Mesozooplankton impacts on lower trophic levels from freshwater, marine and brackish systems in spring – a comparative study

Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität
zu Kiel

vorgelegt von
Heidrun Feuchtmayr

Kiel, Mai 2004

Referent: Prof. Dr. W. Lampert
Korreferent: .....

Tag der mündlichen Prüfung: 09.07.04

Zum Druck genehmigt: Kiel, ...

.....

Der Dekan

Contents

Introduction

Introduction

Mesozooplankton (0.2 to 2 mm) exist in almost all aquatic environments ranging from freshwater and brackish water to marine systems. The major components of the mesozooplankton community in lakes as well as in the sea are copepods and cladocerans. These two zooplankton guilds are important components in the aquatic food web since they link lower autotrophic organisms (e. g. phytoplankton) to higher animals such as planktivorous fish. However, there are important differences between cladocerans and copepods, especially regarding their impact on the lower trophic levels, either directly via feeding or indirectly by nutrient availability. Both guilds are well studied individually, particularly with regard to their life cycle (Lampert & Sommer, 1997; Mauchline, 1998), morphology (e.g. Brendelberger, 1991; Einsle, 1993), migratory behaviour (Ringelberg *et al.*, 1991; Maier, 1993), N:P stoichiometry (Sterner, 1990; Hessen & Lyche, 1991) and grazing behaviour (e.g. DeMott, 1995). However, comparisons between the two guilds are rare and thus will be focussed on in this thesis.

Life cycles

Cladocerans (Picture 0-1) have a faster metabolism and faster life cycle compared to copepods, due to mainly parthenogenetic reproduction. Further, fast development of the eggs in a brood chamber and no development of a larval stage contribute to the fast life cycle of cladocerans. These characteristics are particularly advantageous in temperate lakes during spring, when freshwater cladocerans are able to build up large populations in a short period of time as response to a phytoplankton spring bloom, which will result in a clear water phase (Lampert, Winfried, 1978; Sommer *et al.*, 1986; Lampert, 1988). In comparison, copepods reproduce sexually and have a slower life cycle with 12 life stages (6 naupliar, 5 copepodite, and a reproductive adult stage). After fertilization, calanoid freshwater copepods (Picture 0-1) typically carry their eggs in egg sacks attached to the body until hatching, while most marine species are free-spawners. Some copepod species are able to avoid food competition, or bridge seasonal food shortage, by diapausing in the sediment (Santer & Lampert, 1995). This strategy allows them to synchronize growth with periods of abundant food.

Freshwater cladocera:
Daphnia hyalina x galeata

Freshwater calanoid copepod:
Eudiaptomus graciloides

Picture 0-1: Examples of a freshwater cladoceran and a copepod species: *Daphnia hyaline x galeata* and *Eudiaptomus graciloides*, respectively.

Freshwater vs. marine zooplankton

While cladocerans typically are more abundant in freshwater, copepods dominate in the sea. Thus, in this study, I aimed at comparing fresh-, salt- and brackish water copepods and cladocerans. Most knowledge on cladocerans derives from freshwater studies, since cladocerans can build up huge populations in lakes. Further, over 600 freshwater cladoceran species are known, outnumbering marine cladocerans, where 8 species are recorded. Restricted knowledge on marine cladocerans is mainly due to their fragility and the associated problems to set up laboratory cultures. Due to the dominance of copepods in the sea, knowledge mainly derives from marine species. Copepods are the most abundant metazoan taxa on the planet, existing even in extreme environments such as under the frozen surface of Antarctic lakes or in ~6,000 m height in volcano lakes (Dussart & Defaye, 2001). Copepods are the most diversified class of crustaceans, over 14,000 species are known.

Grazing behaviour

Cladocerans are known to induce strong reductions of phytoplankton biomass in freshwater (Lampert, Winfried, 1978; Sommer *et al.*, 1986; Lampert, 1988), while very little information on the impact of marine cladocerans on lower trophic levels is available. In contrast, knowledge of the impact of freshwater copepods is very limited, while copepods can considerably reduce phytoplankton in the sea (Bautista *et al.*, 1992). Copepods and cladocerans differ in their feeding behaviour. *Daphnia*, the most common genus and best studied freshwater cladoceran, is a very efficient filter feeder, typically regarded as unselective, unable to discriminate between similar sized food particles. However, their filtering apparatus determines the maximum size of ingestible particles, which is generally reported at ~30 µm in diameter, whilst smallest ingested particles are reported at ~1 µm

