey & Hendzel 1980). All the plankton communities exposed to grazers had C:N ratios >8.3, indicating moderate N-limitation according to Healey & Hendzel (1980) and Hecky et al. (1993).

In size classes smaller and larger than 15 to 70 µm, grazers supported particle sizes outside their specific grazing spectra (Fig. 2). Unexpected distributions compared with controls occurred in chemostats influenced by doliolids, where large food-item levels (>100 µm) decreased, and in treatments with copepods, where picoplankton levels decreased. Doliolids efficiently decimated organisms >100 µm, although the maximum food size ingestible for *Doliolum denticulatum* was 75 µm in grazing experiments with natural plankton communities of Blanes Bay (Katechakis 1999). This can be explained by the circumstance that in this size class only long-chain diatoms (*Rhizosolenia* spp. and *Skeletonema costatum*) occurred, whose ingestibility depends on their orientation in the filtration stream.

Since their valve diameters are between 10 and 20 µm, it is possible that they were ingested.

The same grazing experiments showed that copepods were not able to pick up particles <7.5 µm. Yet temporarily, the proportion of picoplankton was lowest under the influence of copepods. This may be due to trophic cascade effects and will be discussed in the following section together with the implications for the microbial food web.

Measurements of similarity suggest that the potential to modify a given algal population increases with increasing selectivity of the grazer (Table 4).

Changes in composition of microbial food web (1st stage flasks)

The abundances of solitary bacteria determined in the initial samples correspond well with results of Vaqué (1996) for Blanes Bay. During the course of the experiment HNF abundances influenced the abundance of solitary bacteria. High HNF densities were accompanied by low densities of solitary bacteria, including coccal cyanobacteria (Fig. 4). The inability of copepods to ingest particles <7.5 µm led to higher HNF densities than in other treatments. This explains the low bacterial abundances in chemostats with copepods. Conversely, *Penilia avirostris* exerted the largest grazing pressure on HNF of all grazers, although this species cannot graze on the bacteria themselves (Turner at al. 1988, Katechakis 1999). Accordingly, the cladoceran treatment resulted in the highest bacteria numbers. Doliolids caused medium HNF and bacteria densities. Jürgens et al. (1994), Jürgens (1995), Jürgens & Jeppesen (2000) described similar cascading effects for limnic systems. In lakes, strong top-down effects in the pelagic are well known (Carpenter at al. 1985). It is still not clear whether such trophic cascades occur in the marine pelagic. The interactions in our experiments between mesozooplankton and the microbial food web suggest that a top-down transmission of effects can occur, at least in the lower trophic levels. Recent enclosure experiments with carnivorous mesozooplankton and natural algal communities in the NE Atlantic indicate that such effects can also occur at higher trophic levels (H.S. et al. unpubl. data).

The differences in the appearance of particle-bound bacteria and filamentous cyanobacteria between the treatments arise from an adaptation to the different kind of grazing pressures exerted by copepods, cladocerans or doliolids. Abundances of both bacterial groups increased most under the influence of doliolids (Fig. 3). Filamentous cyanobacteria of all sizes (7 to 140 µm) lay inside the food size spectrum of cladocerans and copepods, but not inside the food size spectrum of doliolids, as evaluated in grazing experiments with natural phytoplankton assemblages from Blanes Bay (Katechakis 1999). Indeed 18.9% (± 5.8% SE of the mean) of the total filamentous cyanobacterial biovolume lay over the maximum size doliolids can manipulate. As well, we found many more particles in treatments with doliolids than in other chemostats. The combination of a higher density of particles in chambers with doliolids and the production of particles larger than the ingestible food size for doliolids may explain the differences in particle-bound bacteria numbers between the doliolid treatments and the other chemostats. An elongated shape and attachment to particles can be an effective bacterial defence against grazing. Various authors have documented this for freshwater systems with respect to bacterivorous protists (e.g. Güde 1989, Šimek & Chrzanowski 1992, Jürgens et al. 1994, Jürgens 1995, Jürgens & Jeppesen 2000) and metazoan predation (Langenheder & Jürgens 2001). Little is known about similar processes in marine environments. In particular, the importance of bacterivorous metazooplankton such as appendicularians and doliolids may be underestimated.

The general increase of cyanobacteria in all 1st stage chemostats (Fig. 3) may be explained by the fact that the high light intensity was advantageous to cyanobacteria (Sommer 1994c).

The ratio of autotrophic to heterotrophic flagellates was highest in chemostats with doliolids, and these were the only treatments that differed significantly from controls (Fig. 3). The annual average autotrophic nanoflagellates (ANF) is 50.2% of the nanoflagellate community in Blanes Bay (Vaqué 1996). Under the influence of doliolids, the proportion was 13% higher. One conceivable cause is that doliolids competed with HNF for bacteria, so that the relative proportion of ANF increased. Comparable changes *in situ* might have consequences for the trophic interactions at lower trophic levels, as the grazing pressure on the microbial food web would be altered. Carbon demands should rise relative to production and, hence, lead to the potential for top-down control of bacterial biomass and production. Changes in food chain length and energetic transfer efficiency might follow.

Our results show that in marine systems direct and indirect effects of herbivores can result in trophic cascades and that the effects of herbivores on phyto-, protozoo- and bacterioplankton strongly depend on the taxonomic compostion of the herbivores. This has implications for the modeling of grazing effects in marine pelagic ecosystems.

Acknowledgements. This article is a contribution to the MAST III-project COMWEB founded by the EU (MAST3-CT96-0052).

LITERATURE CITED

Alldredge AL, Madin LP (1982) Pelagic tunicates: unique herbivores in the marine plankton. BioScience 32(8):655–663

Andersen T (1997) Pelagic nutrient cycles: herbivores as sources and sinks. Ecol Stud 129:1–280

Andreu P, Duarte CM (1996) Zooplankton seasonality in Blanes Bay (northwest Mediterranean). Publ Espec Inst Esp Oceanogr 22:47–54

Bjørnsen PK (1986) Automatic determination of bacterioplankton biomass by image analysis. Appl Environ Microbiol 51(6):1199–1204

Booth BC, Lewin J, Norris RE (1982) Nanoplankton species predominant in the subarctic Pacific in May and June 1978. Deep-Sea Res Part I 29(2):185–200

Bøsheim KY, Bratbak G (1987) Cell volume to carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar Ecol Prog Ser 8:211–223

Cadée GC, Hegeman J (1991) Historical phytoplankton data for the Marsdiep. Hydrobiol Bull 29:111–119

Carpenter SR, Kitchell JF, Hodgson DR (1985) Cascading trophic interactions and lake productivity. BioScience 35: 634–639

Copin-Montegut C, Copin-Montegut G (1983) Stoichiometry of carbon, nitrogen, and phosphorus in marine particular matter. Deep-Sea Res 30:31–46

Crocker KM, Alldredge AL, Steinberg DK (1991) Feeding rates of the doliolid, *Dolioletta gegenbauri*, on diatoms and bacteria. J Plankton Res 13(1):77–82

Cushing DH (1975) Marine ecology and fisheries. Cambridge University Press, Cambridge

DeBiase AER, Sanders RW, Porter KG (1990) Relative nutritional value of ciliate protozoa and algae as food for *Daphnia*. Microb Ecol 19:99–210

Deibel D (1982a) Laboratory determined mortality, fecundity, and growth rates of *Thalia democratica* Forskal and *Dolioletta gegenbauri* Uljanin (Tunicata, Thaliacea). J Plankton Res 4:143–153

Deibel D (1982b) Laboratory-measured grazing and ingestion rates of the salp, *Thalia democratica* Forskal, and the doliolid, *Dolioletta gegenbauri* Uljanin (Tunicata, Thaliacea). J Plankton Res 4(2):189–202

Deibel D (1998) The abundance, distribution and ecological impact of doliolids. In: Bone Q (ed) The biology of pelagic tunicates. Oxford University Press, Oxford, p 171–186

Droop MR (1974) The nutrient status of algal cells in continuous culture. J Mar Biol Assoc UK 54:825–855

Droop MR (1975) The nutrient status of algal cells in batch culture. J Mar Biol Assoc UK 55:541–555

Escaravage V, Prins TC, Smaal AC, Peeters JHC (1996) The response of phytoplankton communities to phosphorous input reductions in mesocosm experiments. J Exp Mar Biol Ecol 198:55–79

Fuhrmann JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters off British Columbia, Antarctica and California. Appl Environ Microbiol 39: 1085–1095

Gismervik I, Andersen T, Vadstein O (1996) Pelagic food webs and eutrophication of coastal waters: impact of grazers on algal communities. Mar Pollut Bull 33(1–6): 22–35

Granéli E (ed) (1990) Toxic marine phytoplankton. Elsevier, New York

Grasshoff K, Kremling K, Ehrhardt M (1983) Methods of seawater analysis. Wiley-VCH, Weinheim

Güde H (1989) The role of grazing on bacteria in plankton succession. In: Sommer U (ed) Plankton ecology, succession in plankton communities. Springer-Verlag, Berlin, p 365–374

Healey FP (1978) Physiological indicators of nutrient deficiency in algae. Mitt Int Ver Theor Angew Limnol 21: 34–41

Healey FP, Hendzel LL (1980) Physiological indicators of nutrient deficiency in lake phytoplankton. Can J Fish Aquat Sci 37:442–453

Hecky RE, Campbell P, Hendzel LL (1993) The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. Limnol Oceanogr 38:709–724

Hessen DO, Andersen T (1992) The algae-grazer interface: feedback mechanisms linked to elemental ratios and nutrient cycling. Ergeb Limnol 35:111–120

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35(2):403–424

Iverson RL (1990) Control of marine fish production. Limnol Oceanogr 35:1593–1604

Jürgens K (1995) Die Bedeutung heterotropher Nanoflagellaten als Bakterienkonsumenten sowie deren Regulation durch Prädation und Ressourcen. Berichte aus der Biologie, Shaker, Aachen

Jürgens K, Jeppesen E (2000) The impact of metazooplankton on the structure of the microbial food web in a shallow, hypertrophic lake. J Plankton Res 22(6):1047–1070

Jürgens K, Arndt H, Rothhaupt KO (1994) Zooplankton-mediated change of bacterial community structure. Microb Ecol 27(1):27–42

Katechakis A (1999) Nischenüberlappung zwischen herbivorem gelatinösen- und Crustaceen-Zooplankton im NW-Mittelmeer (Catalanisches Meer). Thesis, Universität Kiel

Kiørboe T (1998) Population regulation and role of mesozooplankton in shaping marine pelagic food webs. Hydrobiologia 363:13–27

Langenheder S, Jürgens K (2001) Regulation of bacterial biomass and community structure by metazoan and protozoan predation. Limnol Oceanogr 46(1):121–134

Lund JWG, Kipling C, LeCren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11: 147–170

Miralto A, Barone G, Romano G, Poulet AS and 7 others (1999) The insidious effect of diatoms on copepod reproduction. Nature 402:173–176

Mura MP, Agusti S, Cebrian J, Satta-MP (1996) Seasonal variability of phytoplankton biomass and community composition in Blanes Bay (1992–1994). Publ Espec Inst Esp Oceanogr 22:23–29

Officer CB, Ryther JH (1980) The possible importance of silicon in marine eutrophication. Mar Ecol Prog Ser 3:83–91

Paffenhöfer GA, Knowles SC (1978) Feeding of marine planktonic copepods on mixed phytoplankton. Mar Biol 48(2): 143–152

Paffenhöfer GA, Stewart TB, Youngblouth MJ, Bailey TG (1991) High resolution vertical profiles of pelagic tunicates. J Plankton Res 13:971–981

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948

Redfield AC (1958) The biological control of the chemical factors in the environment. Am Sci 46:205–221

Reid FMH (1983) Biomass estimation of components of the marine nannoplankton and picoplankton by the Utermoehl settling technique. J Plankton Res 5(2):235–252

Ryther JH, Sanders JG (1980) Experimental evidence of zooplankton control of the species composition and size distri-

bution of marine phytoplankton. Mar Ecol Prog Ser 3(4): 279–283
Satta MP, Agusti S, Mura MP, Duarte CM (1996) Seasonal variability of planktonic gross primary production in Blanes Bay (March 1992–March 1994). Publ Espec Inst Esp Oceanogr 22:31–38
Schöllhorn E, Granéli E (1996) Influence of different nitrogen to silica ratios and artifical mixing on the structure of a summer phytoplankton community from the Swedish west coast (Gullmar Fjord). J Sea Res 35:159–167
Šimek K, Chrzanowski TH (1992) Direct and indirect evidence of size-selective grazing on pelagic bacteria by freshwater nanoflagellates. Appl Environ Microbiol 58: 3715–3720
Simon M, Azam F (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar Ecol Prog Ser 51:201–213
Sommer U (1983) Nutrient competition between phytoplankton species in multispecies chemostat experiments. Arch Hydrobiol 96:399–416
Sommer U (1985) Comparison between steady state and non steady state competition: experiments with natural phytoplankton. Limnol Oceanogr 30(2):335–346
Sommer U (1988) Phytoplankton succession in microcosm experiments under simultaneous grazing pressure and resource limitation. Limnol Oceanogr 33:1037–1054
Sommer U (1994a) Are marine diatoms favored by high Si:N ratios? Mar Ecol Prog Ser 115:309–315
Sommer U (1994b) The impact of light intensity and daylength on silicate and nitrate competition among marine phytoplankton. Limnol Oceanogr 39(7):1680–1688
Sommer U (1994c) Planktologie. Springer-Verlag, Berlin, Heidelberg
Sommer U (1996) Nutrient competition experiments with periphyton from the Baltic Sea. Mar Ecol Prog Ser 140: 161–167
Sommer U (1998a) Silicate and the functional geometry of marine phytoplankton. J Plankton Res 20(9):1853–1859
Sommer U (1998b) From algal competition to animal production: enhanced ecological efficiency of *Brachionus plicatilis* with a mixed diet. Limnol Oceanogr 43(6):1393–1396
Sommer U, Stibor H, Katechakis A, Hansen T (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. Hydrobiologia (in press)
Strickland J, Parsons TR (1972) A practical handbook of seawater analysis, 2nd edn. Bull Fish Res Board Can 167:1–310
Tang KW, Dam HG (2001) Phytoplankton inhibition of copepod egg hatching: test of an exudate hypothesis. Mar Ecol Prog Ser 209:197–202
Tilman D (1982) Resource competition and community structure. Princeton University Press, Princeton, NJ
Tilman D, Kiesling R, Sterner RW, Kilham SS, Johnson FA (1986) Green, blue-green and diatom algae: taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Arch Hydrobiol 106:473–485
Turner JT, Tester PA, Ferguson RL (1988) The marine cladoceran *Penilia avirostris* and the 'microbial loop' of pelagic food webs. Limnol Oceanogr 33(2):245–255
Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt Int Verein Theor Angew Limnol 9:1–38
Vaqué D (1996) Seasonal dynamics of planktonic microbial communities on the coast of the northwest Mediterranean Sea. Publ Espec Inst Esp Oceanogr 22:39–46
Verity PG, Smetacek V (1996) Organism life cycles, predation, and the structure of marine pelagic ecosystems. Mar Ecol Prog Ser 130:277–293

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: April 25, 2001; Accepted: December 5, 2001
Proofs received from author(s): April 26, 2002

PAPER A3

Feeding selectivities of the marine cladocerans *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni*

Katechakis A, Stibor H (2004)

***Marine Biology* 145:529-539**

Marine Biology (2004) 145: 529–539
DOI 10.1007/s00227-004-1347-1

RESEARCH ARTICLE

A. Katechakis · H. Stibor

Feeding selectivities of the marine cladocerans *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni*

Received: 28 November 2002 / Accepted: 27 February 2004 / Published online: 1 April 2004

Abstract We conducted grazing experiments with the three marine cladoceran genera *Penilia*, *Podon* and *Evadne*, with *Penilia avirostris* feeding on plankton communities from Blanes Bay (NW Mediterranean, Spain), covering a wide range of food concentrations (0.02–8.8 $mm^3\ l^{-1}$, plankton assemblages grown in mesocosms at different nutrient levels), and with *Podon intermedius* and *Evadne nordmanni* feeding on the plankton community found in summer in Hopavågen Fjord (NE Atlantic, Norway, 0.4 $mm^3\ l^{-1}$). *P. avirostris* and *P. intermedius* showed bell-shaped grazing spectra. Both species reached highest grazing coefficients at similar food sizes, i.e. when the food organisms ranged between 15 and 70 μm and between 7.5 and 70 μm at their longest linear extensions, respectively. *E. nordmanni* preferred organisms of around 125 μm, but also showed high grazing coefficients for particles of around 10 μm, while grazing coefficients for intermediate food sizes were low. Lower size limits were >2.5 μm, for all cladocerans. *P. avirostris* showed upper food size limits of 100 μm length (longest linear extension) and of 37.5 μm particle width. Upper size limits for *P. intermedius* were 135 μm long and 60 μm wide; those for *E. nordmanni* were 210 μm long and 60 μm wide. Effective food concentration (EFC) followed a domed curve with increasing nutrient enrichment for *P. avirostris*; maximum values were at intermediate enrichment levels. The EFC was significantly higher for *P. intermedius* than for *E. nordmanni*. With increasing food concentrations, the clearance rates of *P. avirostris* showed a curvilinear response, with a narrow modal range; ingestion rates indicated a rectilinear functional response. Mean clearance rates of *P. avirostris*, *P. intermedius* and *E. nordmanni* were 25.5, 18.0 and 19.3 ml $ind.^{-1}\ day^{-1}$, respectively. Ingestion rates at similar food concentrations (0.4 $mm^3\ l^{-1}$) were 0.6, 0.8 and 0.9 μg C $ind.^{-1}\ day^{-1}$.

Communicated by O. Kinne, Oldendorf/Luhe

A. Katechakis (✉) · H. Stibor
Institute of Marine Sciences, Experimental Ecology, Christian-Albrechts-University, Düsternbrooker Weg 20, 24105 Kiel, Germany
E-mail: Katechakis@zi.biologie.uni-muenchen.de
Tel.: +49-89-5902265
Fax: +49-89-5902461

Present address: A. Katechakis
Department Biology II, Aquatic Ecology, Ludwigs-Maximilians-University, Karlstraße 23–25, 80333 Munich, Germany

Introduction

The grazing behaviour of herbivorous mesozooplankton is one of the critical factors structuring pelagic food webs. Herbivores distribute the organic matter synthesised by autotrophs towards higher trophic levels. Herbivorous cladocerans are considered filter feeders (Brendelberger et al. 1986) and form the most well-studied group of mesozooplankton in lakes. In contrast to the multitude of over 600 recorded freshwater cladoceran species (Schram 1986), only eight cladoceran species have been reported to be truly marine (Onbé 1977). These belong to the three genera *Penilia*, *Podon* and *Evadne* (Baker 1938; Onbé 1977). *Penilia* only exists in temperate waters (Della Croce and Venugopal 1973; Grahame 1976), while *Podon* and *Evadne* are mainly found in boreal oceans (Raymont 1983). In contrast to limnic systems, cladocerans play a less important role in marine pelagic systems compared to copepods (Raymont 1983; Egloff et al. 1997). Nevertheless, cladocerans sporadically consume a substantial portion of the primary production in marine environments (Bosch and Taylor 1973; Turner et al. 1988; Kim et al. 1989). Besides other factors, food size is fundamentally important for feeding relationships in the pelagic. It is the only community that follows the rule "smaller organisms are eaten by larger ones", except for parasitic fungi and a few naked protozoa (Sommer and Stibor 2002). The selectivity-size range of marine cladocerans is not well known. Moreover, published studies are to some extent contradictory. This may be due to the use of different methods by different authors. Feeding experiments with *Penilia avirostris* showed that this species does not generally graze upon particles >15 μm (Gore 1980;

Paffenhöfer and Orcutt 1986; Turner et al. 1988). Nival and Ravera examined the morphology of the feeding appendages of *Podon* (Nival and Ravera 1981) and *Evadne* (Nival and Ravera 1979), and suggested that these animals probably consume food particles with sizes up to 250 μm and between 20 and 170 μm, respectively. Based on gut content analyses, Kim et al. (1989) have reported a size range of food organisms from 4 to 115 μm for marine cladocerans in general, with no significant differences between *Penilia*, *Podon* and *Evadne*. To resolve the contradictory evidence presented in the literature, we chose similar techniques to investigate the selectivity-size spectra of *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni* feeding on natural plankton communities.

Materials and methods

Grazing experiments with *Penilia avirostris* were conducted in the NW Mediterranean (Blanes Bay, Catalan Sea, Spain), those with *Podon intermedius* and *Evadne nordmanni* in the NE Atlantic (Hopavågen, Trondheim Fjord, Norway). Experiments were performed as batch cultures in 100-ml glass jars. The jars were placed randomly in a water bath at a temperature around 22°C in Spain and around 20°C in Norway. The in situ surface temperature in Blanes Bay was 25–26°C (measured with a WTW LF 20 temperature sensor) and 17–18°C in Hopavågen Fjord. We filled the jars with natural plankton assemblages, including bacterioplankton, protozoa and phytoplankton, found in summer in Blanes Bay or in Hopavågen Fjord. Plankton assemblages for the grazing experiments with *P. avirostris* were grown in mesocosms 0.5 sea miles (~0.93 km) off-shore, at different nutrient levels. The mesocosm units (33 m^3) received N, Si and P at a stoichiometric ratio of 20 N:7 Si:1 P, at the normal nutrient loading rate at the site (5 mmol N m^{-2} day^{-1} and 0.25 mmol P m^{-2} day^{-1}) and at 0.5–16 times the normal nutrient loading rate (for more details see Duarte et al. 2000). Enrichment resulted in nine different food densities, covering a biovolume range between 0.02 and 8.8 mm^3 l^{-1} (Table 1) and a range of seston food sizes from < 1 to 300 μm at the longest linear extension (Tables 1, 2). For the grazing experiments with *P. intermedius* and *E. nordmanni* we used the natural phytoplankton community found in the summer in Hopavågen Fjord, with a density of 0.4 mm^3 l^{-1} and a size range of < 1 to 380 μm (Tables 1, 3).

To exclude extraneous metazoan grazers from the jars, water was filtered through a plankton net with a mesh size of 100 μm. Filtration let sufficient needle-shaped algae > 100 μm pass. For each of the nine food densities in the experiments with *P. avirostris*, five adult cladocerans (mean ± SEM: 680 ± 44 μm, $n = 25$) were incubated once for 6 h in the dark. Experiments with *P. intermedius* (569 ± 48 μm, $n = 15$) and with *E. nordmanni* (690 ± 31 μm, $n = 17$) were replicated three times. Here also five individuals were incubated for each treatment. The cladocerans were collected with surface tows using a mesozooplankton net with a mesh size of 250 μm, and returned to the laboratory inside a cooler within 1 h of collection. We sorted experimental grazers with a wide-bore pipette, placed them into filtered seawater and allowed them to acclimate to the laboratory conditions for 1 h, before we incubated them for the experiments. We visually controlled whether grazers were intact at the beginning of the experiments and several times during the experimental terms by observing their swimming behaviour. To prevent food plankton sedimentation, we also mixed the vessels gently on this occasion. In addition, mesozooplankton swimming caused some turbulence in the flasks. To correct for possible changes in the food guild during the experiment, we took initial samples and compared them with controls without mesozooplankton. We did this once for each of the nine food densities in the experiments with *P. avirostris* and in triplicate for the experiments with *P. intermedius* and with *E. nordmanni*. After the incubation period, experiments were terminated by addition of Lugol's iodine (5 g I_2 + 10 g KI ad 100 ml aq. dest.) to all vessels.