(Gliwicz, 1977; Gliwicz & Siedlar, 1980; Geller & Müller, 1981; Gophen & Geller, 1984; Brendelberger, 1985). Cladocerans are known to be able to detect food patches and are able to reject already captured food particles clogging their filtering apparatus (Cuddington & McCauley, 1994; Jensen, Larsson & Hogstedt, 2001). In contrast, cyclopoid and calanoid copepods actively select and catch their food particles (Koehl, 1984; DeMott, 1986) although calanoid copepods have long been thought to feed unselectively. Copepods create a feeding current out of which they select certain particles before ingestion (Vanderploeg, 1981; Paffenhöfer, 1982; Cowles & Strickler, 1983). The particle selection is believed to be judged upon palatability by chemoreceptors (Poulet & Marsot, 1978). This selectivity allows copepods to be able to discriminate between phytoplankton and non-edible particles (e.g. plastic beads) (e.g. Donaghay & Small, 1979; Fernandez, 1979; DeMott, 1988a), phytoplankton and detritus (e.g. Roman, 1977; DeMott, 1988b), phytoplankton of different food quality (e.g. Cowles, Olson & Chisholm, 1988), and toxic versus non-toxic species or strains (e.g. Huntley, Barthel & Star, 1983; Turriff, Runge & Cembella, 1995). Alike cladocerans, copepods are able to reject already captured particles, supposed to depend on chemical characteristics (Ong, 1969; Friedman & Strickler, 1975; Frost, 1977). Copepods are known to prefer larger particles over small ones, but an overlap between copepods and cladocerans in the food size spectra is known for cells ~10-30 µm in diameter (Geller & Müller, 1981; Kleppel, 1993; Adrian & Schneider-Olt, 1999). These different grazing behaviors of cladocerans and copepods can have important and contrasting impacts on the composition of the plankton community and will be compared in this thesis.

Effects on nutrients

Further, copepods and cladocerans induce changes on elemental concentrations by preferential excretion since they differ in their nutrient stoichiometry. For metabolism and growth, zooplankton need certain resources and essential nutrients. Some of these nutrients are carbon (C), nitrogen (N) and phosphorus (P), the elements most focused on for stoichiometric analyses of aquatic systems and determination of elemental limitation. Numerous investigations studied ecological stoichiometry in phytoplankton as well as in zooplankton, mainly regarding nutrient limitations (e.g. Sterner & Hessen, 1994; Gismervik, 1997). According to Redfield (1958), a ratio of C:N:P of 106:16:1 is believed to allow for phytoplankton growth not limited in nitrogen or phosphorus (Sterner & Elser, 2002).

In marine systems which are mainly dominated by copepods, nitrogen is often reported to be the primary limiting nutrient for phytoplankton (Vitousek & Howarth, 1991). Copepods are relatively rich in nitrogen (N) compared to other nutrients (Elser & Hassett, 1994; Gismervik, 1997), and are able to maintain their mineral composition, e.g. C:N ratio, even if the stoichiometry of their food changes, an effect known as homeostasis (Sterner, 1990). As

a result, copepods can alter the relative availability of N for phytoplankton by defecation of nitrogen depleted material (Elser & Foster, 1998).

On the contrary, freshwater cladocerans are reported to be relatively rich in phosphorus, *Daphnia* retain phosphorus in their biomass while they preferentially recycle nitrogen (Andersen & Hessen, 1991; Hessen & Lyche, 1991). Thus, *Daphnia* excrete a higher N:P ratio compared to copepods (Touratier, Field & Moloney, 2001), which has important implications for phytoplankton. In order to fulfil their stoichiometric demands, *Daphnia* can even shift phytoplankton growth towards P limitation (Rothhaupt, 1997). As lakes are often reported to be limited in phosphorus in spring and summer, *Daphnia* frequently face food P deficiency, because they require relatively high amounts of phosphorus compared to phytoplankton. As a consequence of P limited food, growth of *Daphnia* is reported to be restricted (Elser & Hassett, 1994), as *Daphnia* need phosphorus not only for skeletal tissue, ATP and phospholipids, but also for RNA and DNA production. Elser *et al.* (2000) stated that *Daphnia magna* face phosphorus deficiency when the C:P ratio of their food exceeds about 300, because this leads to a reduced gross growth efficiency. Thus, copepods and cladocerans can have an impact on nutrient stoichiometry of sediment particles (often consisting mainly of faecal pellets), the dissolved fraction and food particles in contrasting ways. By nutrient cycling and feedback mechanisms, copepods and cladocerans worsen the nutritional situation for their own body stoichiometry.