To determine grazer-induced changes in the abundance, the species composition, and the biovolume of the food guild, we counted samples using an inverted microscope (Leica DMIL), counting settling chambers with a volume of 10 or 30 ml, depending on the food guild's density (Utermöhl 1958). Sedimentation time lasted at least 24 h. If present, we counted at least 400 cells of each species to ensure an error margin of < 10% (Lund et al. 1958). Biovolumes were calculated using the

Table 1 Densities, size spectra, main food size and relative biovolume of main food size of plankton communities offered as food in grazing experiments with *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni*

Plankton community	Density ($mm^3 l^{-1}$)	Size spectrum (μm)	Main size class (interval means in μm)	Relative biovolume of main size class (%)
Blanes Bay				
1	0.02	< 1 to 50	2.5	36.0
2	0.07	< 1 to 60	2.5	41.6
3	0.09	< 1 to 85	10	59.5
4	0.36	< 1 to 125	40	54.6
5	0.36	< 1 to 125	85	35.1
6	1.29	< 1 to 205	85	57.9
7	5.46	< 1 to 250	125	86.9
8	5.94	< 1 to 250	175	78.9
9	8.81	< 1 to 300	> 210	53.9
Hopavågen Fjord	0.38	< 1 to 380	10 and 40	24.7 and 25.5

Table 2 Taxonomic list of all plankton from Blanes Bay (NW Mediterranean) offered as food in grazing experiments with *Penilia avirostris*. Biovolumes were calculated using the equations of Hillebrand et al. (1999). Carbon contents were estimated after Nalewajko (1966) for phytoplankton, after Bøsheim and Bratbak (1987) for flagellates, and after DeBiase et al. (1990) for ciliates (*ANF* autotrophic nanoflagellates; *HNF* heterotrophic nanoflagellates)

Taxon	Cell dimension (μm)		Biovolume ($\mu m^3 cell^{-1}$)	Biomass (pg C $cell^{-1}$)
	Longest extension	Width or diameter		
Picoplankton				
~1 μm	1	1	0.52	0.05
Nanoplankton				
~2.5 μm	2.5	2.5	8.2	0.82
~5 μm	5	5	65	6.5
Cyanobacteria				
Filamentous	20	2.5	98	9.8
Bacillariophyceae				
Centrales				
Bacteriastrum sp.	30	20	9,425	943
Coscinodiscus sp.	12.5–150	12.5–150	920–530,144	92–53,014
Chaetoceros socialis	17.5–52.5	10–30	221–663	22–66
Chaetoceros sp. A	25	15	295	30
Chaetoceros sp. B	75	62.5	2,356	236
Rhizosolenia alata	60–125	5–15	1,178–22,089	118–2,209
Rhiosolenla fragilissima	125–250	15	22,089–44,178	2,209–4,418
Rhizosolenia shrubsolei	300	7.5–20	5,890	589
Skeletonema costatum	32.5–195	7.5	1,436–8,616	144–862
Stephanopyxis sp.	120	30	85,765	8,577
Thalassiosira sp.	42.5–255	15	7,510–45,060	751–4,506
Pennales				
Licmophora sp.	70–120	15–30	13,779–94,162	1,378–9,416
Navicula sp.	15	5	147	15
Nitzschia longissima	75–200	2.5–7.5	125–2,344	13–234
Pleurosigma sp.	50	2.5	1,094	109
Dinophyceae				
Dinophysiales				
Dinophysis rotundata	40	12.5	4712	471
Dinophysis sp.	25	15	2,045	205
Peridiniales				
Ceratium lineatum	100	30	8,357	836
Ceratium longipes	180	165	74,286	7,429
Gymnodinium sp.	15	10	785	79
Heterocapsa triguetra	20	15	1,178	118
Peridinium sp. A	15	15	1,767	177
Peridinium sp. B	55	37.5	40,497	4,050
Prorocentrales				
Prorocentrum micans	30	15	2,209	221
Prymnesiophyceae				
Coccolithus sp.	7.5	7.5	221	22
Other flagellates				
ANF spp.	2.5–10	2.5–10	8.2–523	1.8–73
HNF spp.	2.5–10	2.5–10	8.2–523	1.8–73
Ciliata				
Ciliate sp. A	15	10	4,712	660
Ciliate sp. B	25	20	29,452	4,123

equations of Hillebrand et al. (1999). For this purpose, we measured the linear dimensions of 20 specimens of each species. Carbon contents were estimated after Nalewajko (1966) for phytoplankton, after Bøsheim and Bratbak (1987) for flagellates, and after DeBiase et al. (1990) for ciliates (Tables 2, 3).

For all following analyses, we subdivided the plankton community into nine size classes with interval means of 1, 2.5, 5, 10.25, 42.5, 85, 125, 175 and 205 μm and into organisms ≥210 μm. Food species were assigned to classes according to the longest linear dimension of cells or colonies. By the designations pico-, nano- and microplankton we mean food sizes ranging from around 0.2 to 2, 2 to 20 and 20 to 200 μm, respectively.

Selectivity coefficients and effective food concentrations

Grazing coefficients g (day^{-1}) of *P. avirostris*, *P. intermedius* and *E. nordmanni* were calculated using the equations of Frost (1972):

$$g = \mu - \frac{\ln C_1^* - \ln C_0^*}{t_1 - t_0} \text{ with } \mu = \frac{\ln C_1 - \ln C_0}{t_1 - t_0} \quad (1)$$

where μ is the gross growth rate of food organisms, C_1 and C_0 are the food concentrations (μm^3 ml^{-1}) at the end (t_1) and at the beginning (t_0) of the experiment in the controls, and C_1* and C_0* are the food concentrations in treatments with grazers.

Table 3 Taxonomic list of all plankton from Hopavågen Fjord (NE Atlantic) offered as food in grazing experiments with *Podon intermedius* and *Evadne nordmanni*. Biovolumes were calculated using the equations of Hillebrand et al. (1999). Carbon contents were estimated after Nalewajko (1966) for phytoplankton, after Bøsheim and Bratbak (1987) for flagellates, and after DeBiase et al. (1990) for ciliates

Taxon	Cell dimension (μm)		Biovolume ($\mu m^3 cell^{-1}$)	Biomass (pg C $cell^{-1}$)
	Longest extension	Width or diameter		
Picoplankton				
1 μm	1	1	0.52	0.05
Nanoplankton				
2.5 μm	2.5	2.5	8.2	0.82
5 μm	5	5	65	6.5
7.5 μm	7.5	7.5	221	22
Cyanobacteria				
Filamentous	15	2.5	74	7.4
Bacillariophycese				
Centrales				
Coscinodiscus sp.	20	20	3,770	377
Chaetoceros socialis	15	7.5	147	15
Chaetoceros sp.	60	35	571	57
Leptocylindrus minimus	22.5	5–7.5	442	44
Leptocylindrus danicus	40	10–15	3,142	314
Rhizosolenia sp.	15	7.5–10	663	66
Skeletonema costatum	12.5	7.5	773	77
Pennales				
Licmophora sp.	90	25	14,456	1,446
Nitzschia longissima	70–130	2.5–10	104–525	10–53
Pleurosigma sp.	95	2.5–5	2,019	202
Pseudonitzschia pungens	95	2.5–5	742	74
Pseudonitzschta sp.	75–105	2.5–5	468–1,181	47–118
Thalassionema nitzschioides	25	2.5–7.5	313	31
Thalssiosira sp.	15	5	295	29
Dinophyceae				
Diaophysiales				
Dinophysis acuminata	55	50	71,995	7,199
Dinophysis acuta	45	27.5	17,819	1,782
Dinophysis norvegica	65	45	68,919	6,892
Perdiniales				
Alexandrium sp.	25	20	3,272	327
Ceratiumfurca	105–320	44–125	113,097–2,208,932	11,310–220,893
Ceratiumfusus	380	30	62,177	6,218
Ceratium tripos	305	255	452,389	45,239
Dinoflagellate sp.	25	10	1,309	131
Eutreptiella sp.	40	10	1,047	105
Gymnodinium sp.	30	10	1,571	157
Peridinium sp.	22.5–35	20–30	4,712–16,493	471–1,649
Protoperidinium sp.	30	25	4,909	491
Scrippsiella sp.	32.5	13.75	3,514	351
Prorocentrales				
Prorocentrum micans	57.5	30	14,137	1,414
Prymnesiophyceae				
Coccolithus sp.	7.5	7.5	221	22
Phaeocystis pouchetii	7.5	7.5	221	22
Chrysophyceae				
Distephanus speculum	42.5	40	4,189	419
Cryptophyceae				
Rhodomonas sp.	15	7.5	276	28
Ciliata				
Ciliate spp.	20–55	15–55	3,142–39,597	314–3,960
Others				
Cyst	10–20	7.5–15	295–2,356	30–236

Selectivity was studied through the normalised selectivity coefficient W'' defined by Vanderploeg and Scavia (1979) and modified after Vanderploeg et al. (1984):

$$W' = \frac{g_i}{g_{\max}} \quad (2)$$

where g_i is the grazing coefficient reached for food size class i and $g_{\max}$ is the grazing coefficient for the most preferred size class ($0 < W' < 1$). Confidence intervals were used to estimate which selectivity coefficients differ significantly from zero grazing.

According to Vanderploeg et al. (1984), these W' values were used to estimate the effective food concentrations

(EFC) for every grazer and every plankton community offered as food:

$$\mathrm{EFC} = \sum_{i=1}^{n} W' \cdot X_i \quad (3)$$

where X_i is the concentration of food size class i and n is the total number of size classes.

Clearance rates and ingestion rates

Clearance rates F (ml ind.$^{-1}$ day^{-1}) and ingestion rates I (µg C ind.$^{-1}$ day^{-1}) were calculated according to Frost (1972):

$$F = V \cdot \frac{g}{N_G} \text{ and } I = F \cdot \overline{C} \quad (4)$$

where V is the jar volume (ml), g is the grazing coefficient (day^{-1}), N_G is the number of incubated animals, and $\overline{C}$ is the mean food concentration (µg C ml^{-1}) in the experimental vessel.

Results

Selectivity-size spectra, selectivity profiles and effective food concentrations

Figure 1 shows the selectivity-size spectra of *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni*, based on the grazing coefficients presented in Table 4. In the case of *P. avirostris* values for *W'* are overall means calculated from the single experiments shown in Fig. 2. Not all plankton communities offered as food in the grazing experiments with *P. avirostris* covered the whole size range of all food size classes (Table 1; Fig. 2). Therefore, calculation of means and standard errors (SEM) for *P. avirostris* are based on three to nine measurements (*n* in Table 4). As no organisms ≥210 µm were eaten by any of the investigated cladoceran species, we pooled all plankton offered as food ≥210 µm into one size class. As this size class has no defined upper limit, and thus no defined interval mean, it is not included in Fig. 1.

P. avirostris reached highest grazing coefficients at intermediate food sizes between 15 and < 70 µm. These consisted mainly of diatoms (50.5–100% of the biovolume of the size class) with valve diameters from 2.5 to 20 µm. Lower size limits were > 2.5 µm, thus including nanoflagellates and ciliates. Upper size limits covered particles up to 100 µm long (the dominating length in size class 125 µm) and 37.5 µm wide. The selectivity profile hardly changed from one experiment to another as the shape of the particle size spectrum changed (Fig. 2). *P. avirostris* always showed high selectivity coefficients for intermediate food sizes (nanoplankton and small microplankton), even if other food sizes dominated the community. Only in two cases (TFC 1.3 and 5.5 mm^3 l^{-1}, communities 6 and 7) did *P. avirostris* also express high grazing rates on larger food items of around 85–125 µm (diatoms with valve diameters ≤ 20 µm).

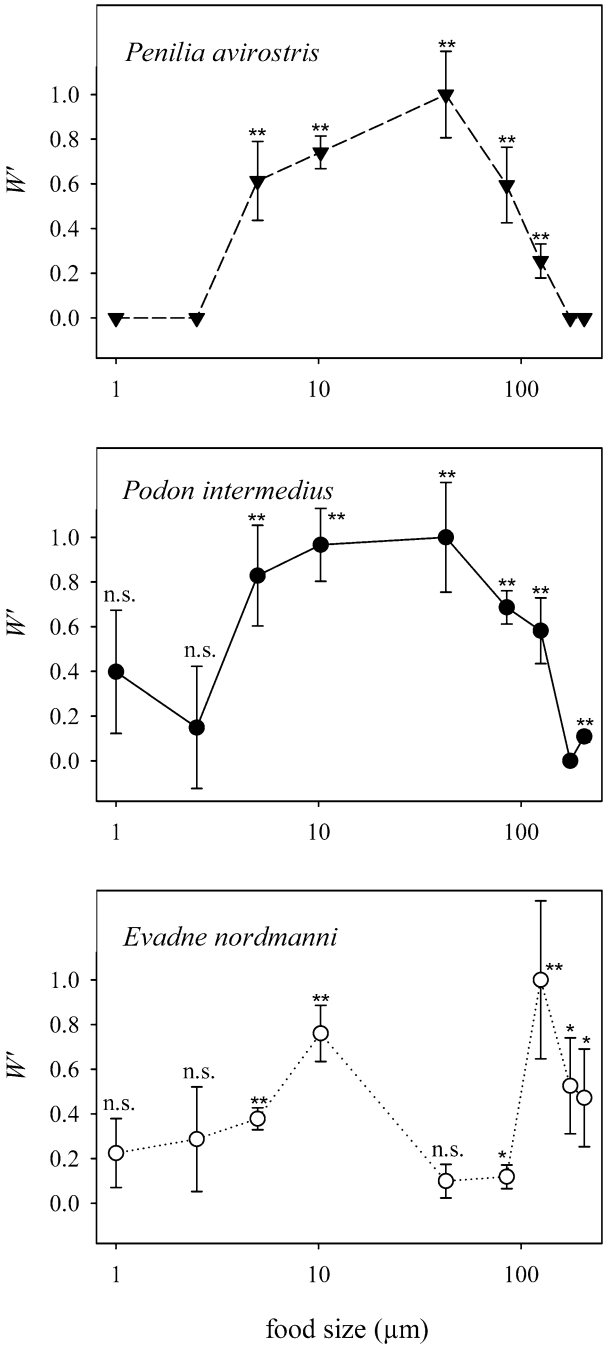

Fig. 1 *Penilia avirostris*, *Podon intermedius*, *Evadne nordmanni*. Food size selectivities, based on the selectivity coefficients *W'* of Vanderploeg and Scavia (1979) presented in Table 4. Data points are means of three to nine measurements for *P. avirostris* (see Table 4 for details) and three measurements each for *P. intermedius* and *E. nordmanni*. *Error bars*: ± SEM. *Asterisks* denote statistically significant differences from zero at $P < 0.05$ (*, 95% CI) and at $P < 0.01$ (**, 99% CI), respectively (*n.s.* not significant). Note logarithmic scale of food size axes

Table 4 *Penilia avirostris*, *Podon intermedius*, *Evadne nordmanni*. Absolute (g, day^{-1}) and relative (W', %) grazing coefficients for different food sizes. Means (±SEM) are based on three to nine measurements (n) for *P. avirostris* (see "Materials and methods" for details) and three measurements each for *P. intermedius* and *E. nordmanni*. *Asterisks* denote statistically significant differences from zero at $P<0.05$ (*, 95% CI) and at $P<0.01$ (**, 99% CI), respectively (*n.s.* not significant)

Food size class (μm)	Interval means (μm)	*Penilia avirostris*				*Podon intermedius*			*Evadne nordmanni*		
		n	g	W'	Signif.	g	W'	Signif.	g	W'	Signif.
~1	1	9	0	0		0.08 ± 0.05	0.40 ± 0.28	n.s.	0.06 ± 0.04	0.22 ± 0.16	n.s.
~2.5	2.5	9	0	0		0.03 ± 0.05	0.15 ± 0.27	n.s.	0.07 ± 0.06	0.29 ± 0.23	n.s.
>2.5 to <7.5	5	9	0.05 ± 0.01	0.61 ± 0.18	**	0.16 ± 0.04	0.83 ± 0.23	**	0.09 ± 0.01	0.38 ± 0.05	**
7.5 to <15	10.25	9	0.06 ± 0.01	0.74 ± 0.07	**	0.18 ± 0.03	0.97 ± 0.16	**	0.19 ± 0.03	0.76 ± 0.13	**
15 to <70	42.5	9	0.08 ± 0.01	1.00 ± 0.19	**	0.19 ± 0.05	1.00 ± 0.25	**	0.03 ± 0.02	0.10 ± 0.08	n.s.
70 to <100	85	7	0.05 ± 0.01	0.60 ± 0.17	**	0.13 ± 0.01	0.69 ± 0.08	**	0.03 ± 0.01	0.12 ± 0.05	*
100 to <150	125	6	0.02 ± 0.01	0.26 ± 0.08	**	0.11 ± 0.03	0.58 ± 0.15	**	0.25 ± 0.09	1.00 ± 0.35	**
150 to <200	175	4	0	0		0	0		0.13 ± 0.05	0.53 ± 0.22	*
200 to <210	205	4	0	0		0.02 ± 0.01	0.11 ± 0.03	**	0.12 ± 0.06	0.47 ± 0.22	*
≥210	–	3	0	0		0	0		0	0	

P. intermedius reached highest grazing coefficients at food sizes between 7.5 and <70 μm. These consisted mainly of diatoms and of dinoflagellates (means ± SEM: 43.9 ± 1.3% and 35.9 ± 1.6%, respectively) that were 2.5–50 μm wide. Grazing coefficients for adjacent size classes (unidentified nanoplankton from >2.5 to <7.5 μm and organisms between 70 and <100 μm long, consisting of 93.0 ± 3.1% diatoms) were also high (82.8 ± 22.5% and 68.6 ± 7.5%, respectively, of the maximum grazing coefficient measured). The lower size limit was >2.5 μm, the upper one around 135 μm at the maximum linear extension (the dominating seston size in size class 125 μm from Hopavågen Fjord) and 60 μm particle width. Although statistically not significantly different from zero, grazing also occurred on the smallest sizes detected by counts, and was statistically significant ($P<0.01$, 99% CI) on diatom colonies around 205 μm long and 15 μm wide (Fig. 1; Table 4). The selectivity profile of *P. intermedius* was very similar to the offered food size spectrum (Fig. 3).

In contrast, *E. nordmanni* filtered the entire food size range offered <210 μm, but preferred organisms between 7.5 and <15 μm (Prymnesiophyceae and different kinds of unidentified nanoplankton) and particles between 100 and <150 μm long (diatoms, 5–15 μm in diameter). Grazing coefficients of *E. nordmanni* for intermediate food sizes (diatoms, dinoflagellates, ciliates) were low (9.9 ± 7.5% for sizes between 15 and <70 μm and 11.9 ± 5.3% for sizes between 70 and <100 μm). Statistically feeding on sizes between 15 and <70 μm and on organisms ≤2.5 μm was not significantly different from zero. The upper size limit of ingested food, expressed as particle width, was 60 μm.

Effective food concentration (EFC) ranged from 3% to 71% for *P. avirostris*, depending on total food concentration (TFC), and followed a domed curve with maximum values at intermediate TFCs (for regression equations see Fig. 4). Maximum (±SEM) EFC was 58.8 ± 5.6% (based on the three highest values measured) at TFCs from 0.1 to 0.4 mm^3 l^{-1}, characterised by food particles around 10–85 μm.

Feeding on the same plankton community from Hopavågen Fjord (TFC 0.4 mm^3 l^{-1}, main food sizes around 10 μm and around 40 μm), *P. intermedius* and *E. nordmanni* attained EFCs of 72.3 ± 15.5% and 36.1 ± 3.7%, respectively. Values for *P. intermedius* were significantly higher than for *E. nordmanni* (one-way ANOVA, $P<0.1$, $F_{(1,4)}=5.2$).

Clearance rates and ingestion rates

The clearance rate of *P. avirostris* showed a curvilinear response with increasing food concentration, with a narrow modal range (turning point at around 1 mm^3 l^{-1}, Fig. 5, eye adjustment of the data). No clearance could be detected at food concentrations of 0.02 mm^3 l^{-1} (equivalent to 1.5 μg C l^{-1}) (Figs. 2, 5). The mean clearance rate was 25.5 ± 5.5 ml ind.$^{-1}$ day^{-1}. The maximum value attained was 54.9 ml ind.$^{-1}$ day^{-1}. *P. intermedius* and *E. nordmanni* showed clearance rates of 18.0 ± 5.4 and 19.3 ± 5.5 ml ind.$^{-1}$ day^{-1}, respectively (Fig. 5).

Ingestion rates increased linearly with increasing food supply for *P. avirostris* until a concentration threshold (around 270 μg C l^{-1}, equivalent to 2.8 mm^3 l^{-1}, for *P. avirostris*) beyond which the relation suggests a plateau (Fig. 5). The mean ingestion rate measured was 6.0 ± 2.2 μg C ind.$^{-1}$ day^{-1}. Ingestion rates for *P. intermedius* and *E. nordmanni* at 0.4 mm^3 l^{-1} were 0.8 ± 0.2 and 0.9 ± 0.2 μg C ind.$^{-1}$ day^{-1}, respectively (Fig. 5).

Clearance rates and ingestion rates of *P. avirostris*, *P. intermedius* and *E. nordmanni*, attained at similar food concentrations (0.4 mm^3 l^{-1}), did not differ significantly from each other (one-way ANOVAs, clearance rates $P=0.91$, ingestion rates $P=0.85$).

Discussion

The feeding selectivity of marine cladocerans is not well known, mainly as a result of the difficulties with culturing these animals. During research stays in Spain and

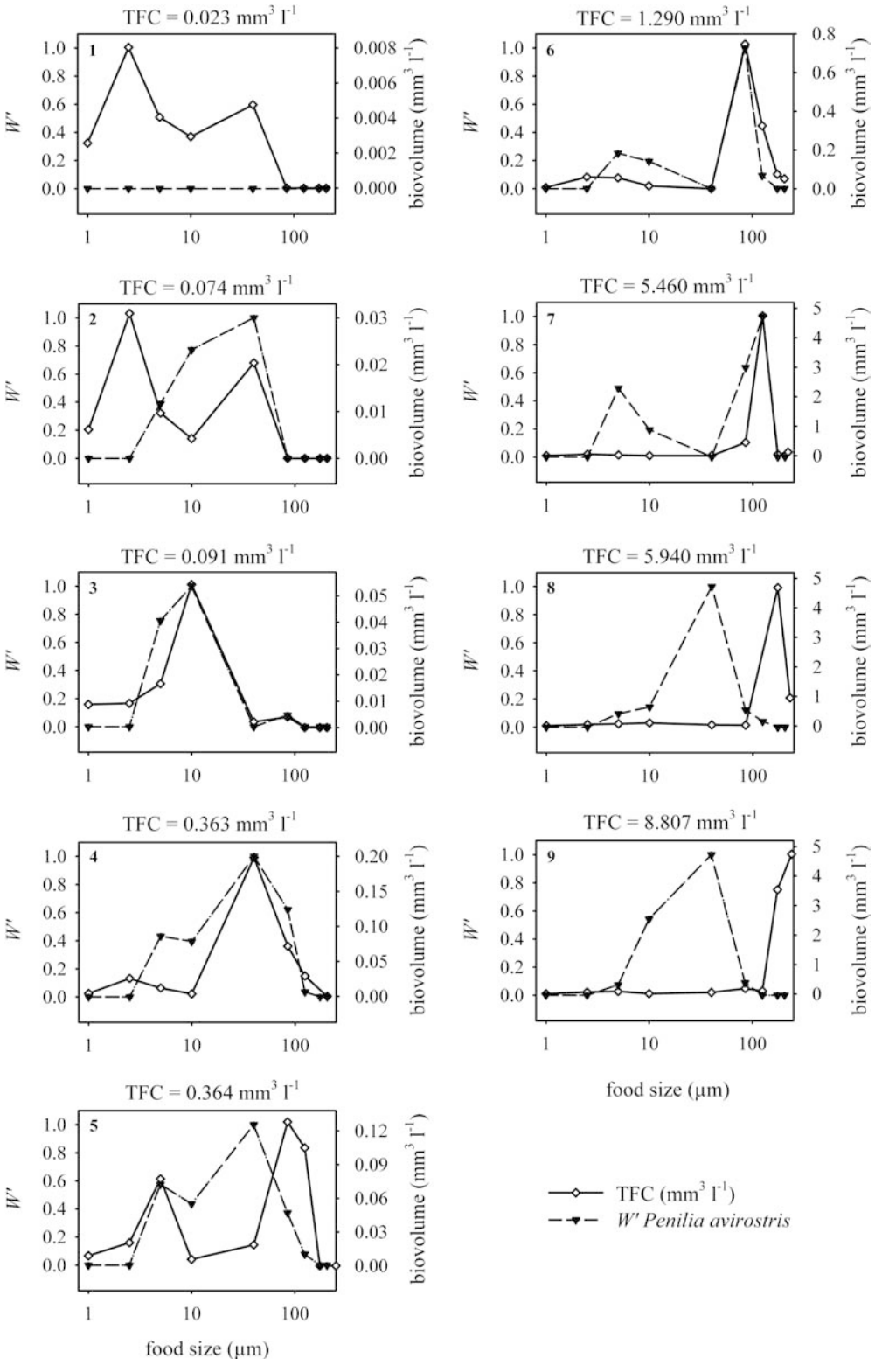

Fig. 2 *Penilia avirostris*. Selectivity coefficient curves *W'* for different food sizes at different total food concentrations (*TFC*) as found in plankton communities (1–9) from Blanes Bay (NW Mediterranean). Note logarithmic scale of food size axis

in Norway, we had the possibility to conduct grazing experiments with animals captured briefly before the experiments started. Our goal was to investigate the food size selectivities of members of all three marine cladoceran genera, *Penilia*, *Podon* and *Evadne*, under similar conditions. For this, we compared *Penilia avirostris*, *Podon intermedius* and *Evadne nordmanni* feeding on natural plankton communities with a broad range of food sizes.

P. avirostris and *P. intermedius* showed similar grazing profiles, with maximum grazing coefficients for intermediate food sizes. *E. nordmanni* deviated from

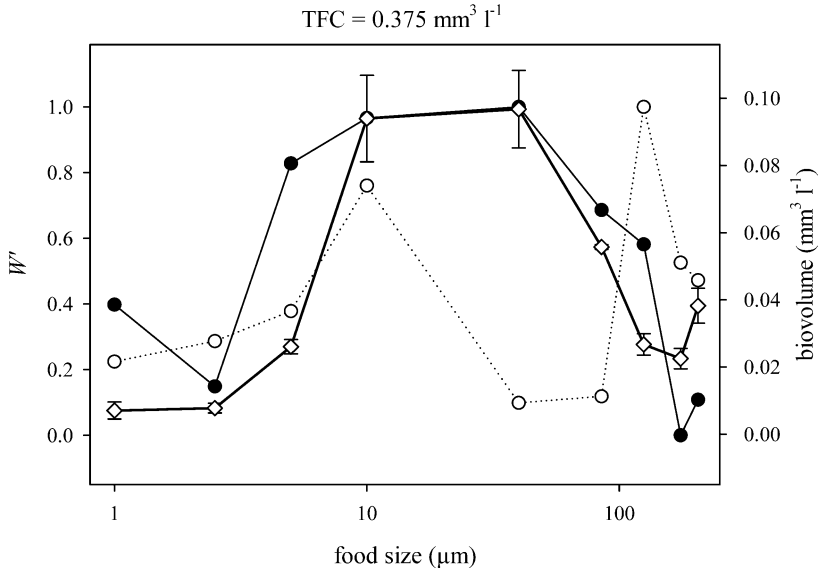

Fig. 3 *Podon intermedius*, *Evadne nordmanni*. Selectivity coefficient curves *W'* of *P. intermedius* (*closed circles*) and *E. nordmanni* (*open circles*) for different food sizes at the total food concentrations (*open diamonds*, *TFC*) found in summer in Hopavågen Fjord (NE Atlantic). *Error bars*: ±SEM, based on three measurements. Note logarithmic scale of food size axis

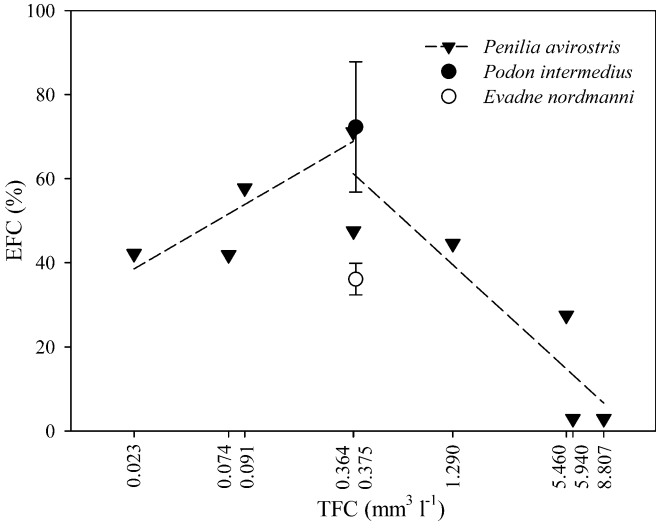

Fig. 4 *Penilia avirostris*, *Podon intermedius*, *Evadne nordmanni*. Effective food concentration (*EFC*) as a function of total food concentration (*TFC*). For *P. avirostris* (Pen) the relationship between EFC and TFC is described by the following linear functions: $EFC_{Pen\ for\ TFC \le 0.4} = 79.89 + 10.88(\ln TFC)$, $r^2 = 0.78$, $F_{(1,2)} = 7.2$, $P < 0.1$; $EFC_{Pen\ for\ TFC \ge 0.4} = 43.84 - 17.12(\ln TFC)$, $r^2 = 0.84$, $F_{(1,4)} = 20.6$, $P < 0.01$. Note logarithmic scale of TFC axis

the others, with high grazing coefficients for both large and small sizes, but low grazing coefficients for intermediate sizes (Fig. 1; Table 4). As we will explain below, the latter indicates that *Evadne* might have problems with motile prey. All investigated cladocerans fed on particle sizes that included components of the microbial food web, that is ciliates and nanoflagellates. There is some evidence, that *P. intermedius* and *E. nordmanni* might even consume picoplankton. Further investigation will be needed to varify these indications.

The EFC varied with the TFC. Nevertheless, *P. avirostris* was able to keep its ingestion rates stable over a wide range of food densities by adjusting its clearance rate to changing TFC. *P. intermedius* reached higher EFCs than *E. nordmanni*, which might be a competitive advantage for *P. intermedius*. On the other hand, clearance and filtration rates were similar for both species, and in accordance to the rates attained by *P. avirostris* at the same food concentrations.

Selectivity-size spectra, selectivity profiles and effective food concentrations

The grazing spectrum of *P. avirostris* covered a food size range of >2.5 to 100 µm. The lower size limit is consistent with the results of Paffenhöfer and Orcutt (1986), who observed a lower size limit of 2.2 µm. In correspondence with Turner et al. (1988), our results indicate no grazing on bacterioplankton. This is in contrast with the findings of Pavlova (1959 and Sorokin et al. (1970). We did not specifically count bacteria in the present experiments. However, Katechakis et al. (2002) documented that *P. avirostris* does not feed on solitary bacteria in any case, but influences the bacterioplankton indirectly via a trophic cascade by grazing on nanoflagellates. Gore (1980), Paffenhöfer and Orcutt (1986), Turner et al. (1988) and Kim et al. (1989) observed upper size limits of 15–50 µm. The difference in the upper size limit between our findings and other studies results from the use of different size scales. Other authors refer to upper size limits as measured by particle width or equivalent spherical diameters (ESD). We based our analyses on the longest linear cell and colony extensions, as the ESD may disguise the real dimensions of particles that can be handled. In our experiments, grazing coefficients for sizes >37.5 µm result exclusively from feeding on needle-shaped (*Nitzschia longissima* and *Rhizosolenia* spp.) and long-chain diatoms (*Skeletonema costatum* and *Thalassiosira* sp.). With valve diameters

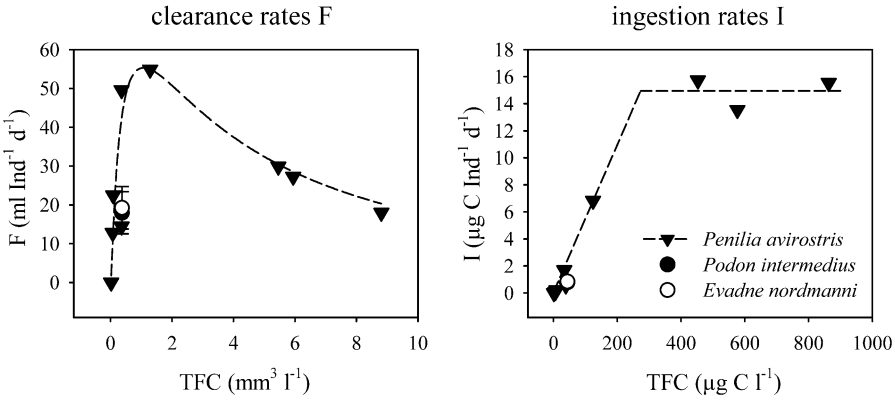

Fig. 5 *Penilia avirostris*, *Podon intermedius*, *Evadne nordmanni*. Clearance rates and ingestion rates; for *P. avirostris* eye adjustments of the data were done. *Error bars*: ±SEM, based on three measurements

between 5 and 20 µm, they could be ingested by *P. avirostris* if orientated longitudinally in the filtering current. Therefore, the upper size limit we found lies within the size spectrum found by other authors, if expressed as particle width.

The grazing profile of *P. avirostris* poorly followed the peak of available particles, but showed relatively invariant patterns. This speaks for a passive, mechanical filtering mode, which would be consistent with considerations in the literature (Pavlova 1959; Paffenhöfer and Orcutt 1986; Lipej et al. 1997). Nevertheless, in some experiments, *P. avirostris* expressed grazing peaks for dominant particles larger than its normally preferred food sizes (Fig. 2, communities 6 and 7). But these additional peaks, separated from the other peaks by zero-grazing values, are based exclusively on the ingestion of needle-shaped diatoms, whose valve diameters are within the normally preferred food size spectrum. Therefore, we do not rate these ingestions as intended captures of beneficial prey, but as accidental ingestions, depending on the alga's orientation in the filtering current.

The selectivity-size spectrum we detected for *P. intermedius* reached from >2.5 to 135 µm. Our results correspond more or less with those of Kim et al. (1989), who reported a size range of food organisms from 4 to 115 µm for marine cladocerans in general. Based on the morphology of the feeding appendages, Nival and Ravera (1981) suggested a maximum food size of 250 µm for *P. intermedius*. The only food organisms reaching these sizes in our experiments were colonies of diatoms and dinoflagellates belonging to the genus *Ceratium* (Table 3); the latter were not eaten by *P. intermedius*. This is in accordance with Jagger et al. (1988), who did not find any remains of *Ceratium* in faecal pellets of *P. intermedius*, although *Ceratium* was very abundant during their sampling. Diatoms showed large linear cell and colony extensions, but valve diameters ≤15 µm. Grazing on sizes >60 µm resulted almost exclusively from feeding on long-chain diatoms (mainly *Leptocylindrus minimus*, *Leptocylindrus danicus* and *Skeletonema costatum*).

Various authors presumed, but could not prove, raptorial feeding modes for *Podon* species (*P. intermedius*: Nival and Ravera 1981; Jagger et al. 1988; *P. polyphemoides*: Nival and Ravera 1981; Kim et al. 1989; Turner and Granéli 1992). Our experiments gave no evidence that *P. intermedius* might actively select beneficial prey.

In contrast to *P. avirostris* and *P. intermedius*, the food-size-based grazing of *E. nordmanni* did not follow a bell-shaped curve. Nival and Ravera (1979) suggested that *Evadne* probably can catch and hold animal prey or large algae. This might be an explanation for the high grazing coefficients in the size classes 125, 175 and 205 µm, and indicates that *E. nordmanni* actively selected for large diatoms. On the other hand, the curve shape for *E. nordmanni* grazing can also be interpreted as an inability to catch motile prey. Except for nanoflagellates, only the size classes 42.5 and 85 µm contained motile plankton organisms, such as ciliates and dinoflagellates. They made up 52.3±1.6% and 27.0±3.1%, respectively, of the size classes' biovolume. *E. nordmanni* showed the lowest grazing coefficients for these size classes. The assumption that *E. nordmanni* might have problems with motile prey is supported by Freyer (1968, 1974), who reports that *Evadne* is quite slow in capturing motile prey.

E. nordmanni filtered the entire food size range offered. For methodical reasons described below, we suggest a selectivity-size spectrum from >2.5 to <210 µm at the longest linear extension. Nival and Ravera (1979) suggested that related *Evadne spinifera* probably consumes food particles between 20 and 170 µm. Thus, the lower size limit detected by us is about one order of magnitude smaller than that predicted by Nival and Ravera (1979), but it corresponds roughly with the results of Kim et al. (1989). An explanation may be that morphology alone generally does not adequately describe the filtering characteristics of filter feeders, but that filtration physics also has to be taken into account (Brendelberger et al. 1986; Jürgens 1994; Acuña 2001). The upper size limit we found is larger than that predicted by Nival and Ravera (1979) and that shown by Kim et al. (1989), but grazing on sizes >60 µm resulted from feeding on the same long-chain diatoms as described for *P. intermedius*.

Although results were not statistically significant, *E. nordmanni* and *P. intermedius* showed some evidence of grazing on food sizes around 1 µm and around 2.5 µm, thus including bacterial sizes. This may have been a result of grazing by nanoflagellates and ciliates in

Table 5 *Penilia avirostris*, *Evadne nordmanni*, *Podon* spp. Clearance rates and ingestion rates

Taxon	Clearance rate (ml ind.$^{-1}$day^{-1})		Ingestion rate (µg C ind.$^{-1}$day^{-1})		Reference
	Range	Mean	Range	Mean	
Penilia avirostris	4.8–26		[a]		Paffenhöfer and Orcutt (1986)
	41–252	101	[a]		Pavlova (1959)
	18–56		[a]		Turner et al. (1988)
	4.8–30	21	[a]		Turner et al. (1998)
	0.1–20	2.2	[a]		Wong et al. (1992)
	0–55	26	0–16	6.0	Present study
Podon polyphemoides	2.9–62		[a]		Turner and Granéli (1992)
Podon intermedius	8.4–28	18	0.4–1.2	0.8	Present study
Evadne nordmanni	9.8–29	19	0.4–1.3	0.9	Present study

[a]Rates measured by author(s), but conversion of units not possible

our experiments. We did not find any indications of a relationship between the abundances of ciliates, nanoflagellates and picoplankton; however, inverse microscopy is not the proper method to investigate pico- and small nanoplankton abundances (Booth et al. 1982; Reid 1983).

The EFC of *P. avirostris* depended on the TFC and on the food size composition. Both factors depended again on the nutrient conditions under which the food communities grew (Table 1; Fig. 2). The results suggest that oligotrophic to mesotrophic conditions, providing intermediate TFC and food sizes, are advantageous for *P. avirostris*.

Nutrient conditions in Hopavågen Fjord provided higher EFC for *P. intermedius* than for *E. nordmanni*. This might be rated a competitive advantage for *P. intermedius*, and could partly explain the general predominance of *P. intermedius* over *E. nordmanni* in Hopavågen Fjord (O. Vadstein, personal communication). On the other hand, clearance and ingestion rates were the same for both species.

Clearance rates and ingestion rates

All cladocerans investigated reached similar clearance and ingestion rates at comparable food concentrations. Clearance and ingestion rates of *P. avirostris* were within the range of rates found in literature (Table 5). No clearance or ingestion rates have been published for *P. intermedius* or any *Evadne* species so far. The values found for *P. intermedius* are within the range measured by Turner and Granéli (1992) for *Podon polyphemoides*. Nevertheless, a comparison of results was not always possible due to the use of different rate units. If possible, we converted units for clearance rates to millilitres per individual per day and ingestion rates to micrograms of carbon per individual per day. Our results for ingestion rates include a degree of uncertainty, as conversion of phytoplankton biovolume to carbon biomass depends very much on the conversion factor chosen. We decided to follow the estimations of Nalewajko (1966), which treat all phytoplankton species equally. Other computations emphasise small taxa (e.g. Strathmann 1967) or large algal sizes (e.g. Rocha and Duncan 1985) and may lead to deviating results. Moreover, rate measurements are always influenced by a variety of parameters, such as temperature, kind of food source, food density, life history of animals, and choice of experimental method.

The relationship between the clearance rate and TFC followed a bell-shaped curve with narrow modal ranges for *P. avirostris*. A decrease of clearance rates with increasing food concentration has been documented already for this cladoceran species (Pavlova 1959; Paffenhöfer and Orcutt 1986; Wong et al. 1992), but not an initial increase, which would indicate a switching from non-feeding to feeding activities (Marten 1973). Non-feeding activities suggest that TFC or EFC or both are too low to support basic metabolism. Our results indicate that this was the case at TFC ≤ 0.02 mm^3 l^{-1} for *P. avirostris*. Paffenhöfer and Orcutt (1986) observed feeding activities of *P. avirostris* also at lower food concentrations (0.01 mm^3 l^{-1}), but reproduction did not occur at these levels.

Although *P. avirostris* reduced its filtration efforts at higher food concentrations, ingestion rates remained stable. This behaviour points to an optimal adjustment of energy expenses. According to Paffenhöfer (1988), such an ability corresponds to species adapted to varying trophic conditions. Indeed, *P. avirostris* (Paffenhöfer and Orcutt 1986) occurs most commonly in near- and inshore environments that are often subject to fluctuating particulate densities.

For *Podon* and *Evadne* no feeding experiments over a wider range of food concentrations have been published so far. They will be necessary to investigate their functional responses and to examine in more detail the importance of cladocerans in marine pelagic food webs.

Acknowledgements We thank T. Hansen for technical support, C.M. Duarte and the Centro de Estudios Avanzados de Blanes (CEAB, Blanes, Catalunya, Spain) for the supply of laboratory space for our experiments. This article is a contribution to the MAST III project COMWEB founded by the EU (MAST3-CT96-0052). Grazing experiments in Norway were supported by the Improving Human Potential—Transnational Access to Research

Infrastructures Programme of the European Commission (Trondheim Marine Systems R.I.). Our experiments comply with the current laws of Spain and Norway.

References

Acuña JL (2001) Pelagic tunicates: why gelatinous? Am Nat 158:100–107

Baker HM (1938) Studies on the Cladocera of Monterey Bay. Proc Calif Acad Sci (Ser 4) 23:311–365

Booth BC, Lewin J, Norris RE (1982) Nanoplankton species predominant in the subarctic Pacific in May and June 1978. Deep-Sea Res A 29:185–200

Bosch HF, Taylor WR (1973) distribution of the cladoceran *Podon polyphemoides* in the Chesapeake Bay. Mar Biol 19:161–171

Bøsheim KY, Bratbak G (1987) Cell volume to carbon conversion factors for a bacterivorous *Monas* sp. enriched from seawater. Mar Ecol Prog Ser 8:211–223

Brendelberger H, Herbeck M, Lang H, Lampert W (1986) *Daphnia*'s filters are not solid walls. Arch Hydrobiol 107:197–202

DeBiase AER, Sanders RW, Porter KG (1990) Relative nutritional value of ciliate protozoa and algae as food for *Daphnia*. Microb Ecol 19:99–210

Della Croce N, Venugopal P (1973) *Penilia avirostris* Dana in the Indian Ocean (Cladocera). Int Rev Gesamten Hydrobiol Hydrogr 58:713–721

Duarte CM, Agustí S, Agawin NSR (2000) Response of a Mediterranean phytoplankton community to increased nutrient inputs: a mesocosm experiment. Mar Ecol Prog Ser 195:61–70

Egloff DA, Fofonoff PW, Onbé T (1997) Reproductive biology of marine cladocerans. Adv Mar Biol 31:79–167

Freyer G (1968) Evolution and adaptive radiation in the Chydoridae (Crustacea: Cladocera): a study in comparative functional morphology and ecology. Philos Trans R Soc Lond B Biol Sci 254:221–385

Freyer G (1974) Evolution and adaptive radiation in the Macrothricidae (Crustacea: Cladocera): a study in comparative functional morphology and ecology. Philos Trans R Soc Lond B Biol Sci 269:137–274

Frost BW (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol Oceanogr 17:805–815

Gore MA (1980) Feeding experiments on *Penilia avirostris* Dana (Cladocera: Crustacea). J Exp Mar Biol Ecol 44:253–260

Grahame J (1976) Zooplankton of a tropical harbour: the numbers, composition and response to physical factors of zooplankton in Kingston Harbour, Jamaica. J Exp Mar Biol Ecol 25:219–237

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403–424

Jagger RA, Kimmerer WJ, Jenkins GP (1988) Food of the cladoceran *Podon intermedius* in a marine embayment. Mar Ecol Prog Ser 43:245–250

Jürgens K (1994) Impact of *Daphnia* on planktonic microbial food webs—a review. Mar Microb Food Webs 8:295–324

Katechakis A, Stibor H, Sommer U, Hansen T (2002) Changes in the phytoplankton community and in the microbial food web of Blanes Bay (Catalan Sea, NW Mediterranean) under prolonged grazing pressure by doliolids (Tunicata), cladocerans or copepods (Crustacea). Mar Ecol Prog Ser 234:55–69

Kim SW, Onbé T, Yoon YH (1989) Feeding habits of marine cladocerans in the Inland Sea of Japan. Mar Biol 100:313–318

Lipej L, Mozetic P, Turk V, Malej A (1997) The trophic role of the marine cladoceran *Penilia avirostris* in the Gulf of Trieste. Hydrobiologia 360:197–203

Lund JWG, Kipling C, LeCren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11:147–170

Marten GG (1973) An optimization equation for predation. Ecology 54:92–101

Nalewajko C (1966) Dry weight, ash and volume data for some freshwater planktonic algae. J Fish Res Board Can 23:1285–1287

Nival S, Ravera S (1979) Morphological studies of the appendages of the marine cladoceran *Evadne spinifera* Müller by means of the scanning electron microscope. J Plankton Res 1:207–213

Nival S, Ravera S (1981) Evaluation des potentialités alimentaires chez deux cladocères planctoniques, *Podon intermedius* Lilljeborg et *Podon* (*Pleopsis*) *polyphemoïdes* Leuckart (Crustacea, Branchiopoda), par une étude de morphologie fonctionnelle des structures cuticulaires au microscope électronique a balayage. Ann Inst Oceanogr 57:31–40

Onbé T (1977) The biology of marine cladocerans in a warm temperate water. In: Proc Symp Warm Water Zoopl. Special publication, UNESCO/NIO, Goa, pp 383–398

Paffenhöfer GA (1988) Feeding rates and behaviour of zooplankton. Bull Mar Sci 43:430–445

Paffenhöfer GA, Orcutt JD (1986) Feeding, growth and food conversion of the marine cladoceran *Penilia avirostris*. J Plankton Res 8:741–754

Pavlova EV (1959) On grazing by *Penilia avirostris* Dana. Trans Sevastopol Biol Stn Akad Nauk Ukr SSR 11:63–71 (English translation in Fish Res Board Can Trans 967)

Raymont JEG (1983) Plankton and productivity in the oceans: zooplankton, vol 2, 2nd edn. Pergamon, New York

Reid FMH (1983) Biomass estimation of components of the marine nanoplankton and picoplankton by the Utermoehl settling technique. J Plankton Res 5:235–252