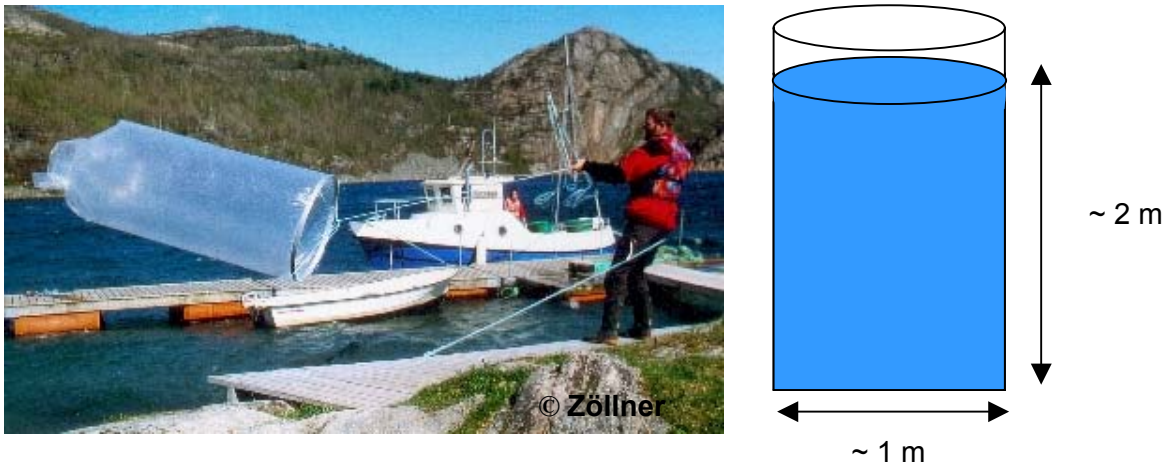
Stable Isotopes

Furthermore, I aimed to investigate feedback mechanisms of lower trophic levels on zooplankton as well as interactions between the different zooplankton guilds and species. It is difficult to assess feedback impacts on zooplankton using conventional techniques and therefore I used stable isotope ratios. Analysis of stable isotope ratios (SIA) in basal resource and consumer tissues has proved a powerful ecological tool to assess food web structure, trophic interactions and pathways of energy flow in a wide variety of ecosystems (reviewed by Peterson & Fry, 1987) and particularly in aquatic environments (e.g. Fry, 1988; Grey, Jones & Sleep, 2001; Jones & Waldron, 2003). I assume that any differences in isotopic signature at the end of the experiment, not only between zooplankton guilds or species, but also between density treatments of the same guild, might reveal changes in trophic behaviour otherwise overlooked. I expected different and density dependent impacts of cladocerans and copepods on the lower food web, differences in their utilised carbon source, as well as in their trophic level.

Outline of this project

All described differences between cladocerans and copepods were expected to impact lower trophic levels, mainly the phytoplankton community, either directly via feeding or indirectly by

nutrient availability. In order to study the different impacts, we carried out mesocosm experiments by exposing enclosure bags *in situ* (Picture 0-2). Food web studies are a classic focus of aquatic mesocosm experiments, to examine 'top-down' or 'bottom-up' controls such as the impacts of fish or nutrients (e.g. Spencer & Ellis, 1998; McKee *et al.*, 2003). Investigations of impacts on lower trophic levels such as zooplankton-phytoplankton interactions, are typically conducted in smaller scale laboratory experiments, and often with individual species (e.g. Durbin & Durbin, 1992; Hansen *et al.*, 1993). An exception are large indoor mesocosm experiments in high tanks or columns (e.g. the plankton towers in Plön (Lampert & Loose, 1992)). Despite the problems associated with extrapolating data derived from mesocosm studies to a natural situation, they provide an intermediate scale between laboratory and natural environmental conditions, and are a widely appreciated tool in ecological studies. Most *in situ* enclosure experiments have been conducted in lakes, as lakes provide advantageous conditions. Compared to the sea, lakes are often less exposed to wind, have less waves and a lower depth (regarding anchorage of racks). *In situ* enclosure studies may be particularly useful to investigate impacts of zooplankton, in particular the differing impacts of cladocerans and copepods, on lower trophic levels within a food web. However, this has rarely been performed yet. In order to allow quantitative investigations, we created differently scaled densities of the two zooplankton guilds.



Picture 0-2: An empty mesocosm bag before the start of the experiment and a simplified scheme of a bag and its approximate size.