Rocha O, Duncan A (1985) The relationship between cell carbon and cell volume in freshwater algal species used in zooplankton studies. J Plankton Res 7:279–294

Schram FR (1986) Crustacea. Oxford University Press, New York

Sommer U, Stibor H (2002) Copepoda—Cladocera—Tunicata: the role of three major mesozooplankton groups in pelagic food webs. Ecol Res 17:161–174

Sorokin YI, Petipa TS, Pavlova EV (1970) Quantitative estimate of marine bacterioplankton as a source of food. Oceanology 10:253–260

Strathmann RH (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12:411–418

Turner JT, Granéli E (1992) Zooplankton feeding ecology: grazing during enclosure studies of phytoplankton blooms from the west coast of Sweden. J Exp Mar Biol Ecol 157:19–31

Turner JT, Tester PA, Ferguson RL (1988) The marine cladoceran *Penilia avirostris* and the ''microbial loop'' of pelagic food webs. Limnol Oceanogr 33:245–255

Turner JT, Hopcroft RR, Lincoln JA, Huestis CS, Tester PA, Roff JC (1998) Zooplankton feeding ecology: grazing by marine copepods and cladocerans upon phytoplankton and cyanobacteria from Kingston Harbour, Jamaica. Mar Ecol 19:195–208

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt Int Ver Theor Angew Limnol 9:1–38

Vanderploeg HA, Scavia D (1979) Calculation and use of selectivity coefficients of feeding: zooplankton grazing. Ecol Model 7:135–149

Vanderploeg HA, Scavia D, Liebig JR (1984) Feeding rate of *Diaptomus sicilis* and its relation to selectivity and effective food concentration in algal mixtures in Lake Michigan. J Plankton Res 6:919–941

Wong CK, Chan ALC, Tang KW (1992) Natural ingestion rates and grazing impact of the marine cladoceran *Penilia avirostris* Dana in Tolo Harbour, Hong Kong. J Plankton Res 14:1757–1765

PAPER B1

Limnology and Oceanography

Mixotrophic vs. obligately autotrophic algae as food for zooplankton – the light:nutrient hypothesis might not hold for mixotrophs

Katechakis A, Haseneder T, Kling R, Stibor H (2005)
***Limnology and Oceanography* 50:1290-1299**

Deutsche Forschungsgemeinschaft DFG

Limnol. Oceanogr., 50(4), 2005, 1290–1299

Mixotrophic versus photoautotrophic specialist algae as food for zooplankton: The light : nutrient hypothesis might not hold for mixotrophs

Alexis Katechakis,[1] *Tanja Haseneder, Robert Kling, and Herwig Stibor*
Ludwigs-Maximilians-University, Department Biology II—Aquatic Ecology, Groβhadener Str. 2, 82152 Planegg-Martinsried, Germany

Abstract

We reared mixotrophic (*Ochromonas tuberculata* and *Cryptomonas* sp.) and photoautotrophic specialist algae (*Scenedesmus obliquus*) at different light : phosphorus supplies and compared their effects as food for zooplankton (*Daphnia magna*). According to the light : nutrient hypothesis (LNH), biomass and nutrient stoichiometry of phototrophic specialists depend strongly on light : phosphorus supplies. If this is true, herbivore growth and fecundity should be limited by food quantity at low light intensities and by stoichiometric food quality at high light intensities. In turn, phosphorus fertilization should cause a transition from limitation by food quality to limitation by food quantity. In contrast to the LNH, biomass and nutrient stoichiometry of mixotrophs were almost unaffected by alterations in the supply of light and dissolved nutrients. Bacterial counts indicate that mixotrophs compensated for light or phosphorus deficiency by heterotrophic nutrition. Compared to phototrophic specialists, a diet of *Cryptomonas* sp. therefore enabled a similar or higher and more stable secondary production at most light : nutrient supplies. *O. tuberculata*, however, appeared to be toxic. Our results indicate that mixotrophs might have a balancing effect on variations in transfer efficiency caused by perturbations to light and nutrient supplies.

Global perturbations to solar insolation and to biogeochemical cycles are altering the inputs of light and nutrients to ecosystems, thus influencing primary and secondary production (e.g., Lindroth et al. 1993; Schindler 1998). Studies in this context have traditionally focused on the role of food quantity and have suggested that high primary production and biomass should yield high secondary production and biomass and therefore potentially also sustain a higher biomass of top predators (e.g., Begon et al. 1996). More recently, however, it has become increasingly clear that food quality in terms of elemental nutrient composition may be a key determinant with regard to trophic efficiency in food webs (e.g., Hessen 1992; Gulati and DeMott 1997) and that food chain production varies with the degree of mismatch between the carbon : nutrient ratios of autotrophs and their consumers (e.g., Sterner et al. 1998; Hessen and Faafeng 2000).

A compilation of stoichiometric data in terrestrial and aquatic food webs indicates that carbon : nutrient ratios of autotrophs are suboptimal for herbivores in many ecosystems (Elser et al. 2000). In aquatic systems, for example, the mismatch between the cellular carbon : phosphorus (C : P) ratios of algae and their consumers can be very high. While the C : P ratios of phytoplankton may range from 100 to ~1,000 (e.g., Gächter and Bloesch 1985; Elser and Hassett 1994), C : P ratios of herbivorous zooplankton are typically much smaller and less variable. The total range of body C : P in crustacean zooplankton taxa studied to date varies from 50 to 200 (e.g., Andersen and Hessen 1991), showing limited intraspecific variability (strong physiological homeostasis), with most variation associated with differences among species. Hence, algae with low C : P ratios are rated a better food quality for herbivorous mesozooplankton than algae with high C : P ratios (e.g., Sterner et al. 1998; Hessen and Faafeng 2000; Makino et al. 2002).

High C : P ratios in autotrophs have been attributed to a joint effect of high light intensities and low P supplies. At high light : nutrient ratios, higher primary production may, therefore, paradoxically cause lower zooplankton production as a result of a reduction in transfer efficiency caused by low food quality. On the other hand, at low light supply, food quantity may limit secondary production. These relationships have been summarized in the light : nutrient hypothesis (LNH) by Sterner et al. (1997) and seem well supported by recent theoretical (Andersen 1997; Loladze et al. 2000) and empirical studies (e.g., Urabe and Sterner 1996; Hessen et al. 2002; Urabe et al. 2002*a*).

The LNH is based on the assumption that photoautotrophic specialists constitute the base of the food chain. To date, the role of mixotrophic organisms has been neglected within this context. Mixotrophic algae combine phototrophic and phagotrophic production dependent on the availability of light and nutrients (e.g., Sibbald and Albright 1991; Raven 1997) and have been found in several classes of phytoplankton (e.g., Jones 2000). Mixotrophic algae are widespread in pelagic ecosystems and, for the following reasons, mixotrophs can be expected to have different effects on the algae–herbivore interface than predicted by the LNH. First, the ability to use alternative production pathways indicates that the stoichiometric composition of mixotrophs might be less affected by alterations in the supply with light and dissolved nutrients than the stoichiometry of phototrophic specialists. Second, potentially limiting nutrients, particularly P, are often several orders of magnitude more concentrated in the biomass of food organisms of mixotrophs (bacteria and bac-

[1] Corresponding author (alexis.katechakis@gmx.net).

Acknowledgments

We thank Sebastian Diehl for helpful comments on an earlier draft of the manuscript and Angelika Wild and Achim Weigert for technical assistance. We also thank Dag Hessen and two anonymous reviewers for their most helpful comments toward the final version of the article. Experiments were supported by the Deutsche Forschungsgemeinschaft, DFG (project ST180/ 1-1, 1-2).

terial-sized particulate matter) than in the dissolved phase (e.g., Vadstein 2000). C : P ratios of bacteria are indeed relatively low and constrained (~10–500) (e.g., Makino 2003). Heterotrophic nutrition might therefore entail low C : P ratios in mixotrophs, making them a nutrient-rich food source for herbivores at high environmental light : nutrient ratios as well. Third, mixotrophic organisms may dominate phytoplankton biomass under low light conditions and in low-nutrient environments (e.g., Riemann et al. 1995) (i.e., exactly in those environments where, according to the LNH, secondary production may be restricted by autotroph food quantity and quality, respectively).

Based on these expectations, we formulated the following two hypotheses and tested them experimentally in the laboratory: (1) The C : P ratios of mixotrophs are lower and much less dependent on external light : nutrient supply ratios than the C : P ratios of purely phototrophic algae, and (2) compared to photoautotrophic specialists, mixotrophs are a superior food source for herbivorous zooplankton at high light : nutrient supply ratios and in low light environments.

Material and methods

Experimental setup—Experiments were performed in semicontinuous two-stage chemostats consisting of 600-ml tissue culture flasks. We filled all chemostats with sterile-filtered, autoclaved water from oligotrophic Lake Langbürgener See (South Bavaria, Germany). Chemostats were placed in a climate chamber at a temperature of 20° ± 1°C and illuminated with fluorescent bulbs (Osram light code 77 and Osram cool-white 21–840, 36 W each, in equal parts, Osram) in a 16 : 8h light : dark rhythm.

In the first stages, we inoculated equivalent biovolumes (measured with a Casy 1 TTC particle counter, Schärfe Systems) of purely phototrophic *Scenedesmus obliquus* (SAG culture 276-3a, SAG culture collection, Göttingen, Germany), mixotrophic *Ochromonas tuberculata* (CCAP culture 933/27, CCAP culture collection, Ambleside, U.K.), mixotrophic *Cryptomonas* sp. (SAG 19.80), or a mixture of *S. obliquus* and *O. tuberculata.* Algal stock cultures were nonaxenic. *O. tuberculata* and *Cryptomonas* sp. cover the two extremes of mixotrophic strategies documented in literature (e.g., Jones 2000). *Ochromonas* is a predominantly heterotrophic mixotroph (e.g., Sibbald and Albright 1991) that uses phototrophy only when prey concentrations limit heterotrophic growth (Rothhaupt 1996*b*). *Cryptomonas* is rated primarily as a phototroph, ingesting prey only at low rates, for example to meet requirements for cell maintenance during prolonged dark periods or for the uptake of essential organic carbon compounds, such as vitamins (e.g., Sanders and Porter 1988; Tranvik et al. 1989).

In the second-stage flasks, three neonates each of *Daphnia magna* were fed with material from the first-stage flasks. Neonates were born within 12 h before setting up the experiment, gathered from mothers established in stock cultures at our institute.

We conducted two series of experiments: One series in a light gradient (60–345 μmol quanta m^{-2} s^{-1}) at constant nutrient supply (N : P was 16 : 1 in molar units, with P = 0.5 μmol L^{-1}), and a second series in a P gradient (N : P = 16 : 1 to 16 : 20, with P = 0.5 to 10 μmol L^{-1}) at constant light conditions (345 μmol quanta m^{-2} s^{-1}). Light intensity in the first series was adjusted by shading the chemostats with layers of greaseproof paper. We established seven light intensities: 60, 90, 125, 160, 205, 265, and 345 μmol quanta m^{-2} s^{-1} (measured with a LI-1400 DataLogger, Li-Cor). P concentrations in the second series were adjusted by adding the desired concentration of P as NaH_2PO_4. We used seven P concentrations: 0.5, 1, 2.5, 4, 5, 7.5, and 10 μmol L^{-1}. N was added as $NaNO_3$ and NH_4Cl in equal parts to all treatments. All chemostats received a mixture of vitamins (0.02 μmol L^{-1} vitamins H and B, 0.004 μmol L^{-1} vitamin B_{12}, final concentration) and supplementary nutrients (Na_2EDTA, $FeSO_4$, $MnCl_2$, 1 μmol L^{-1} final concentration each). To maintain a sufficient stock of bacterial biomass for potential phagotrophy by mixotrophs, chemostats received additionally 0.1 μg glucose ml^{-1}. Every light : nutrient treatment was replicated in triplicate for every tested algal species.

After inoculation, first-stage chemostats with algal monocultures were allowed to grow for 7 d to attain a sufficient food concentration of a least 10^4 cells ml^{-1} (measured with a Casy 1 TTC particle counter) and to stabilize food quality before starting *Daphnia* performance experiments. Chemostats with mixtures of *S. obliquus* and *O. tuberculata* were run for 3 weeks before experiments started to enable equilibrium of both taxa. From the time of inoculation on, every 2 d, 200 ml of the culture suspension were replaced by fresh medium (sterile-filtered, autoclaved lake water, supplemented with nutrients as described above) and transferred to second-stage flasks, yielding an average dilution rate of the medium of D = 0.17 d^{-1}. *D. magna* individuals remained undiluted. Experiments were terminated when all cladocerans in a chemostat had produced eggs or had reached adulthood (after 6–10 d). Adulthood was judged with regard to instar numbers and size of abdominal appendages (Stibor and Lampert 1993). During the experimental period, we inspected by eye whether grazers were intact several times a day by controlling their swimming behavior in the chemostats. To homogenize the culture suspension, we also mixed the vessels gently on this occasion.

Sample preparation and analysis—All parameters were determined once at the beginning and once at the end of the experiment.

Food quantity and quality—For the examination of algal biomass and C : P stoichiometry, we filtered known aliquots of the culture suspensions from first-stage chemostats on precombusted Schleicher and Schuell GF6 glass-fiber filters. Filters were dried in an oven at 60°C and stored in a desiccator (C) or freezer (P) until analysis. C content was determined with a C-Mat 500 carbon analyzer (Juwe). Algal P concentration was determined by spectrophotometric methods (acid molybdenum-blue technique) after oxidation by persulfate (APHA 1992).

To ascertain the proportions of *S. obliquus* and *O. tuberculata* in the mixed chemostats, we fixed samples with Lugol's iodine (5 g I_2 + 10 g KI in 100 ml distillated water, 1% final concentration), settled samples in Utermöhl cham-

bers (Hydrobios), and counted them in an inverted microscope (Leica DMIL, Leica) according to the method of Utermöhl (1958) and Lund et al. (1958).

To assess *Daphnia magna* performance, we determined the individuals' somatic growth rates and standardized egg numbers. Somatic growth rates, g (per day), were estimated by measuring the rate of change of body lengths, L (in mm), under a dissecting microscope and converting body lengths to body mass, ω (in μg), using conventional length–mass regressions, thus (Stibor 2002):

$$g = (\ln \omega_{adult} - \ln \omega_{neonate})/t,$$

where t is the experimental duration in days, and $\omega = 12.58 L^{2.41}$. Standardized egg number was calculated as the number of produced eggs per adult female divided by individual body length.

Bacterial net growth—To determine possible impacts on the bacterial guild in the chemostats, we preserved samples with 0.2-μm–filtered formaldehyde (2% final concentration), stained them with 4,6-diamidino-2-phenylindol (DAPI; 2 μg DAPI ml^{-1}, sample final concentration), after Porter and Feig (1980), and enumerated samples at $\times$1,000 magnification using an epifluorescence microscope (Zeiss Axioplan, Carl Zeiss). Bacterial net growth rate, r (per day), was calculated as

$$r = (\ln C_1 - \ln C_0)/t$$

where C_1 and C_0 are the bacterial abundances (cells ml^{-1}) at the end and at the beginning of the experiment and where t is the experimental term in days.

Results

In the following, we will initially treat the monospecific cultures and subsequently the mixed treatments. Detailed results are presented first for light manipulations and then for P fertilization.

Food quantity and quality—Purely phototrophic algae reached higher maximum biomasses than mixotrophs in both the light and the P gradient, but generally provided a lower food quality in terms of C : P ratios. Moreover, both parameters were much more affected by changing light or nutrient supplies in phototrophic specialists than in mixotrophs.

The biomass and the C : P ratio of phototrophic specialist *Scenedesmus obliquus* increased considerably with increasing light supply. In contrast, mixotroph biomasses and C : P ratios remained largely constant across the light gradient (Fig. 1A,B, left panel; Table 1). Biomasses and C : P ratios of purely phototrophic algae were higher than values for mixotrophs throughout the light gradient. Only at the lowest light intensity of 60 μmol quanta m^{-2} s^{-1} did biomasses and C : P ratios not differ significantly between purely phototrophic and mixotrophic species (two-way analyses of variance [ANOVAs]; Table 2, and post-hoc Tukey-test analyses, $p < 0.01$).

While the biomass of *Cryptomonas* sp. increased slightly along the entire P supply range, P fertilization left biomasses of *S. obliquus* and *O. tuberculata* largely unaffected, except for an initial increase in *S. obliquus* biomass, from the lowest to the second lowest P concentration (Fig. 1A, right panel; Table 1). Similarly, P fertilization influenced mixotroph C : P ratios only at the lowest P concentrations. In contrast, P fertilization severely affected C : P ratios of phototrophic specialists across the entire P gradient, leading to a more than sixfold decrease from ~800 to ~120 (Fig. 1B, right panel; Table 1). Similar to the light gradient, biomasses of phototrophic specialists were higher throughout the P gradient than values for mixotrophs (two-way ANOVAs, Table 2, and post-hoc Tukey-test analyses, $p < 0.01$). C : P ratios of phototrophic specialists approached mixotrophic C : P ratios with increasing P supply. At P supplies $\geq$4 μmol L^{-1} C : P ratios did not differ significantly between purely phototrophic and mixotrophic species (two-way ANOVAs, Table 2, and post-hoc Tukey-test analyses, $p < 0.01$).

Chemostats with mixtures of *S. obliquus* and *O. tuberculata* developed very similarly to chemostats containing only phototrophic specialists (Fig. 1A,B). Only at low light intensities of $\leq$120 μmol quanta m^{-2} s^{-1} did biomasses and C : P ratios of mixed cultures develop similarly to treatments with mixotrophs (Fig. 1A,B, left panel; two-way ANOVAs, Table 2, and post-hoc Tukey-test analyses, $p < 0.05$). This is because *S. obliquus* generally dominated mixed chemostats (Fig. 2), while *O. tuberculata* reached appreciable proportions of total biomass only at low light intensities of $\leq$120 μmol quanta m^{-2} s^{-1} (between 42.0% $\pm$ 7.9% standard error [SE] of the means and 59.9% $\pm$ 6.4%). Its proportion of total biomass decreased continuously with increasing light supply. At light intensities >120 μmol quanta m^{-2} s^{-1} and throughout the P gradient, *O. tuberculata* was almost completely outcompeted by *S. obliquus* (relative biomass of *O. tuberculata* of <5% and <3%, respectively).

Daphnia magna *performance*—ANOVAs indicate that growth and fecundity of *D. magna* differed for different food types, for different treatments (light supply or P supply), and for interactions between food type and treatments (Table 3). Animals feeding on *O. tuberculata* suffered high mortality during the course of the experiment and died out before they could reproduce. Therefore, they are not further treated here. Growth rates and reproduction were significantly affected by food quantity and quality (Fig. 1C,D).

In the light gradient, *D. magna* juveniles grew more rapidly and reproduced more robustly feeding on the mixotroph *Cryptomonas* sp. relative to the phototrophic specialist diet (two-way ANOVAs, Table 3, and post-hoc Tukey-test analyses, $p < 0.01$). At most light intensities, somatic growth and egg production were higher on *Cryptomonas* sp. than on *S. obliquus* (Fig. 1C,D, left panel), although purely phototrophs provided higher food quantities (Fig. 1A, left panel). This was due to a better food quality of *Cryptomonas* sp., gauged in terms of C : P ratios (Fig. 1B, left panel). Moreover, the constant mixotroph food characteristics across the light gradient (Fig. 1A,B, left panel) produced relatively stable *D. magna* responses. In contrast, somatic growth and egg production were more affected by changing light conditions feeding on *S. obliquus*, with highest performances at intermediate light intensities of between 90 and 160 μmol quanta

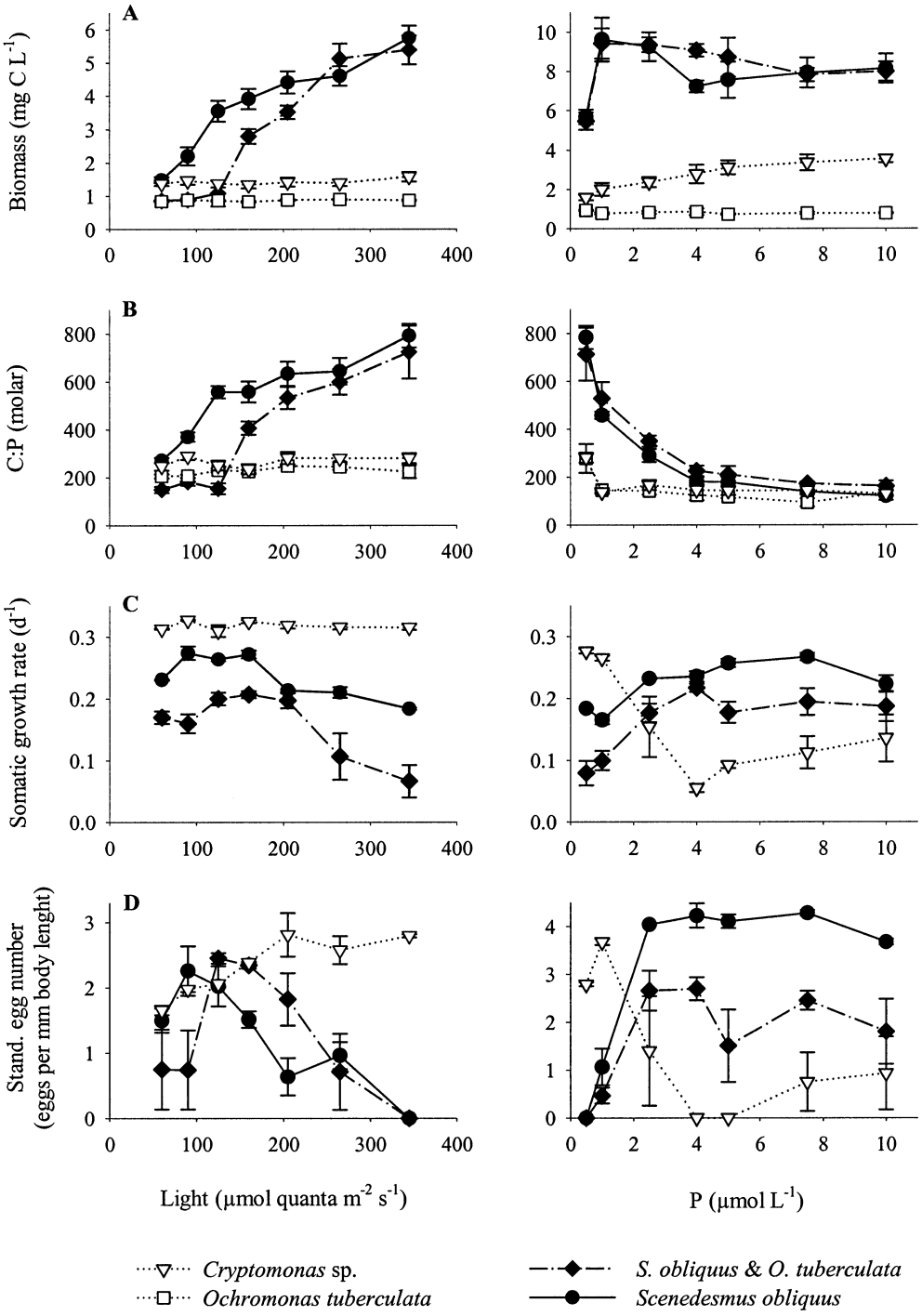

Fig. 1. (A and B) Biomasses (mg C L^{-1}) and C : P ratios (molar) of phototrophic specialist algae (*Scenedesmus obliquus*), mixotrophic algae (*Ochromonas tuberculata, Cryptomonas* sp.), and mixtures of phototrophic specialist and mixotrophic algae (*S. obliquus* and *O. tuberculata*) in a light gradient at constant nutrient supply (left panel) and in a P gradient at constant light conditions (right panel). Data points are means of 3 × 2 (replicates × measurements) = 6 samples (*see* Materials and methods for details). Error bars indicate ±SE of the means. (C and D) Average somatic growth rates (d^{-1}) and average standardized egg numbers (eggs per mm body length) of *Daphnia magna* feeding on phototrophic specialist algae, mixotrophic algae, or mixtures of phototrophic specialist and mixotrophic algae. Data points are means of three replicates. Error bars indicate ±SE of the means.

Table 1. Regression coefficients (linear: $y = y_0 + ax$, or exponential decay: $y = y_0 + a \exp^{-bx}$) of biomass (mg C L^{-1}) and C : P ratio (molar) development for *Scenedesmus obliquus*, *Ochromonas tuberculata*, *Cryptomonas* sp., and a mixture of *S. obliquus* and *O. tuberculata* in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (*see* Materials and methods for details). Sample size for each species in each gradient: 3 × 2 × 7 (replicates × measurements × treatments) = 42. Values in brackets are ±SE of the means.

	Light gradient					P gradient					
Species	y_0	a	r^2	$F_{1,40}$	p	df	y_0	a	b	r^2	F	p
Scenedesmus obliquus												
Biomass (mg C L^{-1})	1.3 (0.3)	0.01 (0.001)	0.70	93.2	<0.0001	1	7.8 (0.6)	0.02 (0.1)	—	0.001	0.1	0.83
C : P (molar)	256.3 (38.8)	1.6 (0.2)	0.64	71.1	<0.0001	2	157.7 (15.9)	1025.1 (104.9)	1.1 (0.2)	0.91	192.8	<0.0001
Ochromonas tuberculata												
Biomass (mg C L^{-1})	0.9 (0.02)	0.0001 (0.0001)	0.02	1.0	0.34	1	0.9 (0.1)	−0.01 (0.01)	—	0.03	1.1	0.30
C : P (molar)	209.7 (11.5)	0.1 (0.1)	0.06	0.3	0.58	1	140.3 (12.9)	−2.9 (2.4)	—	0.35	1.4	0.24
Cryptomonas sp.												
Biomass (mg C L^{-1})	1.3 (0.1)	0.001 (0.001)	0.03	1.1	0.30	1	2.0 (0.2)	0.2 (0.04)	—	0.31	18.0	0.0001
C : P (molar)	249.8 (17.5)	0.1 (0.1)	0.03	1.1	0.29	1	153.5 (12.6)	−1.8 (2.6)	—	0.02	0.7	0.41
S. obliquus + *O. tuberculata*												
Biomass (mg C L^{-1})	−0.9 (0.3)	0.03 (0.002)	0.87	273.3	<0.0001	1	13.9 (0.4)	−0.04 (0.1)		0.01	0.4	0.55
C : P (molar)	−18.5 (50.1)	2.8 (0.3)	0.76	126.1	<0.0001	2	245.7 (48.6)	716.2 (55.8)	0.4 (0.1)	0.81	82.3	<0.0001

m^{-2} s^{-1} (Fig. 1C,D, left panel; two-way ANOVAs, Table 3, and post-hoc Tukey-test analyses, $p < 0.05$).

Results in the P gradient were not as clear. At low P concentrations of ≤1 μmol L^{-1}, *D. magna* growth and fecundity were higher feeding on *Cryptomonas* sp. than on *S. obliquus* (Fig. 1C,D, right panel). With increasing P supply, phototrophic specialists provided the better food source (Fig. 1C,D, right panel; two-way ANOVAs, Table 3, and post-hoc Tukey-test analyses, $p < 0.01$). This is because at P supplies of >2.5 μmol L^{-1}, *S. obliquus* attained food qualities similar to *Cryptomonas* sp. (Fig. 1B, right panel), but simultaneously provided higher food quantities (Fig. 1A, right panel). Although the investigated food characteristics of *Cryptomonas* sp. remained relatively constant across the P gradient (Fig. 1A, B, right panel), *D. magna* performance declined with rising P supply (Fig. 1C,D, right panel), a result that is different from the observations made in the light gradient. As was the case with the light gradient, somatic growth rates and egg production attained on *S. obliquus* were highest at intermediate P supplies from 5 to 7.5 μmol L^{-1} and from 2.5 to 7.5 μmol L^{-1}, respectively (Fig. 1C,D, right panel; two-way ANOVAs, Table 3, and post-hoc Tukey-test analyses, $p < 0.05$).

As *S. obliquus* dominated almost all chemostats with mixtures of *S. obliquus* and *O. tuberculata* (Fig. 2), mixed diets and phototrophic specialist diets caused similar *D. magna* responses in both gradients (Fig. 1C,D). However, cladocerans usually grew and reproduced better on the pure *S. obliquus* diet, although the food characteristics of both kinds of diets were similar (Fig. 1A,B). Despite generally low relative biomasses, *O. tuberculata* may have had a detrimental effect on *D. magna* in the mixed diets.

Bacterial net growth—Two-way ANOVAs indicate that bacterial net growth rates differed for different alga species, but not for different treatments (light supply or P supply) (Table 4). Calculations of bacterial net growth rates are based on the bacterial abundances shown in Fig. 3B–D.

In the light gradient, bacterial net growth rates were influenced differently in the presence of phototrophic specialists or mixotrophic algae (two-way ANOVA, Table 4, and post-hoc Tukey-test analyses, $p < 0.05$). Mean bacterial net growth rates were negative in chemostats with *O. tuberculata* (−0.06 ± 0.02 SE of the means d^{-1}) or *Cryptomonas* sp. (−0.05 ± 0.01 d^{-1}), but positive in chemostats with *S. obliquus* (0.05 ± 0.01 d^{-1}). Linear regressions indicate that bacterial net growth rates increased slightly with light intensity in chemostats with *O. tuberculata* ($y = -0.15 + 0.0005x$, $r^2 = 0.91$, $F_{1,5} = 51.3$, $p < 0.001$), but remained constant throughout the light gradient in treatments with *Cryptomonas* sp. ($p = 0.75$) or *S. obliquus* ($p = 0.92$) (Fig. 3A).

In the P gradient, bacterial net growth rates were influenced differently in the presence of *O. tuberculata* than with *S. obliquus* or *Cryptomonas* sp. (two-way ANOVA, Table 4, and post-hoc Tukey-test analyses, $p < 0.05$). Mean bacterial net growth rates were highest under the influence of *O. tuberculata* (0.24 ± 0.04 d^{-1}), 0.06 ± 0.03 d^{-1} with *S. obliquus*, equivalent to the light gradient, and 0.01 ± 0.01 d^{-1} in chemostats with *Cryptomonas* sp. Bacterial net growth rates

Table 2. Results of analyses of variance (ANOVAs) for biomass (mg C L^{-1}) and C : P ratio (molar) development for *Scenedesmus obliquus*, *Ochromonas tuberculata*, *Cryptomonas* sp., and a mixture of *S. obliquus* and *O. tuberculata* in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (*see* Materials and methods for details). Sample size in each gradient: 3 × 2 × 7 × 4 (replicates × measurements × treatments × species) = 168.

		Light gradient				P gradient			
		Biomass (mg C L^{-1})		C : P ratio (molar)		Biomass (mg C L^{-1})		C : P ratio (molar)	
	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Species	3	379.6	<0.001	117.8	<0.001	793.9	<0.001	123.7	<0.001
Treatment (light or P)	6	106.8	<0.001	53.3	<0.001	15.6	<0.001	62.5	<0.001
Species × treatment	18	41.8	<0.001	15.7	<0.001	5.9	<0.001	7.9	<0.001

were positively correlated to P supply under the influence of *O. tuberculata* ($y = 0.14 + 0.0002x$, $r^2 = 0.52$, $F_{1,5} = 5.5$, $p < 0.1$) and *Cryptomonas* sp. ($y = -0.03 + 0.01x$, $r^2 = 0.67$, $F_{1,5} = 10.0$, $p < 0.05$), but stable in chemostats with *S. obliquus* ($p = 0.89$) (Fig. 3A).

Discussion

Mixotrophs expressed low and remarkably stable C : P ratios between 100 and 300, despite huge variations in absolute and relative light and nutrient supplies. In contrast, C : P ratios of purely phototrophic algae varied between 100 and 800 (Fig. 1B). The response of phototrophic specialist C : P ratios to the manipulations accords well with the LNH (Sterner et al. 1997) and with previous studies, which showed that algal C fixation depends strongly on light intensity, while P acquisition is closely coupled to overall P supply (e.g., Urabe and Sterner 1996; Makino et al. 2002; Urabe et al. 2002*a*).

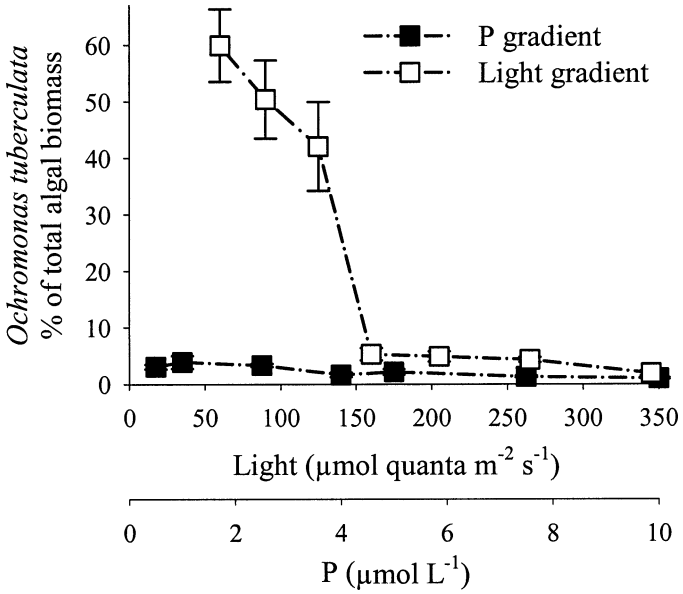

Fig. 2. Proportion of mixotrophic *Ochromonas tuberculata* biomass (%) of total biomass in competition with phototrophic specialist *Scenedesmus obliquus* in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (*see* Materials and methods for details). Data points are means of 3 × 2 (replicates × measurements) = 6 samples. Error bars indicate ±SE of the means.

In contrast to the LNH, changes in light or nutrient supply left the nutrient composition of mixotrophs almost unaffected. *O. tuberculata* and *Cryptomonas* sp. probably compensated for light or P deficiency by heterotrophic nutrition. We did not conduct specific grazing experiments with mixotrophs in the present study. Comparisons of bacterial net growth rates, however, indicate that *O. tuberculata* and *Cryptomonas* sp. ingested bacteria at low light and P supplies and that both mixotrophs became increasingly photoautotrophic at sufficiently high light and nutrient supplies (Fig. 3). As a consequence, at high light : nutrient ratios and at low light conditions, mixotrophs contained on average two to three times as much P per unit carbon as phototrophic specialists and fairly closely resembled the C : P ratios commonly found in bacteria (e.g., Makino et al. 2003). Similarly, algal C : P ratios were low and stable in mixed chemostats, as long as *O. tuberculata* contributed substantially to overall biomass. To the extent mixotrophs were suppressed, community C : P ratio increased rapidly, resembling the treatments containing only *Scenedesmus obliquus* (Figs. 1B, 2).

The observation that *O. tuberculata* was supressed by *S. obliquus* at high light and nutrient levels (Fig. 2) and the finding that mixotrophs generally expressed lower biomasses than purely phototrophic algae (Fig. 1A) can be explained with the energetic costs mixotrophic organisms have to invest in the synthesis and maintenance of both a photosynthetic apparatus and in mechanisms for prey uptake and its subsequent digestion. These energetic costs may lower a mixotroph's resource use efficiency and may lower photosynthetic performance, resulting in a reduced maximum growth rate compared with a phototrophic or heterotrophic specialist. A mixotroph is therefore expected to be inferior if it competes with specialist phototrophs for light or with specialist phagotrophs for prey (e.g., Rothhaupt 1996*a*; Raven 1997; Jones 2000).

Another expectation that our results support is that at high light : nutrient ratios, as at low light conditions, the mixotroph *Cryptomonas* sp. provided the better food source for herbivore production compared to phototrophic specialists. At high light and nutrient supplies, phototrophic specialists presented the more favorable food (Fig. 1C,D).

In accordance with the LNH and numerous experimental studies (e.g., Urabe and Sterner 1996; Hessen et al. 2002;

Table 3. Results of analyses of variance (ANOVAs) for growth rate and reproduction data of *Daphnia magna* feeding either on phototrophic specialist *Scenedesmus obliquus*, mixotrophic *Cryptomonas* sp., or a mixture of phototrophic specialist *S. obliquus* and mixotrophic *Ochromonas tuberculata* in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (*see* Materials and methods for details). Sample size in each gradient: 3 × 7 × 3 (replicates × treatments × species) = 63.

		Light gradient				P gradient			
		Somatic growth rate (d^{-1})		Stand and egg number (eggs per mm body length)		Somatic growth rate (d^{-1})		Stand and egg number (eggs per mm body length)	
	df	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Food type	2	313.9	<0.001	20.0	<0.001	19.7	<0.001	22.3	<0.001
Treatment (light or P)	6	12.0	<0.001	4.4	0.001	0.3	0.909	3.8	0.004
Type × treatment	12	3.8	<0.001	4.3	<0.001	10.7	<0.001	9.5	<0.001

Urabe et al. 2002*a*), the shifts in algal biomass and elemental stoichiometry of purely phototrophic *S. obliquus* caused a trade-off scenario for herbivores in both gradients. Juvenile somatic growth rates and adult fecundity of *D. magna* were limited by food quantity at low light intensities and were limited by food quality at high light intensities (Fig. 1C,D, left panel). In turn, P fertilization caused a transition from limitation by food quality to limitation by food quantity (Fig. 1C,D, right panel).

In contrast to the LNH, the steady food characteristics of mixotrophic *Cryptomonas* sp. enabled a constant herbivore production throughout the light gradient. It is remarkable that at most light : nutrient supply ratios, herbivore growth and fecundity were considerably higher on the mixotroph diet, although food quantity was on average 40% to 70% lower than in monocultures with purely phototrophs. Obviously, transfer efficiency was primarily triggered by food quality. This is consistent with the results received for specialist phototroph and for mixed treatments in the P gradient and sustains recent findings by other authors (e.g., Boersma and Kreutzer 2002; Urabe et al. 2002*a*; Acharya et al. 2004). However, the relative influences of food quality and quantity on zooplankton production are still controversial. Our results from pure and mixed cultures containing *S. obliquus* indicate that increases in food quantity stimulate secondary production only at low C : P ratios of <300, whereas improvements in food quality always enhance transfer efficiency (Fig. 1). This outcome is supported by various estimates of stoichiometric food quality thresholds, which show that the transition from C limitation to P limitation in the growth and reproduction of *D. magna* takes place when food has a C : P ratio of about 250 to 300 (e.g., Hessen 1992; Hessen and Faafeng 2000; Urabe et al. 2002*b*).

In contrast to our expectations and the LNH, the performance of *D. magna* declined under P fertilization with *Cryptomonas* sp. as food, despite low C : P ratios of around 160 and an increasing food quantity (Fig. 1, right panel). The strongly decreasing responses in *D. magna* growth and fecundity indicate that other factors different from algal nutrient stoichiometry influenced food quality. Recent studies have shown, for example, that biochemical constraints like essential fatty acid deficiency may limit secondary production independent from the elemental nutrient composition of food organisms (e.g., Müller-Navarra et al. 2004), and especially at low C : P ratios of <300 (Boersma 2000; Elser et al. 2001; Urabe et al. 2002*a*,*b*). As explained above, we found indications for a decreasing contribution of phagotrophy to overall production at high light and P supplies in both mixotrophs. It is possible that a lowered intake of bacteria had an impairing effect on the food quality of *Cryptomonas* sp. beyond nutrient stoichiometry.

Despite all the positive effects described so far, our experiments imply that mixotrophs may also have detrimental impacts on the algae–herbivore interface. *O. tuberculata* gave some evidence for having a toxic impact on herbivores. *D. magna* suffered high mortality feeding on *O. tuberculata* shortly after the experiments started. We did not test for toxicity, but toxins have been isolated from ochromonads before (e.g., Spiegelstein et al. 1969), and their harmful effect on *D. magna* has been reported by Leeper and Porter (1995).

In conclusion, our study indicates that shifts in light : nutrient supply ratios must not necessarily be accompanied by shifts in seston nutrient stoichiometry as predicted by the LNH, if mixotrophs contribute substantially to overall seston biomass. Consequently, feeding on mixotrophs might also influence secondary production differently than proposed by the LNH. Our results strongly imply that mixotrophs should be considered in the further development of ecological theories that incorporate stoichiometric effects on food web dynamics. Various authors have suggested that variation in seston C : nutrient ratios may regulate the strength of trophic cascades in aquatic ecosystems, with strong cascades occurring at low particulate C : nutrient ratios (e.g., Elser et al. 1998; Hessen and Faafeng 2000). P limitation, supporting

Table 4. Results of analyses of variance (ANOVAs) for bacterial net growth in chemostats with *Scenedesmus obliquus*, *Ochromonas tuberculata*, or *Cryptomonas* sp. in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (*see* Materials and methods for details). Sample size in each gradient: 1 × 7 × 2 (replicates × treatments × species) for mixotrophs + 1 × 3 × 1 for purely phototrophs = 17.

		Bacterial net growth rate (d^{-1})			
		Light gradient		P gradient	
	df	*F*	*p*	*F*	*p*
Species	2	6.5	<0.05	12.2	<0.01
Treatment (light or P)	6	2.3	0.13	0.6	0.71

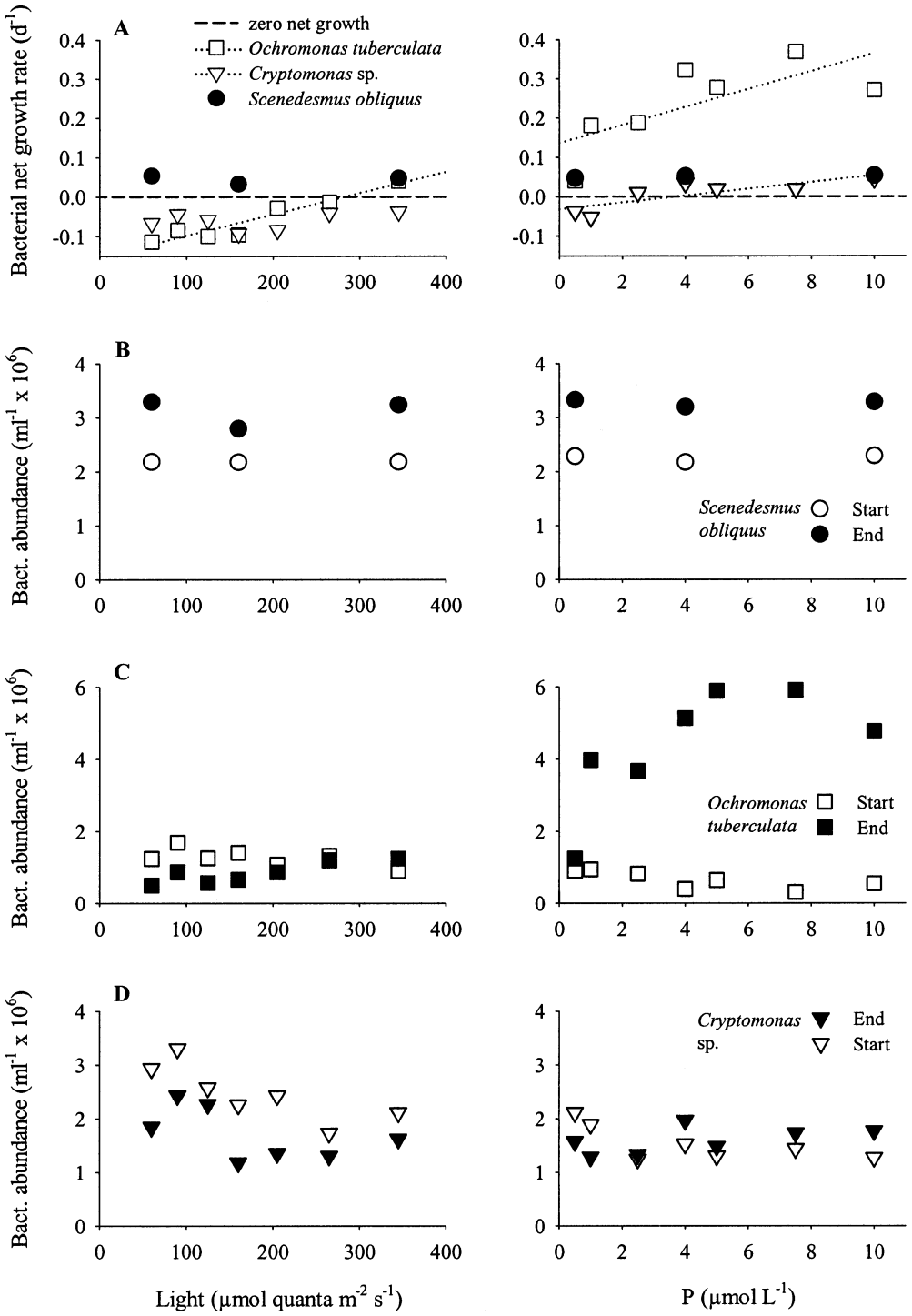

Fig. 3. (A) Bacterial net growth rates (d^{-1}) and (B to D) bacterial abundances ($ml^{-1} \times 10^6$) in the presence of phototrophic specialist algae (*Scenedesmus obliquus*) or mixotrophic algae (*Ochromonas tuberculata* or *Cryptomonas* sp.) in a light gradient at constant nutrient supply and in a P gradient at constant light conditions (*see* Materials and methods for details). Bacterial net growth rates were positively correlated to light supply ($y = -0.15 + 0.0005x$, $r^2 = 0.91$, $F_{1,5} = 51.3$, $p < 0.001$) under the influence of *O. tuberculata* and positively correlated to P supply under the influence of *O. tuberculata* ($y = 0.14 + 0.0002x$, $r^2 = 0.52$, $F_{1,5} = 5.5$, $p < 0.1$) and *Cryptomonas* sp. ($y = -0.03 + 0.01x$, $r^2 = 0.67$, $F_{1,5} = 10.0$, $p < 0.05$).

high C : P ratios, seems to be widespread in lakes today (Elser and Hassett 1994; Elser et al. 2000; Hessen and Faafeng 2000) and might be enhanced in the future (Schindler 1998). Sterner et al. (1997, 1998) predicted an increasing decoupling of higher and lower trophic levels in this case. Indeed, the trophic cascade appears to be muted in P-limited lakes (Carpenter and Kitchell 1993; Pace et al. 1999; Makino et al. 2002). Our results indicate that mixotrophs might act as a buffer within this context. Certainly the hypotheses put forward here merit further exploration in the laboratory and in the field.

References

ACHARYA, K., M. KYLE, AND J. J. ELSER. 2004. Effects of stoichiometric dietary mixing on *Daphnia* growth and reproduction. Oecologia **138:** 330–340.

ANDERSEN, T. 1997. Grazers as sources and sinks for nutrients. Springer.

———, AND D. O. HESSEN. 1991. Carbon, nitrogen, and phosphorus content of freshwater zooplankton. Limnol. Oceanogr. **36:** 807–814.

APHA. 1992. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association.

BEGON, M., J. HARPER, AND C. TOWNSEND. 1996. Ecology: Individuals, populations and communities, 3rd ed. Blackwell.

BOERSMA, M. 2000. The nutritional quality of P-limited algae for *Daphnia*. Limnol. Oceanogr. **45:** 1157–1161.