Within a large scale mesocosm project, we aimed at investigating the impact of mesozooplankton on lower trophic levels and (bio-) chemical characteristics in freshwater, saltwater and brackish water. However, using bulk zooplankton samples for the experiments would result in a comparison of mainly cladoceran impacts in freshwater and copepod impacts in salt- and brackish water, as cladocerans dominate in lakes and copepods in the sea. This procedure would miss out the impacts of the less abundant mesozooplankton guilds. In order to study and compare differences and impacts of copepods and cladocerans

in marine and freshwater habitats, we intended to investigate both zooplankton guilds in all different experimental sites. Furthermore, the project aimed at comparing two seasons, spring and summer, the main growing seasons of primary production. Along with growth of phytoplankton, mesozooplankton is abundant and likely to exhibit main impacts on lower trophic levels. In spring, we tried to synchronise our mesocosm experiments with the phytoplankton bloom, and follow the successional development of a different phytoplankton community in summer. While in this study I mainly focus on impacts on phytoplankton and nutrient dynamics (C, N and P), the microbial food web was studied by E. Zöllner (Zöllner, 2004) and food quality was monitored by C. Becker and D. Brepohl (Becker, 2004). In this thesis I studied the influences of copepods and cladocerans on lower trophic levels and nutrients, and interactions of species by stable isotope signatures in spring experiments. Results of summer mesocosm experiments can be found in Sommer (2003).

The enclosure experiments were designed to compare cladocerans with copepods and to compare the impact of these two guilds between three different environments during springtime: freshwater (Schöhsee in 2001), saltwater (Hopavågen in 2002) and brackish water (Kiel Bight in 2003). The project aimed to answer following questions:

1. How is the phytoplankton community, its diversity, size and species composition affected by different grazing intensities of filtering and particle catching zooplankton?
2. Can cladocerans cause a spring clear-water phase independent of their initial density while copepods are only able to induce it when present in high abundances?
3. Does the different nutrient content of N-rich copepods and P-rich cladocerans affect the nutrient limitation of phytoplankton?
4. Do different zooplankton guilds and species differ in their stable isotope signature, is there a zooplankton density-dependent change, and are there interactions of zooplankton which can be revealed by SIA?
5. How do zooplankton impacts on lower trophic levels differ between a freshwater, brackish water and saltwater system?

In order to examine the impacts of cladocerans and copepods on lower trophic levels within each experimental site, I will focus on the freshwater (chapter 1), saltwater (chapter 2) and brackish water (chapter 3) experiment separately. In Kiel Bight, additional impacts of copepods and cladocerans on ciliates, picophytoplankton and bacteria within the enclosure bags were investigated by flow cytometer analyses. In chapter 4, I will compare the three experiments between each other and include comparisons with the summer enclosure experiments investigated by Sommer (2003), since a similar experimental set up was used.

Freshwater enclosures

Chapter 1

Enclosure experiments

To allow comparability between the three enclosure sites in fresh- (Schöhsee, chapter 1), salt- (Hopavågen fjord, chapter 2) and brackish water (Kiel bight, chapter 3), enclosure bag material, size and design was standardised. Additionally, experimental procedures such as handling of zooplankton, sampling, and the parameters measured were identical. For an overview of the general experimental time course and measured parameters, see Figure 1-1.

1. Freshwater enclosures

1.1. Study site and methods

Site and experimental design

Enclosure bags were exposed in Schöhsee, northern Germany, a small (82 ha) meso- to eutrophic lake with a maximum depth of 30 m (see Figure 1-2). Typically, a spring phytoplankton bloom in Schöhsee occurs in April followed by exponential growth of cladocerans, predominantly *Daphnia hyalina x galeata*, resulting in a clear water phase at the end of May (Rai, 1982). The experiment ran from 3rd to 28th of May 2001. Twenty four polyethylene bags serving as *in situ* enclosures, each being closed at the lower end (~2 m deep), were filled with 1700 litres of 55 µm filtered epilimnetic lake water (see Picture 1-1).