———, AND C. KREUTZER. 2002. Life at the edges: Is food quality really of minor importance at low food quantities? Ecology **83:** 2552–2561.

CARPENTER, S. R., AND J. F. KITCHELL. 1993. The trophic cascade in lakes. Cambridge Univ. Press.

ELSER, J. J., T. H. CHRZANOWSKI, R. W. STERNER, AND K. H. MILLS. 1998. Stoichiometric constraints on food-web dynamics: A whole lake experiment on the Canadian Shield. Ecosystems **1:** 120–136.

———, AND R. P. HASSETT. 1994. A stoichiometric analysis of the zooplankton–phytoplankton interaction in marine and freshwater ecosystems. Nature **370:** 211–213.

———, K. HAYAKAWA, AND J. URABE. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. Ecology **82:** 898–903.

———, AND OTHERS. 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature **408:** 578–580.

GÄCHTER, R., AND J. BLOESCH. 1985. Seasonal and vertical variation in the C : P ratio of suspended and settling seston of lakes. Hydrobiologia **128:** 193–200.

GULATI, R. D., AND W. R. DEMOTT. 1997. The role of food quality for zooplankton: Remarks on the state-of-the-art, perspectives and priorities. Freshwat. Biol. **38:** 753–768.

HESSEN, D. O. 1992. Nutrient element limitation of zooplankton production. Am. Nat. **140:** 799–814.

———, AND B. O. FAAFENG. 2000. Elemental ratios in freshwater seston: Implications for community structure and energy transfer in food webs. Arch. Hyrobiol. Special Issues Adv. Limnol. **55:** 349–363.

———, P. FÆRØVIG, AND T. ANDERSEN. 2002. Light, nutrients and P : C-ratios in algae: Grazer performance related to food quality and quantity. Ecology **83:** 1886–1898.

JONES, R. I. 2000. Mixotrophy in planktonic protists: An overview. Freshwat. Biol. **45:** 219–226.

LEEPER, D. A., AND K. G. PORTER. 1995. Toxicity of the mixotrophic chrysophyte *Proterioochromonas malhamensis* to the cladoceran *Daphnia magna*. Arch. Hydrobiol. **134:** 207–222.

LINDROTH, R. L., K. K. KINNEY, AND C. L. PLATZ. 1993. Responses of deciduous trees to elevated atmospheric CO_2-productivity, phytochemistry, and insect performance. Ecology **74:** 763–777.

LOLADZE, I., Y. KUANG, AND J. J. ELSER. 2000. Stoichiometry in producer-grazer systems: Linking energy flow and element cycling. Bull. Math. Biol. **62:** 1137–1162.

LUND, J. W. G., C. KIPLING, AND E. D. LECREN. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia **11:** 147–170.

MAKINO, W., J. B. COTNER, R. W. STERNER, AND J. J. ELSER. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C : N : P stoichiometry. Funct. Ecol. **17:** 121–130.

———, J. URABE, J. J. ELSER, AND C. YOSHIMIZU. 2002. Evidence of phosphorus-limited individual and population growth of *Daphnia* in a Canadian Shield lake. Oikos **96:** 197–205.

MÜLLER-NAVARRA, D. C., M. T. BRETT, S. PARK, S. CHANDRA, A. P. BALLANTYNE, E. ZORTTA, AND C. R. GOLDMAN. 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature **427:** 69–72.

PACE, M. L., J. J. COLE, AND S. R. CARPENTER. 1999. Trophic cascades revealed in diverse ecosystems. TREE **14:** 483–488.

PORTER, K. G., AND Y. S. FEIG. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:** 943–948.

RAVEN, J. A. 1995. Comparative aspects of chrysophyte nutrition with emphasis on carbon, nitrogen and phosphorus, p. 95–118. *In* C. D. Sandgren, J. P. Smol, and J. Kristiansen [eds.], Chrysophyte algae: Ecology, phylogeny and development. Cambridge Univ. Press.

———. 1997. Phagotrophy in phototrophs. Limnol. Oceanogr. **42:** 198–205.

RIEMANN, B., H. HAVSKUM, F. THINGSTAD, AND C. BERNARD. 1995. The role of mixotrophy in pelagic environments, p. 87–114. *In* I. Joint [ed.], Molecular ecology of aquatic microbes. NATO ASI Series G 38. Springer.

ROTHHAUPT, K. O. 1996*a*. Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. Ecology **77:** 716–724.

———. 1996*b*. Utilizations and substitutable carbon and phosphorus sources by the mixotrophic chrysophyte *Ochromonas* sp. Ecology **77:** 706–715.

SANDERS, R. W., AND K. G. PORTER. 1988. Phagotrophic phytoflagellates. Adv. Microb. Ecol. **10:** 167–192.

SCHINDLER, D. W. 1998. A dim future for boreal waters and landscapes: Cumulative effects of climate warming, stratospheric ozone depletion, acid precipitation and other human activities. BioScience **48:** 157–164.

SIBBALD, M. J., AND L. J. ALBRIGHT. 1991. The influence of light and nutrients on phagotrophy by the mixotrophic nanoflagellate *Ochromonas* sp. Mar. Microb. Food Webs **5:** 39–47.

SPIEGELSTEIN, M., K. REICH, AND F. BERGMANN. 1969. The toxic principals of *Ochromonas* and related chrysomonadinia. Verh. Int. Ver. Limnol. **17:** 778–783.

STERNER, R. W., J. CLASEN, W. LAMPERT, AND T. WEISSE. 1998. Carbon : phosphorus stoichiometry and food chain production. Ecol. Lett. **1:** 146–150.

———, J. J. ELSER, E. J. FEE, S. J. GUILFORD, AND T. H. CHRZANOWSKI. 1997. The light : nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. Am. Nat. **150:** 663–684.

STIBOR, H. 2002. Yolk protein dynamics and predator released kairomones in *Daphnia magna*. Ecology **83:** 362–369.

———, AND W. LAMPERT. 1993. Estimating the size and maturity in field populations of *Daphnia* (Cladocera). Freshwat. Biol. **30:** 433–438.

TRANVIK, L. J., K. G. PORTER, AND J. MCN. SIEBURTH. 1989. Occurrence of bacterivory in *Cryptomonas*, a common freshwater phytoplankter. Oecologia **78:** 473–476.

URABE, J., M. KYLE, W. MAKINO, T. YOSHIDA, T. ANDERSEN, AND J. J. ELSER. 2002*a*. Reduced light increases herbivore production due to stoichiometric effects of light/nutrient balance. Ecology **83:** 619–627.

———, W. MAKINO, K. HAYAKAWA, AND J. J. ELSER. 2002*b*. Food quality determinants for *Daphnia* growth in P-limited lakes. Verh. Int. Ver. Limnol. **28:** 1–6.

———, AND R. W. STERNER. 1996. Regulation of herbivore growth by the balance of light and nutrients. Proc. Natl. Acad. Sci. USA **93:** 8465–8469.

UTERMÖHL, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. **9:** 1–38.

VADSTEIN, O. 2000. Heterotrophic planktonic bacteria and cycling phosphorus. Phosphorus requirements, competitive ability and food web interactions, p. 115–167. *In* B. Schink [ed.], Advances in microbial ecology. Kluwer.

Received: 11 November 2004
Accepted: 7 March 2005
Amended: 22 March 2005

PAPER B2

The mixotroph *Ochromonas tuberculata* may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions

Katechakis A, Stibor H (2005)

***Oecologia* (submitted)**

Deutsche Forschungsgemeinschaft DFG

COMMUNITY ECOLOGY

The mixotroph *Ochromonas tuberculata* may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions

Alexis Katechakis and Herwig Stibor

Ludwigs-Maximilians-University
Department Biology II – Aquatic Ecology
Großhadener Str. 2
D-82152 Planegg-Martinsried
Germany

Correspondence to: Alexis Katechakis
Phone: +49 89 2180 74 215
Fax: +49 89 2180 74 204
Email: alexis.katechakis@gmx.net

ABSTRACT

Mixotrophic organisms combine light, mineral nutrients, and prey as substituable resources. Based on theoretical assumptions and field observations, we tested experimentally the hypothesis that mixotrophs may invade established plankton communities depending on the trophic status of the system and investigated possible effects on food web structure, species diversity, and nutrient dynamics. Oligotrophic systems fascilated the invasion of mixotrophic organisms in two different ways. First, the combination of photosynthesis and phagotrophy gave them a competitive advantage over specialist phototrophs and specialist phagotrophs. Second, low nutrient supplies supported the growth of small plankton organisms that fall into the food size spectrum of mixotrophs. Conversely, high nutrient supplies prevented mixotrophs from successfully invading the food webs. Two important conclusions were derived from our experiments. First, in contrast to ecology paradigm, specialization may not necessarily be the most successful strategy for survival under stable conditions. Indeed, the use of several resources with lower efficiency can be an equally or even more successful tactic in nature. Second, when limiting nutrients are linked to the bacterio- and picophytoplankton, invading mixotrophs may have a habitat-ameliorating effect for higher trophic levels, gauged in terms of food quantity and quality. Using given resources more efficiently, mixotrophs generated higher biomasses and expressed a superior nutritional value for potential planktivores compared to specialized plankton taxa. Our findings may help to explain why energy transfer efficiency between phytoplankton and higher trophic levels is generally higher in oligotrophic systems than in nutrient rich environments.

Key Words

food size – intraguild predation – mechanistic resource competition theory – nutrient stoichiometry – transfer efficiency

INTRODUCTION

Enrichment with nutrients and organic compounds that limit primary or secondary production is one of the most pervasive human alterations of the environment and profoundly affects community structure, species diversity, and ecosystem functioning (DeAngelis 1992; Rosenzweig 1995; Polis et al. 1997). Accurately predicting the consequences of such enrichment requires a better understanding of the influence of trophic structure on community dynamics and ecosystem processes. Studies in this context have traditionally focused on purely photoautotrophic and purely heterotrophic organisms. The role of mixotrophic organisms is still poorly understood. Mixotrophy in the restricted sense is defined as the combination of photosynthesis and phagotrophy in the same individual (Sanders 1991). Mixotrophs have been found in several classes of single-celled aquatic organisms (flagellates, ciliates, and radiolarians) (e.g. Jones 2000). They are widespread in pelagic ecosystems and may compose a considerable portion of planktonic communities in many kinds of waters (e.g. Sanders 1991; Riemann et al. 1995; Isaksson 1998). The ability of mixotrophs to combine light, mineral nutrients, and prey as substituable resources (Nygaard and Tobiesen 1993; Rothhaupt 1996a) suggests, that they react differently on alterations to the inputs of nutrients to ecosystems than specialist phototrophs and specialist phagotrophs do. Indeed, mixotrophs generally express lower and more stable carbon (C):nutrient ratios than specialist phototrophs (Katechakis et al. 2005). Where mixotrophs are common, they may therefore have a balancing effect on variations in nutrient dynamics caused by perturbations to nutrient supplies. While algae with low C:nutrient ratios are rated a better food quality for higher trophic levels than algae with high C:nutrient ratios (e.g. Sterner et al. 1998; Hessen and Faafeng 2000; Makino et al. 2002), mixotrophs may, moreover, have a balancing effect on variations in transfer efficiency (Katechakis et al. 2005).

In general, mixotrophs are most common in natural oligotrophic environments (see Riemann et al. 1995; Isaksson 1998; Jones 2000 for reviews). However, the mechanisms underlaying the succession or possible invasion of mixotrophs in aquatic systems are hardly known. The potential of mixotrophs to invade natural-like plankton communities consisting of specialist phototrophs and specialist phagotrophs has never been examined experimentally. Mechanistic resource competition theory (Tilman 1982; Tittel et al. 2003) predicts that mixotrophs can take full advantage of their strategy, first, if significant losses to higher trophic levels do not occur, second, if organic carbon (prey items) is available to mixotrophs, and third, if the mixotrophs are able to combine light and organic carbon resource use. The first prerequisite is rather given in oligotrophic areas, because predation generally increases with enrichment. Also the second prerequisite should be rather given in oligotrophic areas, due to the fact that small plankton organisms that fall into the food size spectrum of mixotrophs normally seem to dominate nutrient-poor environments, at least in marine systems (e.g. Sommer 2000; Katechakis et al. 2002, 2004). In accordance with the third prerequisite, the combined

use of light and organic carbon resources by mixotrophs has been exemplified under laboratory and field conditions (e.g. Rothhaupt 1996a; Tittel et al. 2003).

In the present study we tested the ability of mixotrophs to invade established plankton communities consisting of a variety of specialized phototrophic and phagotrophic plankton taxa grown at different supplies of dissolved inorganic nutrients and dissolved organic carbon (DOC). Based on the explanations made above, we formulated the following hypotheses: (1) the potential of mixotrophs to invade an existing plankton community decreases with nutrient enrichment, and (2) the C:nutrient ratio of a plankton community decreases as the proportion of mixotrophs increases.

MATERIAL AND METHODS

Experimental setup

Experimental containers. Experiments were performed in semicontinuous chemostats consisting of 30 L polypropylene buckets. We filled all chemostats with 25 L of water from oligotrophic Lake Langbürgener See, S Bavaria, Germany. The water was filtered by a 0.45 µm filter capsule (Sartorius Sartobran-P, Sartorius Göttingen, Germany) to exclude all eukaryotic protists, but possibly allowed the passing of smaller bacteria from the natural bacterial assemblage. To avoid contamination, all buckets were covered with a transparent lid. Atmospheric air was pumped into the airspace between the lid and the water surface. A filter located at the connection point between the tube and the lid prevented air flow contamination. To reduce sedimentation and to enhance dispersion of nutrients and organisms, the water was continuously mixed by a propeller at the bottom of each container. A faucet mounted in midheight of every bucket allowed water sampling. Chemostats were placed randomly in a climate chamber at a temperature of 20 ± 1 °C and illuminated with fluorescent bulbs (Osram light code 77 and Osram cool-white 21-840, 36 W each, in equal parts, Osram, München, Germany) in a 16:8 hours light:dark rhythm. The photon flux density was 134 ± 14 µmol m^{-2} s^{-1} SE of the means (n = 4) at the surface and 101 ± 4 SE µmol m^{-2} s^{-1} at the bottom of the containers under pure water (measured with a sphaerical Li-Cor light probe, Li-Cor, Lincoln, Nebraska, USA).

Food webs. Initial food webs consisted of bacteria, specialist phagotrophs (heterotrophic nanoflagellates, HNF, and ciliates), and purely phototrophic algae (siliceous and non-siliceous) covering a wide range of plankton sizes from pico- to microphyto- and -zooplankton (Table 1). The food webs were built up successively over a period of ten days. First we inoculated specialist phototrophs, then HNFs and finally the ciliates. Thereby, we took care to inoculate equivalent biovolumes of all organisms (~0.03 mm^3 L^{-1} final concentration in each bucket, measured with a Casy 1 TTC particle counter, Schärfe Systems, Reutlingen, Germany). Algal stock cultures were non-axenic. After the completion of all food webs, we allowed the plankton communities to establish themselves for another two weeks before invasion experiments with mixotrophic *Ochromonas tuberculata* started. We simulated invasion by inoculating equivalent biovolumes of *O. tuberculata* in all treatments (~0.02 mm^3 L^{-1} final concentration).

Contaminations. Although containers were covered with a transparent lid, purely phototrophic *Monoraphidium minutum* was transferred to the chemostats one week after invasion experiments with *O. tuberculata* had started, probably from an adjacent climate chamber. At this point in time, some of the plankton communities were already dominated by mixotrophs (see results). Hence, unintentionally we additionally tested, if purely phototrophic organisms can 'recapture' plankton communities dominated by mixotrophs. A scheme of the final resulting food web is illustrated in Fig. 1. Feeding relationships were verified by grazing experiments and microscopical observations (Katechakis and Stibor, unpublished).

Enrichment. Overall system production was manipulated by three levels of enrichment (low – moderate – high). For this, the chemostats received nitrogen (N), silicon (Si) and phosphorus (P) at a stoichiometric ratio of 16:16:1, with P = 0.1, 1, and 10 µmol L^{-1} (final concentrations), respectively. N was added as $NaNO_3$ and NH_4Cl in equal parts, Si was added as Na_2O_3Si, P was added as NaH_2PO_4. Bacterial production was fueled by the addition of 0.1, 1, and 10 mg glucose L^{-1}, respectively. Furthermore, all chemostats received a mixture of vitamins (0.02 µmol L^{-1} vitamin H and B, 0.004 µmol L^{-1} vitamin B_{12}, final concentrations) and supplementary nutrients (Na_2EDTA, $FeSO_4$, $MnCl_2$, 1 µmol L^{-1} each, final concentrations). From the time of first inoculation on, every five days 2.5 L of the culture suspension in the chemostats were replaced by fresh medium (sterile filtered, autoclaved lake water, supplemented with nutrients as described above) within a clean bench, yielding an average dilution rate of the medium of D = 0.2 day^{-1}. Every treatment was replicated four times.

Sample preparation and analysis

Plankton composition. To ascertain the development of the plankton communities in the chemostats over time, we regularly fixed samples with Lugol's iodine (5 g I_2 + 10 g KI in 100 ml distillated water, 1 % final concentration), settled samples in Utermöhl chambers (Hydrobios, Kiel, Germany), and counted them in an inverted microscope (Leica DMIL, Leica, Wetzlar, Germany) according to the method of Utermöhl (1958) and Lund et al. (1958). Biovolumes were calculated by approximation to simple geometrical bodies using the equations of Hillebrand et al. (1999). We started sampling one day after the inoculation of *O. tuberculata* (defined as 'day 1' in the presented figures) and then ran the experiment for another 30 days.

Bacterial numbers were quantified by means of epifluorescence microscopy (Zeiss Axioplan, Carl Zeiss, Oberkochen, Germany) after staining with DAPI (4,6-diamidino-2-phenylindol, 2 µg DAPI ml^{-1} sample final concentration, Porter and Feig 1980), once at the beginning and once at the end of the experiment.

Plankton nutrient stoichiometry. For the examination of plankton C:P ratios we filtered known aliquots of each chemostat on precombusted Schleicher and Schuell GF6 glass fiber filters. Filters were dried in an oven at 60 °C and stored in a desiccator (C) or freezer (P) until analysis. C content was determined with a C-Mat 500 carbon analyser (Juwe, Viersen, Germany). Algal P concentration was determined by spectrophotometric methods (acid molybdenum-blue technique) after oxidation by persulfate (APHA 1992). For the determination of particulate C:N ratios we filtered samples onto precombusted Whatman GF/C filters and measured them with a Fisons CN-Analyser (NA 1500N). Particulate nutrient ratios were determined once at the beginning and once at the end of the experiment.

RESULTS

Food web production and community size structure. Overall system production and food web size structure were clearly determined by the supply of dissolved inorganic nutrients and DOC to the chemostats. At the end of the experiment, overall biovolume, which is a good measure of biomass, was more then ten times higher in highly fertilized systems (47.8 ± 9.1 mm^3 L^{-1} SE of the means) than under moderate nutrient supplies (4.5 ± 0.8 mm^3 L^{-1}), and around 16 times higher than in oligotrophic treatments (2.9 ± 0.4 mm^3 L^{-1}) (Fig. 2). Oligotrophic systems were initially dominated by picoplankton around 2 µm, namely *C. minor*, and small nanoplankton around 5 µm (*C. pseudostelligaria* and *B. saltans*). At high nutrient supplies, the slightly larger *M. minutum* (~8.5 µm) and the much larger *S. delicatissima* (>60 µm) dominated the community. Mesotrophic systems developed the most balanced food webs regarding both community size structure and taxonomical composition (Fig. 3).

Taxonomical composition. Mixotrophic *O. tuberculata* was able to invade all kinds of systems, but only persisted at low and moderate nutrient supplies. At the end of the experiment, mixotrophs clearly dominated oligotrophic systems presenting 91.6 ± 2.7 % of the overall biovolume. Similarly, mixotrophs represented the most important plankton guild at moderate nutrient supplies (72.9 ± 3.9 % of overall biovolume) (Figs. 2 and 3). Although *O. tuberculata* was not completely outcompeted under eutrophic conditions, its success here was marginal in terms of both absolute (0.2 ± 0.04 mm^3 L^{-1}) and relative biovolumes (0.4 ± 0.1 %) at the end of the experiment. Instead, purely phototrophic algae controlled the community at high nutrient supplies, where they made up 96.3 ± 2.3 % of the overall biovolume (Fig. 3), with *M. minutum* and *S. delicatissima* as the prevailing species in equal parts (one-way ANOVA, $F_{1,6} = 0.4$, n.s.) (Fig. 2). In the same way *O. tuberculata* was able to invade all chemostats, also purely phototrophic *M. minutum* invaded all systems. However, in contrast to *O. tuberculata*, *M. minutum* was able to build up noteworthy abundances only at high nutrient supplies (Figs. 2 and 3). Likewise, the relative importance of specialist phagotrophs increased slightly with nutrient enrichment from 0.7 ± 0.3 % at oligotrophic conditions to 2.2 ± 0.7 % in mesotrophic systems up to 3.6 ± 1.6 % at highest nutrient supplies. However, neither relative (one-way ANOVA, $F_{2,9} = 2.0$, n.s.) nor absolute biovolumes (one-way ANOVA, $F_{2,9} = 1.8$, n.s.) differed significatly between treatments. Whereas the overall biovolume of specialist phagotrophs was not much influenced, their composition changed considerably in such a way that *Cyclidium* sp. gained relative importance over *B. saltans* with increasing nutrient enrichment (one-way ANOVAs: oligotrophic $F_{1,6} = 12.5$, $P = 0.01$; mesotrophic $F_{1,6} = 0.6$, n.s.; eutrophic $F_{1,6} = 11.3$, $P < 0.05$) (Fig. 2).

Bacterial numbers. Prior to the invasion of mixotrophs, overall bacterial abundances were highest in low nutrient systems (2.2 ± 0.2 x 10^6 cells ml^{-1}), followed by moderately (2.0 ± 0.4 cells ml^{-1} x 10^6) and highly fertilized (1.4 ± 0.3 x 10^6 cells ml^{-1}) treatments. Differences among treatments, however, were not significant (one-way ANOVA, $F_{2,9} = 1.6$, n.s.). During the experiment overall bacterial abundances decreased significantly in oligotrophic sytems and increased significantly in

eutrophic sytems. Changes at moderate nutrient supplies were not substantial (paired *t*-tests: oligotrophic $t_3 = 3.4$, $P = 0.01$; mesotrophic $t_3 = 0.9$, n.s.; eutrophic $t_3 = -2.0$, $P < 0.1$) (Fig. 4). Consequently, at the end, bacterial abundances were considerably higher at high nutrient supplies (4.9 ± 1.7 x 10^6 cells ml^{-1}) compared to moderate (1.5 ± 0.4 x 10^6 cells ml^{-1}) and low (1.2 ± 0.2 x 10^6 cells ml^{-1}) nutrient conditions (one-way ANOVA, $F_{2,9} = 4.0$, $P = 0.05$, and post hoc Tukey-test analyses, $P < 0.05$). The differences observed among treatments were mainly due to changes in numbers of rod-shaped and coccal bacteria. While abundances of filamentous bacteria increased in all chemostats (paired *t*-tests: oligotrophic $t_3 = -7.5$, $P < 0.01$; mesotrophic $t_3 = -6.8$, $P < 0.01$; eutrophic $t_3 = -4.3$, $P < 0.05$), rod-shaped and coccal bacteria decreased in oligotrophic treatments (paired *t*-tests: $t_3 = 3.6$, $P < 0.05$ and $t_3 = -3.3$, $P < 0.05$, respectively) and in mesotrophic systems ($t_3 = 4.1$, $P < 0.05$ and $t_3 = 3.2$, $P < 0.05$, respectively) but increased under high nutrient supplies ($t_3 = -2.6$, $P < 0.1$ and $t_3 = -2.8$, $P < 0.1$, respectively) (Fig. 4).

Plankton nutrient stoichiometry. Overall plankton C:N ratios were significantly higher in eutrophic systems than in other treatments (one-way ANOVAs: start $F_{2,9} = 7.9$, $P < 0.05$; end $F_{2,9} = 5.8$, $P < 0.05$; post hoc Tukey-test analyses, $P < 0.05$) and remained unaffected throughout the experiment in all chemostats (paired *t*-tests: oligotrophic $t_3 = 0.01$, n.s.; mesotrophic $t_3 = 0.3$, n.s.; eutrophic $t_3 = 0.8$, n.s.) (Fig. 5). C:P ratios were generally lowest in oligotrophic systems, followed by mesotrophic and eutrophic treatments. These differences were not significant at the beginning (one-way ANOVA, $F_{2,9} = 0.8$, n.s.) but intensified during the experiment. C:P ratios decreased considerably in oligotrophic and mesotrophic systems (paired *t*-tests, $t_3 = 2.6$, $P < 0.1$ and $t_3 = 5.1$, $P = 0.01$, respectively) but remained constant in eutrophic systems ($t_3 = 0.2$, n.s.). Consequently, at the end, C:P ratios were significantly lower at low and moderate nutrient supplies (one-way ANOVA, $F_{2,9} = 6.1$, $P < 0.05$ and post hoc Tukey-test analyses, $P < 0.05$) (Fig. 5).

DISCUSSION

Succession is an important biological parameter that continually changes the structure and functioning of food webs. Because of succession, species are continually invading and being lost from communities. Species that invade may or may not be successful and persist. But when they do persist, they alter the shapes of the food webs, unless they simply replace existing species (Pimm 1982). In our experiments, invading mixotrophs changed plankton and bacterial community structure, species diversity, and nutrient cycling, and hence, probably would have changed ecosystem functioning in situ. The observed processes strongly depended on nutrient availability and thus, support several of our expectations: (1) The potential of mixotrophs to invade established plankton communities and successfully compete with purely auto- and heterotrophic specialists was considerably higher at low nutrient conditions. (2) Plankton communities containing high proportions of mixotrophs expressed much lower overall C:P ratios than plankton communities without mixotrophs. However, C:N ratios remained unaffected.

Plankton community composition

Low and moderate nutrient supplies fascilated the invasion of mixotrophic organisms in two different ways. First, the ability of mixotrophs to combine light, mineral nutrients, and prey as substituable resources (Nygaard and Tobiesen 1993; Rothhaupt 1996a) gave them a competitive advantage over specialist phototrophs and specialist phagotrophs. Second, low nutrient supplies supported the growth of small plankton organisms that fall into the food size spectrum of mixotrophs.

O. tuberculata obviously supplemented nutrient restriction by grazing bacteria and picophytoplankton at oligotrophic and mesotrophic conditions. Abundances of both *C. minor* and edible bacteria (coccal and rod-shaped morphotypes) declined considerably in both kinds of systems as mixotrophs gained importance. Other phagotrophs were of minor significance. Therefore, we rate their influence as comparatively negligible. A decline of bacteria and picophytoplankton due to nutrient competition with other phytoplankton is also improbable. Because of their high surface to volume ratio, bacteria and picophytoplankton are estimated stronger competitiors for inorganic nutrients than bigger algae (e.g. Currie and Kalff 1984; Sommer et al. 2002). The consumption of small phototrophs by mixotrophs has also been suggested by other authors (e.g. Havskum and Hansen 1997; Sanders et al. 2000).

As a consequence of combining alternative production pathways, *O. tuberculata* practically suppressed all other plankton species that were present at the beginning of the experiments. At the end, the ratio of mixotrophs to purely autotrophs (M:A) was 12:1 in oligotrophic systems and 3:1 in mesotrophic treatments. The ratio of mixotrophs to purely heterotrophs (M:H) was 135:1 and 33:1,

respectively. This outcome is in accordance with the mechanistic resource competition theory (Tilman 1982; Rothhaupt 1996b; Tittel et al. 2003). The theory predicts that mixotrophs should compete successfully with specialist phototrophs when light and/or nutrient supplies limit autotrophic growth but particulate prey is available. Similarly, mixotrophs should compete successfully with specialist phagotrophs when prey abundances limit heterotrophic growth but light and nutrient conditions allow photosynthesis.

Still, mixotrophs were not able to completely outcompete specialist photo- and phagotrophs. Probably because of its advantageous surface to volume ratio, *C. minor* was able to maintain stable populations under both nutrient regimes, albeit on very low levels. Additionally, *S. delicatissima* reached some importance towards the end of the experiment at moderate nutrient supplies. As the only unedible alga in our experiments, *S. delicatissima* probably benefited from the decrease of all other phytoplankton taxa. Similarly, ciliates apparently took advantage of the weakening of *B. saltans* in mesotrophic systems. *B. saltans* was exposed to a double pressure. The HNF formed an intermediate prey in the heterotrophic food chain (bacteria/picophytoplankton – HNF – ciliates) and furthermore competed with *O. tuberculata* for bacteria and picophytoplankton. The reduction of *B. saltans* is consistent with the expectation that an omnivorous top consumer (i.e. here the ciliate) reduces its intermediate prey (e.g. Diehl and Feissel 2000; Mylius et al. 2001) by feeding simultaneously on the intermediate prey and on the common basal resource ("strategy of eating your competitor", Thingstad et al. 1996). It is also consistent with the results of Rothhaupt (1996b) who showed that *O. tuberculata* eventually outcompetes *B. saltans* once the bacterial prey has been grazed down to a density that limits the growth of the HNF. However, from our results it cannot be seen which of the two processes contributed to which extent to the decrease of *B. saltans*.

High nutrient supplies prevented mixotrophs from successfully invading the food web. The observation that *O. tuberculata* could not persist in eutrophic systems can be explained by the energetic costs mixotrophic organisms must invest in the synthesis and maintenance of both a photosynthetic apparatus and in mechanisms for prey uptake and its subsequent digestion. These energetic costs may lower a mixotroph's resource use efficiency and may lower photosynthetic performance, resulting in a reduced maximum growth rate compared with a phototrophic or heterotrophic specialist. A mixotroph is therefore expected to be inferior if the environmental conditions sufficiently satisfy the demands of purely phototrophic and heterotrophic specialist, respectively (e.g. Rothhaupt 1996a; Raven 1997; Jones 2000). Prey abundances in eutrophic systems apparently supported the growth of ciliates (M:H = 0.05). Likewise, nutrient concentrations promoted specialist phototrophs, which is especially reflected by the impressive development of *M. minutum* (M:A = 0.002). Both processes together signed for the suppression of mixotrophs in eutrophic systems, an observation that is in accordance with field studies (e.g. Bergström et al. 2003; Tittel et al. 2003) and fertilization mesocosm experiments (e.g. Jansson et al. 1996).

An important conclusion from the results discussed above is that mixotrophs may compete successfully with purely autotrophic and purely heterotrophic organisms at the same time, although ecology paradigm predicts that specialization should be the most successful strategy for survival under stable conditions (MacArthur and Connell 1966; Dall and Cuthill 1997). This indicates that the use of several resources with lower efficiency can be an equally successful strategy in nature. Indeed, at low to moderate nutrient supplies, mixotrophs made much better use of the given resources (in the sense of generating biomass) than purely auto- and heterotrophic specialists. This suggests that in situations when limiting nutrients are linked to the bacterio- and picophytoplankton, invading mixotrophs may have a habitat-ameliorating effect for higher trophic levels. A lake for example may continue to be productive during periods that do not favour the growth of specialist phototrophs – a consideration that is sustained by field data (Hitchman and Jones 2000). Higher trophic level production may not only be supported by mixotrophs in terms of food quantity, but may be additionally promoted with regard to food quality (to be explained in following section).

Community nutrient stoichiometry

The elemental nutrient stoichiometry of phytoplankton is an important characteristic for food quality for higher trophic levels such as herbivorous mesozooplankton and fish (Sterner et al. 1998). In this context, algae with low C:P ratios <300 are rated a better food quality than algae with high C:P ratios (e.g. Elser et al. 1998; Hessen and Faafeng 2000; Hessen et al. 2002). Katechakis et al. (2005) showed recently, that when using different nutrient sources, mixotrophs generally express lower and more stable C:P ratios than purely phototrophic algae, and that mixotrophs may therefore enhance transfer efficiency towards herbivores. The present study illustrates that invading mixotrophs may even improve a whole plankton community's food quality, gauged in terms of seston nutrient stoichiometry. The overall C:P ratio was considerably lower where mixotrophs were common. This speaks for a scarcity of dissolved inorganic P that was compensated by additional P uptake by mixotrophs using particular P from prey as P source. In this way, mixotrophs may make nutrient sources available for higher trophic levels, that would not be accessible to them otherwise. On the contrary, N obviously was obtainable in excess in our experiments as C:N ratios remained unaffected.

The outcome that mixotrophs have relevant competition advantages in oligotrophic systems together with the observation that mixotrophs may upgrade plankton food quality for higher trophic levels, might help explain why trophic transfer efficiency and food web strength are generally higher in low nutrient environments than in eutrophic systems (Carpenter and Kitchell 1984; McQueen et al. 1986). Thus far, there is no clear understanding of the reasons for this observation. Müller-Navarra et

al. (2004) showed, however, that differences in ω3-polyunsaturated fatty acid (PUFA)-associated food quality at the plant-animal interface play a role. If confirmed in situ, our results could complement these findings.

Implications for natural aquatic food webs

According to the presented results, one would expect mixotrophs to be especially invasive in steady-state like situations where light is sufficient, but dissolved nutrients are limiting and overall productivity is rather low, as is the case in surface layers after a longer period of stratification. Under such conditions, external import of nutrients is low, and recycling is the primary source for mineral nutrients. Growth rates of pure autotrophs are well below their possible maxima, and mixotrophs might take full advantage of their strategy. We are aware, however, that our experiments are subject to different restrictions. First, compared to nature, we chose a system with a low-diversity food web. As community invasibility may vary with species diversity (Bruno et al. 2003), the susceptibility of communities to invasion might be different in situ. Second, our conclusions are based on investigations having only one mixotrophic species. *Ochromonas* is a predominantly heterotrophic mixotroph (e.g. Sibbald and Albright 1991). Other mixotrophs may behave in another way and hence lead to deviating insights. Finally, the exclusion of higher trophic levels such as herbivorous mesozooplankton and fish provokes questions about the generality of the findings presented here. Nevertheless, our results may help explain observations in very different aquatic habitats. A number of studies have shown, that mixotrophs are abundant and active in illuminated oligotrophic surface marine (e.g. Arenovski et al. 1995; Dolan and Marrase 1995; Havskum and Riemann 1996; Pitta and Giannakourou 2000; Sanders et al. 2000; Pitta et al. 2001) and freshwater systems (e.g. Sandgren 1988; Berninger et al. 1992; Sommer et al. 1993; Tittel et al. 2003). Additionally, the strength of mixotrophic nutrition of mixotrophic algae has been shown to depend on the trophic status of the environment (Stibor and Sommer 2003). Thus, the hypotheses put forward here certainly merit further exploration in the laboratory and in the field.

ACKNOWLEDGEMENTS

We thank Torsten Stadthagen for technical assistance, Thomas Hansen for C:N analyses, Achim Weigert for C:P measurements, and Barbara Santers for providing us with HNF cultures. Our experiments were financially supported by the Deutsche Forschungsgemeinschaft, DFG (project ST180/ 1-1, 1-2).

REFERENCES

APHA (1992) Standard Methods for the Examination of Water and Wastewater, 18th ed. American Public Health Association

Arenovski AL, Lim EL, Caron DA (1995) Mixotrophic nanoplankton in oligotrophic surface waters of the Sargasso Sea may employ phagotrophy to obtain major nutrients. J Plankton Res 17:801-820

Bergström AK, Jansson M, Drakare S, Blomqvist P (2003) Occurrence of mixotrophic flagellates in relation to bacterioplankton production, light regime and availability of inorganic nutrients in unproductive lakes with differing humic contents. Freshwat Biol 48:868-877

Berninger UG, Caron DA, Sanders RW (1992) Mixotrophic algae in three ice-covered lakes of the Pocono mountains, USA. Freshwat Biol 28:263-272

Bruno JF, John J. Stachowicz JJ, Bertness MD (2003) Inclusion of facilitation into ecological theory. TREE 18:119-125

Carpenter SR, Kitchell JF (1984) Plankton community structure and limnetic primary production. Am Nat 124:159-172

Currie DJ, Kalff J (1984) Can bacteria outcompete phytoplankton for phosphorus? A chemostat test. Microb Ecol 10:205-216

Dall SRX, Cuthill IC (1997) The information costs of generalism Oikos 80:197-202

DeAngelis DL (1992) Dynamics of nutrient cycling and food webs. Chapman & Hall, London

Diehl S, Feissel M (2000) Effects of enrichment on three-level food chains with omnivory. Am Nat 155:200-218

Dolan JR, Marrase C (1995) Planktonic ciliate distribution relative to a deep chlorophyll maximum: Catalan Sea, N.W. Mediterranean, June 1993. Deep Sea Res 42:1965-1987

Elser JJ, Chrzanowski TH, Sterner RW, Mills KH (1998) Stoichiometric constraints on food-web dynamics: a whole lake experiment on the Canadian Shield. Ecosystems 1:120-136

Havskum H, Hansen AS (1997) Importance of pigmented and colourless nano-sized protists as grazers on nanoplankton in a phosphate-depleted Norwegian fjord and in enclosures. Aquat Microb Ecol 12:139-151

Havskum H, Riemann B (1996) Ecological importance of bacterivorous, pigmented flagellates (mixotrophs) in the Bay of Aarhus, Denmark. Mar Ecol Progr Ser 137:251-263

Hessen DO, Faafeng BO (2000) Elemental ratios in freshwater seston: implications for community structure and energy transfer in food webs. Arch Hyrobiol Special Issues Adv Limnol 55:349-363

Hessen DO, Færøvig P, Andersen T (2002) Light, nutrients and P:C-ratios in algae: grazer performance related to food quality and quantity. Ecology 83:1886-1898

Hillebrand H, Dürselen CD, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. J Phycol 35:403-424

Hitchman RB, Jones HLJ (2000) The role of mixotrophic protists in the population dynamics of the microbial food web in a small artificial pond. Freshwat Biol 43:231-241

Isaksson A (1998) Phagotrophic phytoflagellates in lakes – a literature review. Arch Hydrobiol Arch Hyrobiol Special Issues Adv Limnol 51:63-90

Jansson M, Blomqvist P, Jonsson A, Bergström AK (1996) Nutrient limitation of bacterioplankton, autotrophic and mixotrophic phytoplankton and heterotrophic nanoflagellates in Lake Örträsket. Limnol Oceanogr 41:1552-1559

Jones RI (2000) Mixotrophy in planktonic protists: an overview. Freshwat Biol 45:219-226

Katechakis A, Haseneder T, Kling R, Stibor H (2005) Mixotrophic vs. photoautotrophic specialist algae as food for zooplankton: The light:nutrient hypothesis might not hold for mixotrophs. Limnol Oceanogr 50:1290-1299

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

Katechakis A, Stibor H, Sommer U, Hansen T (2004) Feeding selectivities and food niche separation of Acartia clausi, Penilia avirostris (Crustacea) and Doliolum denticulatum (Thaliacea) in Blanes Bay (Catalan Sea, NW Mediterranean). J Plankton Res 26:589-603

Lund JWG, Kipling C, LeCren ED (1958) The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiol 11:147-170

MacArthur RH, Connell JH (1966) The Biology of Populations. Wiley, New York

Makino W, Urabe J, Elser JJ, Yoshimizu C (2002) Evidence of phosphorus-limited individual and population growth of Daphnia in a Canadian Shield lake. Oikos 96:197-205

McQueen DJ, Post JR, Mills EL (1986) Trophic relationships in freshwater pelagic ecosystems. Can J Fish Aquat Sci 43:1571-1581

Müller-Navarra DC, Brett MT, Park S, Chandra S, Ballantyne AP, Zorta E, Goldman CR (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427:69-72

Mylius SD, Klumpers K, de Roos AM, Persson L (2001) Impact of intraguild predation and stage structure on simple communities along a productivity gradient. Am Nat 158:259-276

Nygaard K, Tobiesen A (1993) Bacterivory in algae: a survival strategy during nutrient limitation. Limnol Oceanogr 38:273-279

Olrik K (1998) Ecology of mixotrophic flagellates with special reference to Chrysophyceae in Danish lakes. Hydrobiologia 369/370:329-338

Pimm SL (1982) Food webs. Chapman & Hall, London

Pitta P, Giannakourou A (2000) Planktonic ciliates in the oligotrophic Eastern Mediterranean: vertical, spatial distribution and mixotrophy. Mar Ecol Prog Ser 194:269-282

Pitta P, Giannakourou A, Christaki U (2001) Planktonic ciliates in the oligotrophic Mediterranean Sea: longitudinal trends of standing stocks, distributions and analysis of food vacuole contents. Aquat Microb Ecol 24:297-311

Polis GA, Anderson WB, Holt RD (1997) Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs. Ann Rev Ecol Sys 28:289-316

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943-948

Raven JA (1997) Phagotrophy in phototrophs. Limnol Oceanogr 42:198-205

Riemann B, Havskum H, Thingstad F, Bernard C (1995) The role of mixotrophy in pelagic environments. In: Joint I (ed) Molecular Ecology of Aquatic Microbes. NATO ASI Series G 38, Springer, Berlin Heidelberg New York, pp 87-114

Rosenzweig ML (1995) Species diversity in space and time. Camebridge Univ Press, Camebridge

Rothhaupt KO (1996a). Utilizations and substitutable carbon and phosphorus sources by the mixotrophic chrysophyte *Ochromonas* sp.. Ecology 77:706-715

Rothhaupt, KO (1996b). Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. Ecology 77:716-724

Sanders RW (1991) Mixotrophic protists in marine and freshwater ecosystems. J Protozool 38:76-81

Sanders RW, Berninger UG, Lim EL, Kemp PF, Caron DA (2000) Heterotrophic and mixotrophic nanoplankton predation on picoplankton in the Sargasso Sea and on Georges Bank. Mar Ecol Prog Ser 192:103-118

Sandgren CD (1988) The ecology of chrysophyte flagellates: their growth and perennation strategies as freshwater phytoplankton. In: Sandgren CD (ed) Growth and Reproductive Strategies of Freshwater Phytoplankton. Cambridge University Press, Cambridge, pp 9-104

Sibbald MJ, Albright LJ (1991) The influence of light and nutrients on phagotrophy by the mixotrophic nanoflagellate *Ochromonas* sp.. Mar Microb Food Webs 5:39-47

Sommer U (2000) Scarcity of medium-sized phytoplankton in the northern Red Sea explained by strong bottom-up and weak top-down control. Mar Ecol Progr Ser 197:19-25

Sommer U, Stibor H, Katechakis A, Sommer F, Hansen T (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. Hydrobiologia 484:11-20

Sterner RW, Clasen J, Lampert W, Weisse T (1998) Carbon:phosphorus stoichiometry and food chain production. Ecol Lett 1:146-150

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

Thingstad TF, Havskum H, Garde K, Riemann B (1996) On the strategy of "Eating your competitor': A mathematical analysis of algal mixotrophy. Ecology, 77, 2108-2118

Tilman D (1982) Resource competition and community structure. Princeton Univ Press, Princeton

Tittel J, Bissinger V, Zippel B, Gaedke U, Bell E, Lorke A, Kamjunke N (2003) Mixotrophs combine resource use to outcompete specialists: Implications for aquatic food webs. Proc Nat Acad Sci 100:12776–12781

Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt int Verein theor angew Limnol 9:1-38

Table 1 Plankton taxa used for experiments. Given cell dimensions are means of 20 measurements each. Biovolumes were calculated using the equations of Hillebrand et al. (1999)

Taxon	Cell dimensions (µm)		Biovolume (μm^3 $cell^{-1}$)	Strain
	Longest extension	Width or diameter		
Specialist phototrophs				
Bacillariophyceae				
Cyclotella pseudostelligaria	4.9	4.8	86.1	CCAP[a] 1070/3
Synedra delicatissima	64.0	4.5	857.0	CCAP 1080/11
Chlorophyta				
Choricystis minor	2.0	1.7	2.8	SAG[b] 17.98
Monoraphidium minutum	8.5	3.2	22.8	SAG 243-1
Specialist phagotrophs				
Heterotrophic nanoflagellates (HNF)				
Bodo saltans	5.5	4.2	35.2	MPI[c]-*Bodo saltans*
Ciliates				
Cyclidium sp.	25.4	12.8	8140.2	LMU[d]-*Cyclidium* sp.
Mixotrophs				
Ochromonas tuberculata	10.3	8.5	355.3	CCAP 933/27

[a]Culture Collection of Algae and Protozoa, Argyll, UK
[b]Experimental Phycology and Culture Collection of Algae, Göttingen, Germany
[c]Max-Planck-Institute for Limnology, Plön, Germany
[d]Ludwigs-Maximilians-University, Aquatic Ecology, Munich, Germany

Fig. 1 Experimental food web consisting of specialist phototrophs (grey underlayed), specialist phagotrophs, mixotrophs (cross-striped) and bacteria. Solid lines represent major links, dashed lines represent minor links

Fig. 2 Changes over time in the absolute biovolumes of specialist phototrophs (left panel), specialist phagotrophs (middle panel) and mixotrophs (right panel) at low, moderate and high nutrient supplies. Data points are means of four replicates, error bars represent SE of the means. Note different y-axes scaling

Fig. 3 Changes over time in the relative biovolumes of specialist phototrophs, specialist phagotrophs and mixotrophs at low, moderate and high nutrient supplies. Data represent means of four replicates

Fig. 4 Changes in the abundance and the composition of the bacterial community under the influence of different food web compositions (see Figs. 2 and 3) at low, moderate and high nutrient supplies. Rod-shaped bacteria were devided into three size classes: <1.5 µm, 1.5 to 3.0 µm, and >3.0 µm; mean length (N = 20) in each size class: 1.0 ± 0.04 µm SE of the means, 2.3 ± 0.1 µm, and 3.5 ± 0.2 µm, respectively. Coccal bacteria had a mean diameter of 0.7 ± 0.03 µm. Filamentous cyanobacteria were devided into three size classes: 3 to 10 µm, 10 to 20 µm, and 20 to 40 µm; mean length (N = 20) in each size class: 5.6 ± 0.4 µm SE, 13.0 ± 0.4 µm, and 33.7 ± 1.1 µm, respectively. Data points are means of four replicates, error bars represent SE of the means. Note different y-axes scaling

Fig. 5 . Changes in the overall C:N and C:P stoichometry of the plankton community depending on the development of the food web composition (see Figs. 2 and 3) at low, moderate and high nutrient supplies. Data points are means of four replicates, error bars represent SE of the means. Note different y-axes scaling