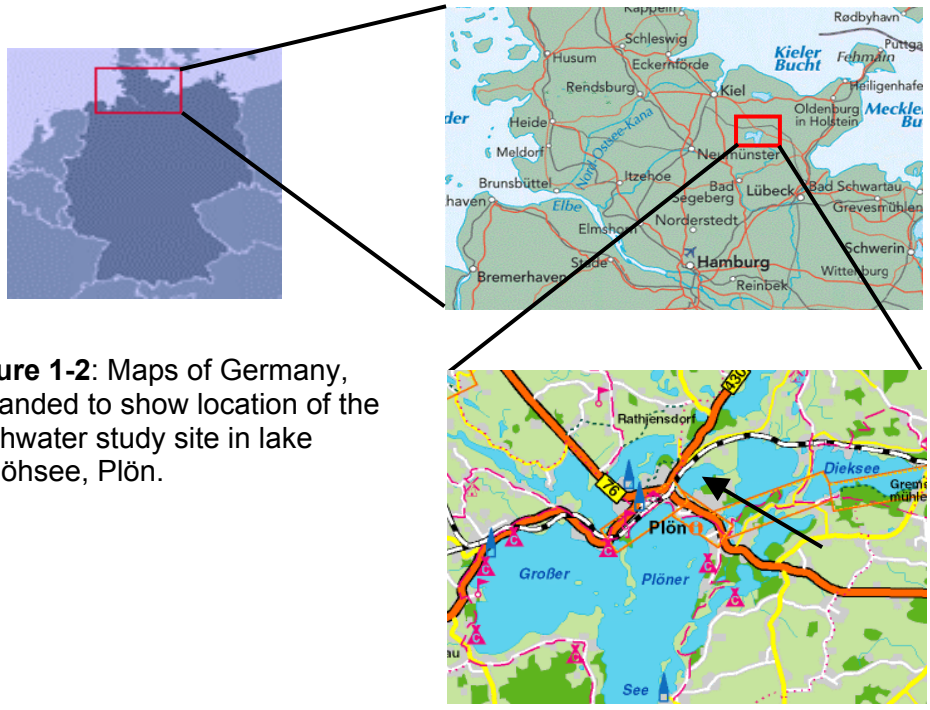
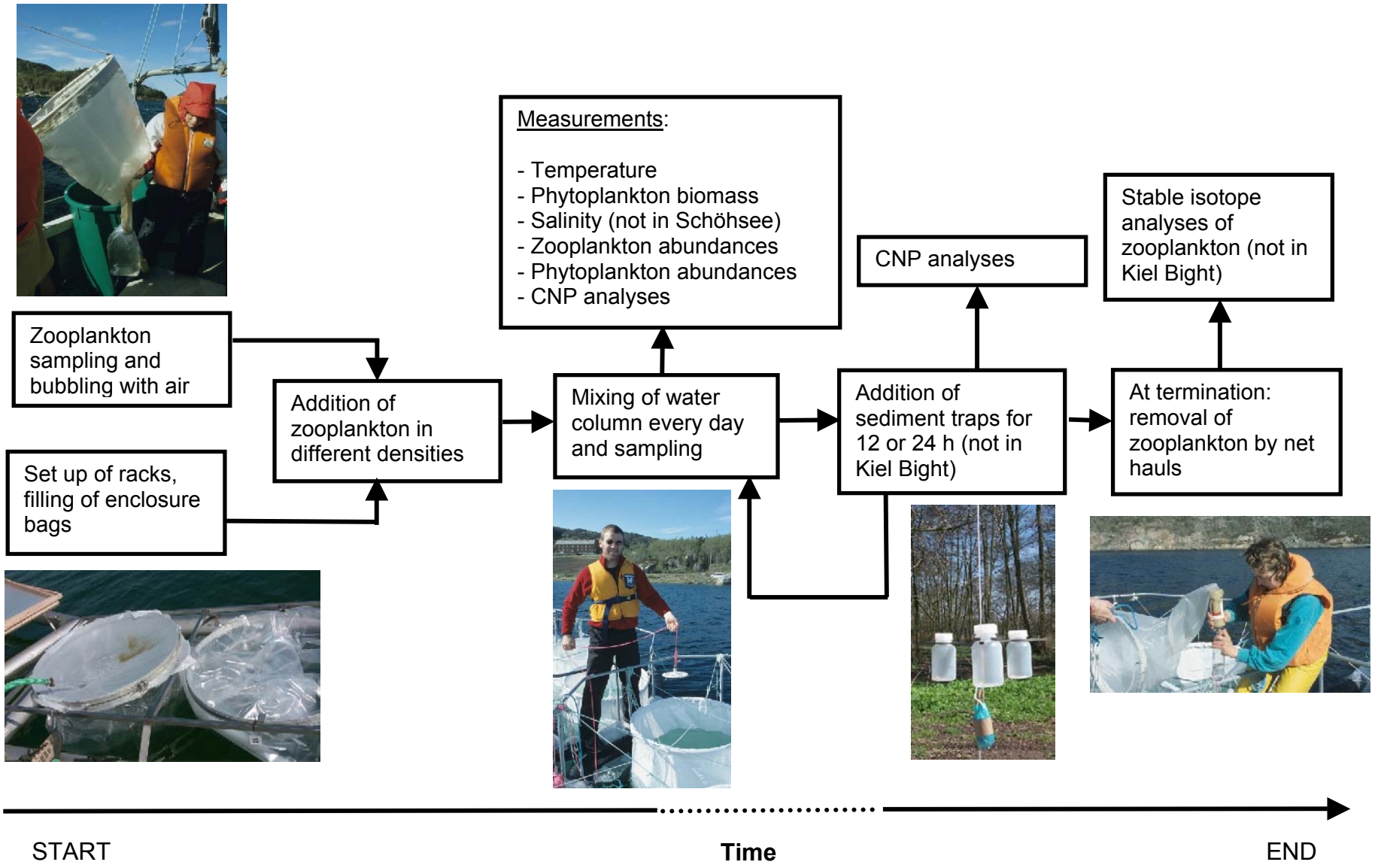