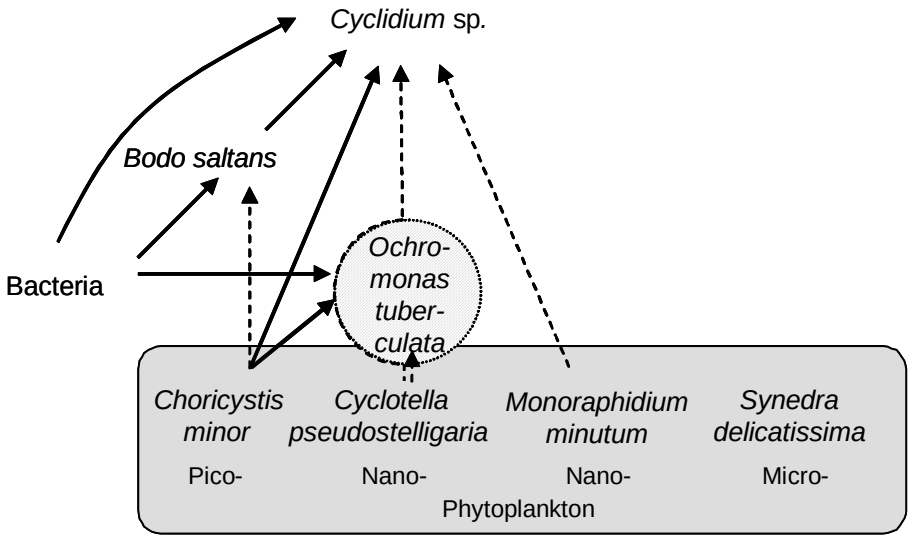

Fig. 1 Katechakis and Stibor – Food web

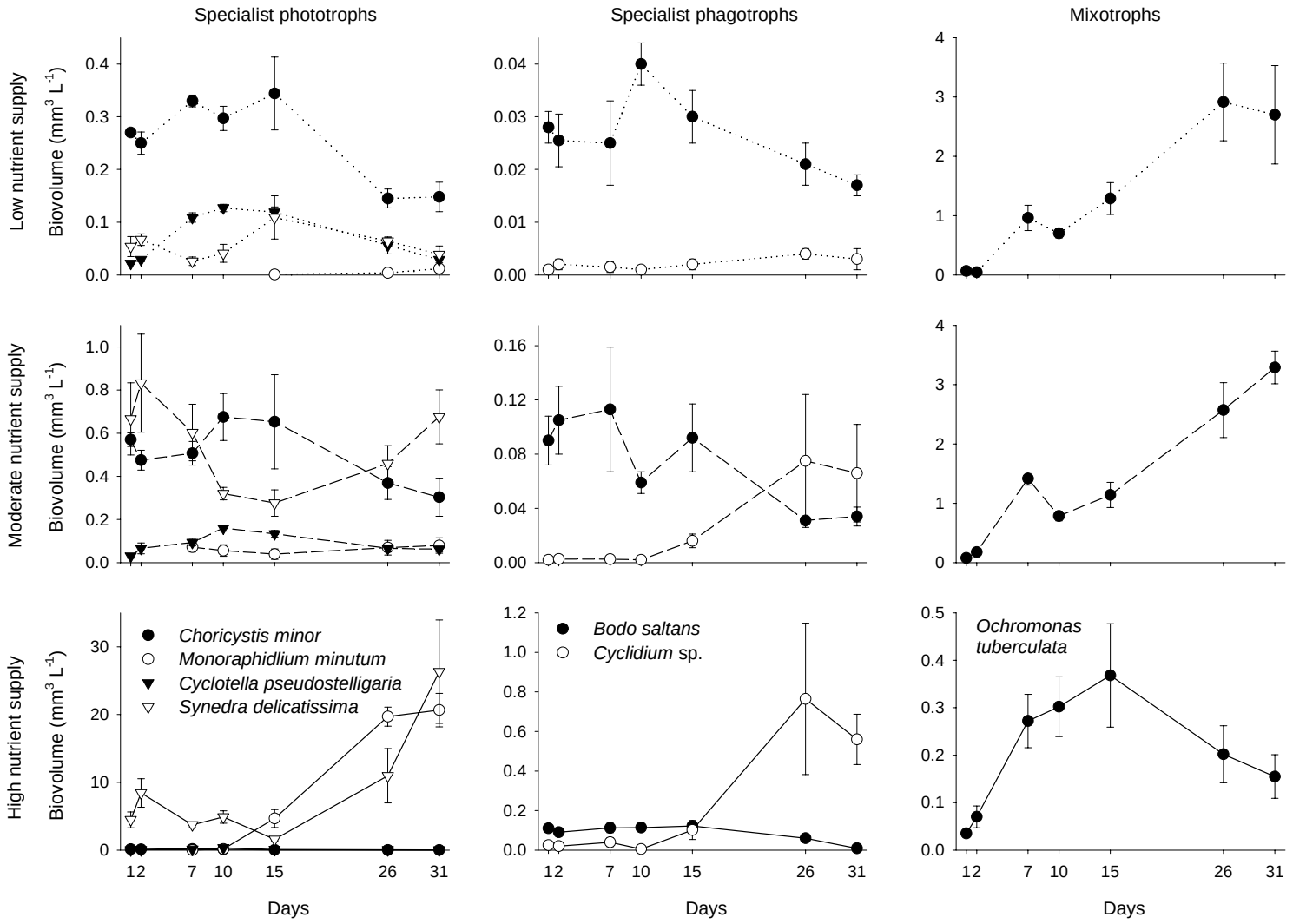

Fig. 2 Katechakis and Stibor – Absolute biovolumes

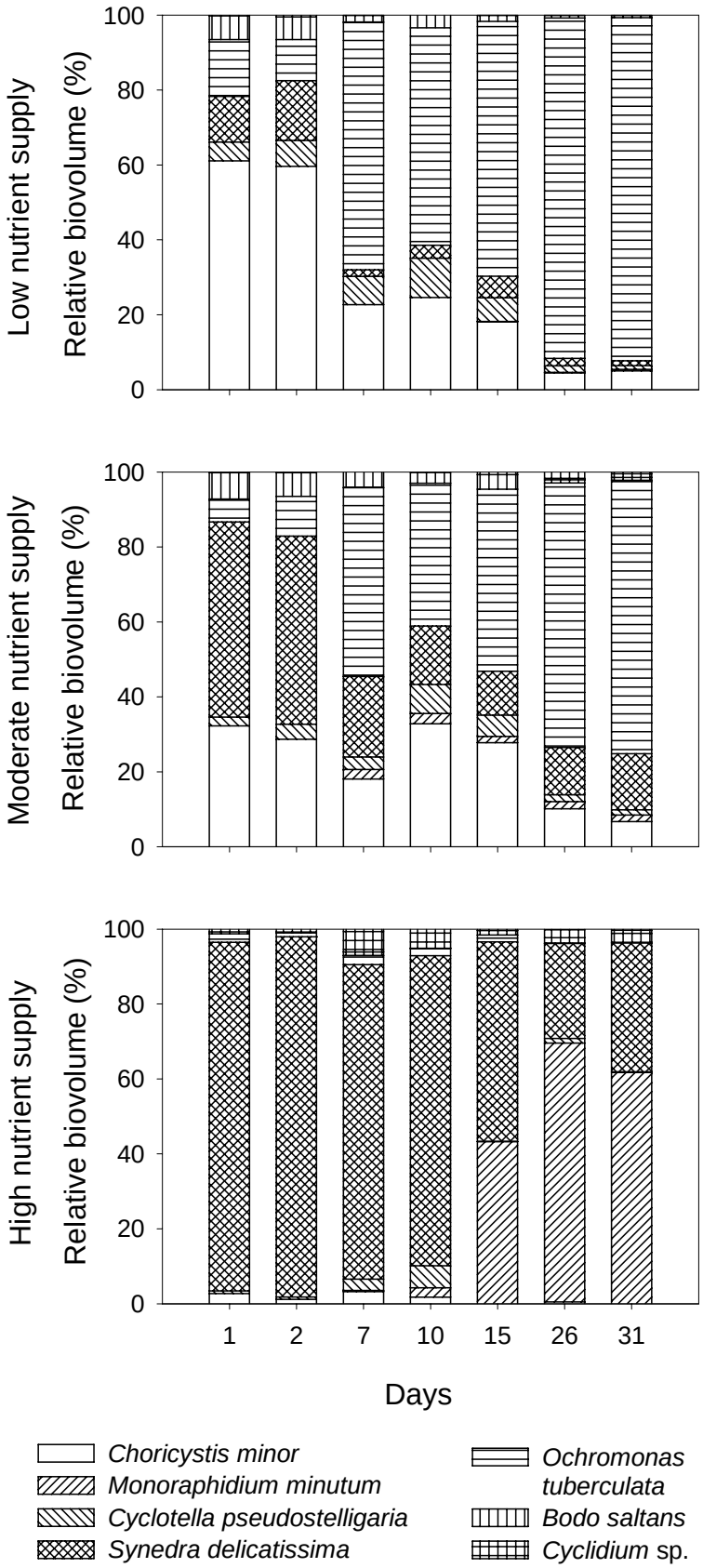

Fig. 3 Katechakis and Stibor – Relative biovolumes

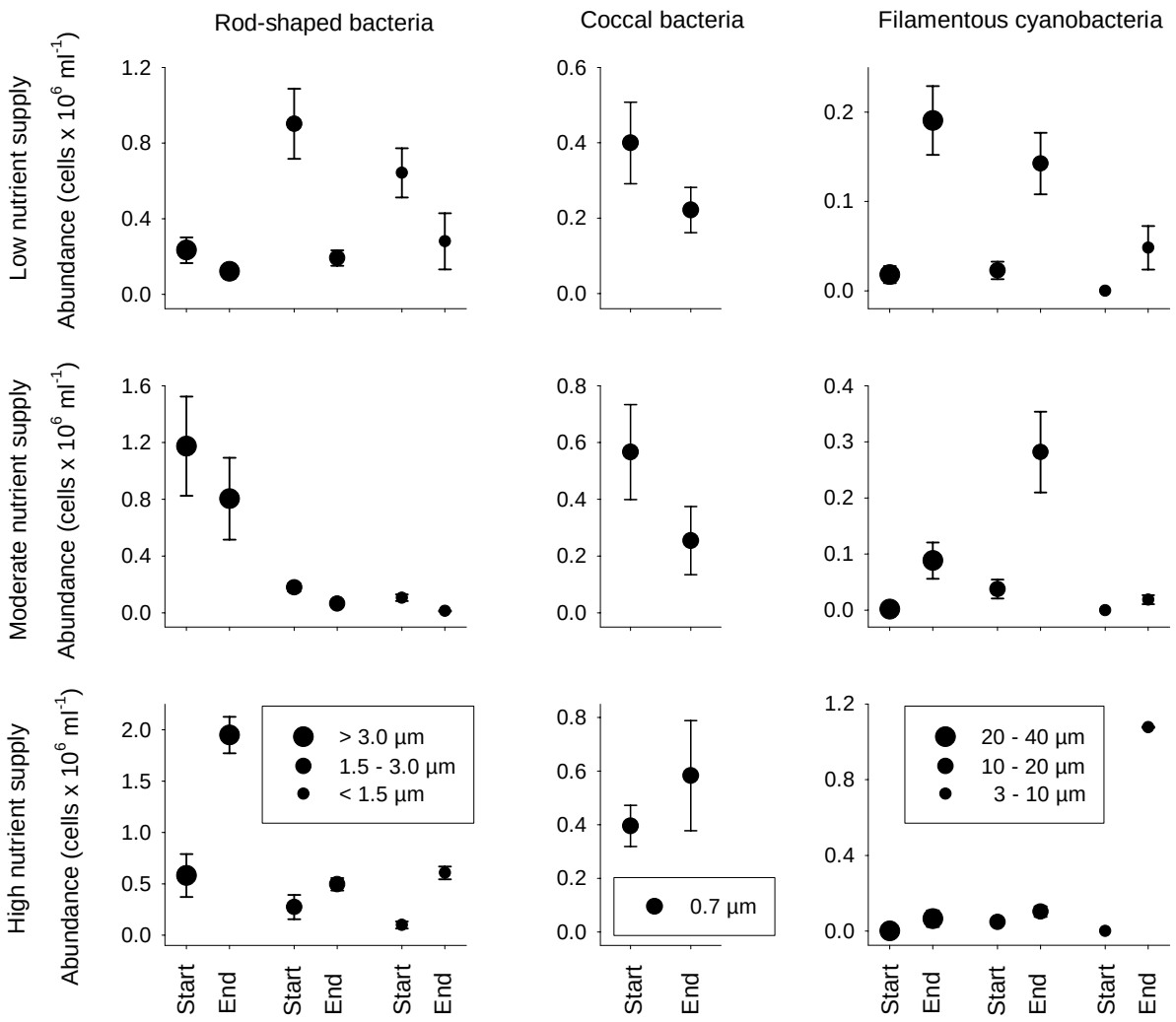

Fig. 4 Katechakis and Stibor – Bacterial abundances

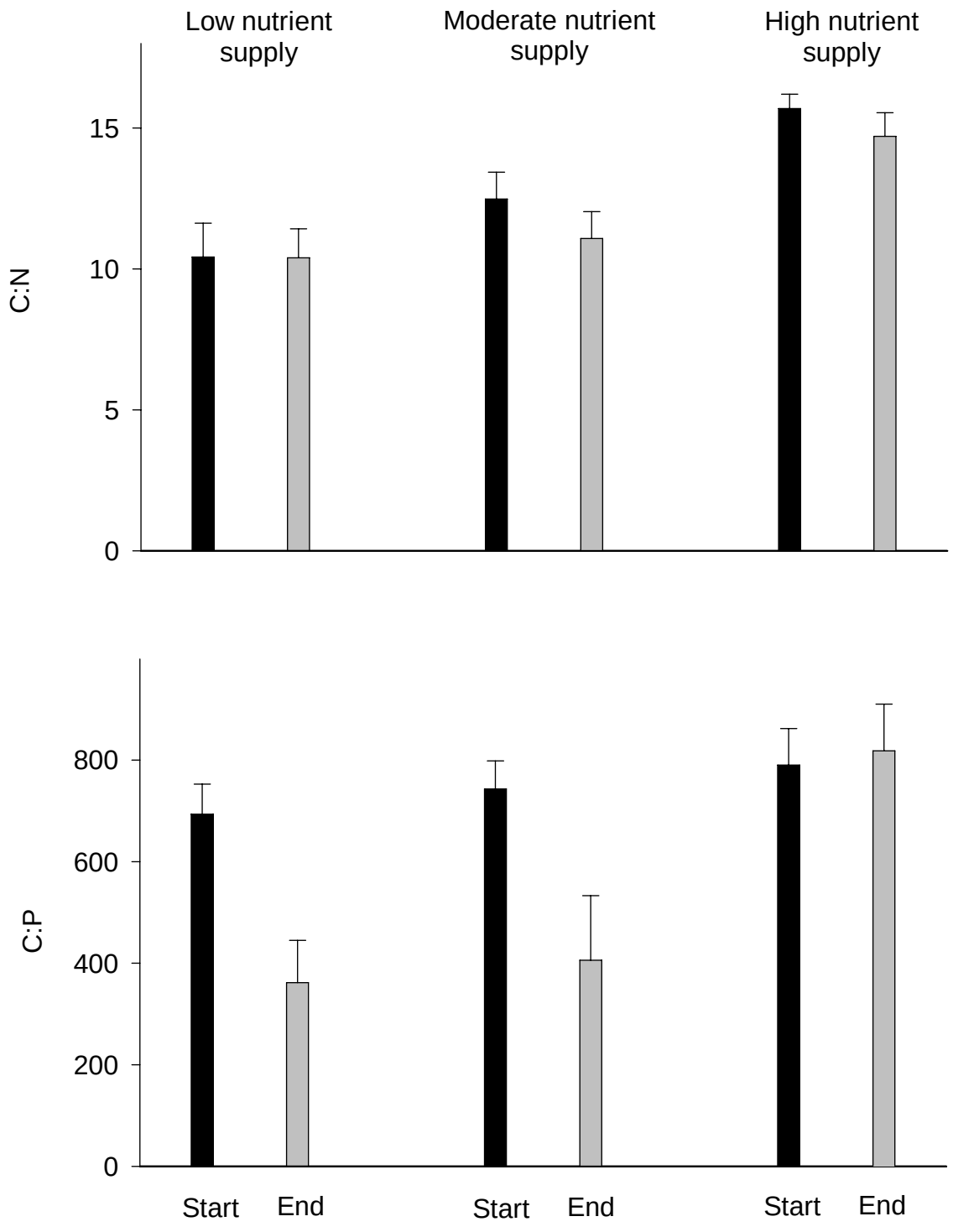

Fig. 5 Katechakis and Stibor – Seston nutrient ratios

CURRICULUM VITAE

Personal data

Date of birth: September 27, 1971 in Bamberg, Germany
Nationalities: German and Greek

School education

1990 Matriculation standard: Abitur

Military service

1990 – 1991 Military service in Regensburg, Germany, and Chania, Crete, Greece (NATO air base)

University education

1991 – 1992 Major/Course of Study: European Economy at Otto-Friedrich-University, Bamberg

1992 – 1994 Major/Course of Study: Biology (basic level) at Georg-August-University, Göttingen

1994 – 1998 Major/Course of Study: Biological Oceanography, Physical Oceanography, Zoology and Microbiology (advanced level) at Christian-Albrechts-University of Kiel

1999 Scientific Degree: Diplom-Biologist (equivalent to Master of Science Degree in Biology)

Professional experience

1989 – 1991 Sales agent at Goebel Publishing House, Bamberg

1992 Internship at Lindner GmbH, Bamberg (controlling and marketing)

1994 – 1998 Scientific collaborator at the following institutions:

- Department of Experimental Ecology (Leibniz Institute for Marine Sciences, IfM)
- Department of Marine Microbiology (IfM)
- Institute for Polar Ecology (IPOE)
- Research Center for Marine Geosciences (GEOMAR)

1999 Sales agent at Mobilcom AG, Kiel

1999 – 2000 Research scientist at Leibniz Institute for Marine Sciences (IfM), Kiel

2000 – 2003 Research scientist at Department of Aquatic Ecology, Ludwig-Maximilian-University (LMU), Munich

2003 – 2004 Consultant at ECOSSA (ECOlogical Sediment & Soil Assessment), Munich

Since 2004 Consultant at KETCHUM Munich for Biogen Idec, GlaxoSmithKline, Novartis, Altana, Organon, Procter & Gamble, Nycomed, and Roche

PUBLICATION LIST / GRANTS

Publications in peer-reviewed journals

1. **Katechakis A**, Stibor H, Sommer U, 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

2. Sommer U, Stibor H, **Katechakis A**, Sommer F, Hansen T (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. ***Hydrobiologia*** 484:11–20

3. **Katechakis A**, Stibor H (2004) Feeding selectivities of the marine cladocerans *Penilia avirostris*, Podon intermedius and *Evadne nordmanni*. ***Marine Biology*** 145:529-539

4. **Katechakis A**, Stibor H, Sommer U, Hansen T (2004) Feeding selectivities and food niche separation of *Acartia clausi*, *Penilia avirostris* (Crustacea) and *Doliolum denticulatum* (Thaliacea) in Blanes Bay (Catalan Sea, NW Mediterranean). ***Journal of Plankton Research*** 26:589–603

5. Stibor H, Vadstein O, Diehl S, Gelzleichter A, Hansen T, Hantzsche F, **Katechakis A**, Lippert B, Løseth K, Peters C, Roederer W, Sandow M, Sundt-Hansen L, Olsen Y (2004) Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. ***Ecology Letters*** 7:321–328

6. **Katechakis A**, Haseneder T, Kling R, Stibor H (2005) Mixotrophic vs. obligately autotrophic algae as food for zooplankton – the light:nutrient hypothesis may not hold for mixotrophs. ***Limnology and Oceanography*** 50:1290-1299

7. **Katechakis A**, Stibor H (2005a) The mixotroph *Ochromonas tuberculata* may invade and suppress specialist phago- and phototroph plankton communities depending on nutrient conditions. ***Oecologia*** (submitted)

Publications in preparation

8. **Katechakis A**, Stibor H (2005b) Effects of productivity and omnivory on the stability of an experimental limnic planktonic food web. (in preparation for submission to ***Ecology Letters***)

9. **Katechakis A**, Stibor H (2005c) Effects of productivity, omnivory and mixotrophy on an experimental limnic planktonic food web. (in preparation for submission to ***Limnology and Oceanography***)

Grants

Articles 1. – 4. were supported by MAST III-project COMWEB funded by the EU.

Article 3. was additionally supported by the "Improving Human Potential – Transnational Access to Research Infrastructures Programme of the European Commission" and the "Trondheim Marine Systems Research Infrastructure".

Articles 6. – 9. were supported by the "Deutsche Forschungsgemeinschaft" (German Research Foundation, DFG).

Other publications, talks, and poster presentations

- **Katechakis A** (1999) Nischenüberlappung zwischen herbivorem gelatinösen- und Crustaceen-Zooplankton im NW Mittelmeer (Catalanisches Meer). ***Diploma Thesis*, *Christian-Albrecht University Kiel, Germany***

- **Katechakis A** (2000) Bottom-up and top-down effects of mesozooplankton on phytoplankton and the microbial food web. ***Institute of Aquatic Ecology, LMU Munich, Germany, oral presentation***

- **Katechakis A**, Stibor H (2001) Auswirkungen von Produktivität und Omnivorie auf die Länge limnischer planktischer Nahrungsnetze. ***German Society of Limnology (DGL), Meeting 2001, Kiel, Germany, oral presentation***

- **Katechakis A**, Stibor H (2002) Effects of productivity and omnivory on the length of limnic planktonic food chains. ***American Society for Limnology and Oceanography (ASLO) summer meeting 2002, Victoria, Canada, oral presentation***

- **Katechakis A** (2003) Food web dynamics in the marine and limnic pelagial. ***Institut de Ciències del Mar (CMIMA), Barcelona, Spain, oral presentation***

- **Katechakis A**, Haseneder T, Kling R, Stibor H (2004) Mixotrophs are different – the light:nutrient hypothesis may not hold for mixotrophs. ***Institute of Aquatic Ecology, LMU Munich, Germany, oral presentation***

- **Katechakis A** (2005) Why is there no fish in the Mediterranean? – Ecological, economical and political constraints (***oral presentation*** in preparation)

ACKNOWLEDGEMENTS

Many thanks to PD Dr. Herwig Stibor who made my research stays in Norway and Spain possible. Dr. Stibor provided many of the underlaying ideas for my work. Together we wrote and submitted the DFG-proposal, on which a part of the presented experiments are based.

Many thanks to Prof. Dr. Sebastian Diehl who let me work in his lab and generously granted additional funds enabling me to continue with my experiments for another six months after they did not run as smoothly as initially planned.

I am also grateful to Prof. Dr. Ulrich Sommer, Prof. Dr. Carlos Duarte, and Prof. Dr. Yngvar Olsen, each of whom provided laboratory space for my experiments at their respective research institutions.

Many friends and colleagues helped me with this study. Thomas Hansen performed most of the nutrient analyses and was a trusted friend in Norway and in Spain. Mechthild Kredler, Torsten Stadthagen, Tanja Haseneder, Robert Kling, Angelika Wild, and Achim Weigert enthusiastically provided continuos support with the preparation and sampling of my experiments in Munich. Margit Feissel successfully managed the administrative work. I would like to thank all of them for their time, expertise and kind support.

Special thanks to my family for their encouragement during the last years. And finally, endless thanks to my companion Anna Benet Pagès for her steadfast support and understanding. This work is dedicated to her.

DECLARATION

I certify that the thesis entitled

"Selected interactions between phytoplankton, zooplankton and the microbial food web: Microcosm experiments in marine and limnic habitats",

submitted for the degree of **"Dr. rer. nat."**,

is the result of my own independent work, that all presented material was written by myself, and that I did not use any other sources of information than those indicated by the given references.

ERKLÄRUNG / EHRENWÖRTLICHE VERSICHERUNG

Hiermit erkläre ich, dass die vorliegende Dissertation das Ergebnis meiner eigenständigen Arbeit ist und dass ich dieses Manuskript persönlich verfasst habe. Zudem habe ich keine anderen als die angegebenen Quellen und Hilfsmittel verwendet.

Ich versichere, dass ich mich anderweitig einer Doktorprüfung ohne Erfolg nicht unterzogen habe.

Starnberg, 20.11.2005

Alexis Katechakis