Figure 1-2: Maps of Germany, expanded to show location of the freshwater study site in lake Schöhsee, Plön.

Figure 1-1: Course of action during enclosure experiments, and parameters measured.



Picture 1-1: Filling of enclosure bags by screening Schöhsee water through a 55µm gauze in order to remove zooplankton. Covers of enclosures are shown mid picture.

The seston community consisted of algae, protozoa, bacteria and some rotifer species, which were able to pass through the gauze. The mesocosms were held in position by a floating framework in a wind-sheltered bay and covered with transparent plastic sheets to avoid fertilization from bird defecation. For zooplankton addition, *Daphnia hyalina x galeata* was collected from Schöhsee and cultured in 250 litre aquaria to produce sufficient quantities for experimental inoculations. *Eudiaptomus gracilis* (Sars) and *E. graciloides* (Lilljeborg) from Schöhsee were also cultured in 200 litre aquaria but reproduction was slow and so inoculations were supplemented by cyclopoid and calanoid copepods collected directly from Schöhsee. Zooplankton was netted (250 µm mesh), placed in 250 litre containers, and bubbled with air for 6 h to remove cladocerans by surface entrapment prior to enclosure inoculation. This procedure produced a copepod community consisting of calanoid and cyclopoid species (*Eudiaptomus spp., Cyclops spp., Mesocyclops leuckarti* (Claus)*, Diacyclops bicuspidatus* (Claus) and *Thermocyclops oithonoides* (Sars), but not all *Daphnia* could be removed. Densities of copepods concentrated in containers were determined and adequate volumes added to the enclosures to obtain five different logarithmically scaled densities: 5, 10, 20, 40 and 80 copepods per litre; and similarly, *Daphnia* were added to achieve 1.25, 2.5, 5, 10 and 20 *Daphnia* per litre (hereafter referred to as copepod or *Daphnia* bags) in randomly selected bags. Copepod abundances were made 4x higher than those of cladocerans to achieve a similar biomass according to mean individual dry mass reported in the literature: *Daphnia hyalina* 17 µg (Santer, 1990); and copepods 4 µg calculated from mean length of *Eudiaptomus* and a length-weight regression (Bottrell *et al.*, 1976; Kiefer, 1978). Highest treatment densities correspond to natural abundances found in Schöhsee at the beginning of May (Fußmann, 1996). Zooplankton treatments were replicated twice, mixed zooplankton of 20 copepods and 5 *Daphnia* per litre was added to two further enclosures, and the remaining two bags were left without any addition to serve as controls. Within the experimental period of 25 days, the natural plankton assemblage served

as the only food resource in order to investigate the different impacts of cladocerans and copepods on the community. For an overview of treatments, zooplankton and environmental conditions, see Table 1-3.

Table 1-3: General overview of the experiment in Schöhsee.

Schöhsee	
Date and (duration) of experiment	4th – 28th May 2001 (25 days)
Treatments (no. of bags) and zooplankton abundances per litre, 2 replicates each	Copepods (10): 5, 10, 20, 40, 80 Cladocerans (10): 1.25, 2.5, 5, 10, 20 Control (2): 0 Mixed bags (2): 20 copepods, 5 cladocerans
Species	Copepods : *Eudiaptomus gracilis* *Eudiaptomus graciloides* *Mesocyclops leuckarti* *Cyclops abyssorum* *Thermocyclops oithonoides* *Diacyclops bicuspidatus* Cladoceran: *Daphnia hyalina x galeata*
Ambient temperature	ø 16.1°C
Water temperature	Increased form 12°C to 18°C

An oxygen probe (WTW, Germany) was used to record oxygen concentrations in each bag every second day upon arrival. The mesocosms were then mixed by lowering and raising a Secchi disk 10 times to create an equal distribution of plankton within the bags prior to sampling of 10 litres. From this volume, sub-samples for phytoplankton and nutrient analyses were collected. Chlorophyll *a* and temperature were measured and zooplankton sampled every few days according to Figure 1-3.

The analyses of phytoplankton samples and nutrient stoichiometry were constrained to the first 14 days because biofilms were observed growing on enclosure bag surfaces and phytoplankton species, that were initially undetected, increased in biomass and hence availability for zooplankton. These effects justify a relatively short experimental time and are presumed to be the reason for the duration of around 11 to 16 days of most enclosure experiments (e.g. Perez-Martinez & Cruz-Pizarro, 1995; Gismervik, Olsen & Vadstein, 2002). The longer duration of my enclosure experiment was to ensure that zooplankton exhibited stable isotope signatures reflecting dietary conditions in the enclosures.

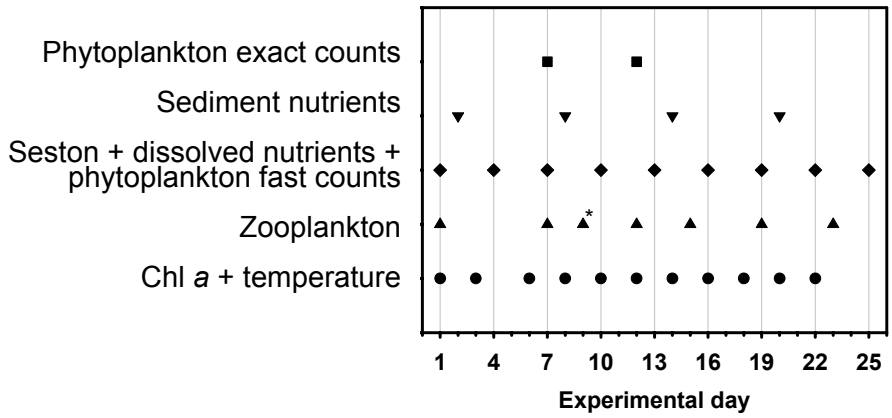


Figure 1-3: Time schedule of sampling, measurements and analyses. On day 12, only cladoceran bags were sampled for zooplankton, indicated with *.

Zooplankton

Samples for calculating copepod and *Daphnia* abundances were collected every third to sixth day (see Figure 1-3) to monitor changes of zooplankton in the enclosures. Two vertical net hauls (55 µm mesh with an aperture of 9 cm) through the water column of the bag resulted in a sampled volume of 13 litres each. Animals from one net haul were killed with hot water or ethanol (10% final concentration) and prepared for counting, while the content of the other net haul was fixed with formaldehyde (4% final concentration) for backup storage. Complete samples were counted or, when densities were too high, sub-samples were taken with a Hensen pipette and up to 100 individuals per taxa were counted. Population density was calculated as individuals per litre in each bag.

Phytoplankton

Every second day, chlorophyll *a* and temperature were measured using an *in situ* fluorescence photometer (Fluoroprobe from BBE Moldaenke, Germany) (Beutler *et al.*, 2002) to determine changes in phytoplankton biomass. In order to compare the impact of the two zooplankton taxa on chlorophyll *a* (chl *a*) over time, logarithmic regressions of the equation $y=y_0+a\ln x$ were calculated using chl *a* values versus nominal zooplankton density gradient for each chl *a* measurement day. Logarithmic regressions were chosen due to logarithmically scaled zooplankton densities.

For phytoplankton counts, 100 ml sub-samples were immediately fixed with Lugol`s solution (1% final concentration). Small sub-samples from every 3rd day were counted by low precision microscopy in order to monitor changes within the phytoplankton composition and abundance and determine dates for exact counts. Detailed phytoplankton counts were performed for day 7 (for comparison to the saltwater experiment, lasting only 7 days) and 12, before *Daphnia* became abundant within the copepod bags. 25 ml were allowed to settle and

400 cells of one algal species counted wherever possible by Utermöhl's technique (1958) with an inverted microscope (Leica DM IRB), providing confidence limits of 95 ± 10% (Frost, 1972). A value for phytoplankton cells per ml for each bag was calculated from the sample volume counted. Biovolume of each algal species was determined from microscopic measurement of 20 individuals per species and calculated according to their geometry (Hillebrand *et al.*, 1999). As size is the factor most influenced by the different grazing behaviour of copepods and cladocerans (e.g. Sommer *et al.*, 2001), phytoplankton was grouped into three different biovolume classes: <100 µm³; 100-1000 µm³; and >1000 µm³ based on effective particle volume.

In order to analyse species-specific impacts of zooplankton, regression analysis was performed using the model $y=ax^b$ for each algal species counted, similar to that described in Sommer *et al.* (2001). Variable y is the algal abundance counted and x is the zooplankton density we aimed to achieve. The exponent b distinguishes positive and negative impacts of copepods or daphniids dependent upon density (for more details see Sommer et al. (2001; 2003)). To examine relationships between phytoplankton size and zooplankton density-dependent impact, exponent b values were plotted against algal biovolume.

Stoichiometry

Impacts of zooplankton on stoichiometry were analysed in more detail on day 7 and day 13, in concordance with phytoplankton counts and for comparison to the saltwater and brackish water experiments (see chapter 2 and 3).

Dissolved nutrients

Subsamples for dissolved nutrient analyses were taken every third day. Total dissolved nitrogen (TDN), phosphorus (TDP), silicate (TDSi) and dissolved inorganic nutrients (orthophosphate PO_4^{3-}, nitrate NO_3^- and nitrite NO_2^- ammonia NH_4^+, and orthosilicate SiO_4^{4-}) of pre-filtered sub-samples (<100 µm) were measured immediately after sampling in an autoanalyser (Skalar SAN^{plus}, Skalar, Breda, the Netherlands according to Grasshoff *et al.* (1999).

Sestonic nutrients

For determination of nutrient stoichiometry of sestonic carbon, nitrogen and phosphorus, 0.5 to 1.5 litres were pre-filtered every 3rd day (Figure 1-3) through 100 µm gauze to remove zooplankton. Seston was collected onto pre-combusted and, for phosphorus, acid washed, Whatman GF/F glass fibre filters and dried overnight at 60°C. Samples were stored in a dessicator until measurement. Total nitrogen and carbon was measured using a FISONS NA2000 elemental analyser, total phosphorus was determined by alkaline persulphate oxidation (Grasshoff *et al.*, 1999) and molar C:N, N:P and C:P ratios calculated.

Sediment nutrients

After sampling of other parameters, sediment traps consisting of three 250 ml bottles were exposed in all bags at maximum depth for 12 h on experimental days 2, 8, 14 and 20. After careful removal, bottles were immediately frozen at –20°C, thawed the next day, mixed thoroughly and pre-screened through 250 µm gauze to remove large zooplankton. From a mixture of the three sediment trap bottles, 300 ml for C, N and P analyses were filtered onto pre-combusted and, for phosphorus, acid washed, Whatman GF/F glass fibre filters. The filters were examined microscopically and any remaining zooplankton removed, prior to drying overnight at 60°C and analysis according to seston samples. For more detailed investigations, the more conservative and recycled elements of nitrogen and phosphorus were chosen, since carbon can enter and exit the system in form of carbon dioxide.

Zooplankton stoichiometry

Zooplankton C:N ratios (molar) were not measured during the experiment, because to do so would have resulted in alteration of copepod and *Daphnia* abundances. Consequently, zooplankton C, N and P were analysed at the end of the experiment, when animals were removed from the enclosure bags. Samples for phosphorus were analysed using the ammonium-molybdate method and subsequent extinction measurements at 720 nm. The C:N ratio of the zooplankton was determined concurrently with stable isotopes in a Carlo Erba NA1500 elemental analyser as follows.

Stable Isotope Analyses

At the end of the experiment (day 25), most zooplankton from each bag was removed by repeated 55 µm net hauls for stable isotope analysis. The copepods and cladocerans were then stored in separate containers with filtered water for 6 to 10 h for gut evacuation before concentrating the animals alive in small vials, shock freezing in liquid nitrogen and storage in the freezer at –20°C (see appendix Feuchtmayr & Grey, 2003). Thawed samples were sorted manually into *Daphnia h. x g.*, cyclopoid copepods consisting of *Cyclops abyssorum, Mesocyclops leuckarti* and *Diacyclops bicuspidatus* and calanoid copepods composed of *Eudiaptomus gracilis* and *E. graciloides* . Animals were concentrated onto pre-combusted Whatman GF/F filters (550°C, 24 h) and rinsed with distilled water. Filters were oven dried at 60°C for at least 6 h and stored in a desiccator. Zooplankton was removed from the filters and up to 150 individuals pooled for one replicate into a tin cup for subsequent analysis of stable carbon and nitrogen isotopes. Three replicates were analysed when sufficient animals were available. Tin cups were oxidised in a Carlo Erba NA1500 elemental analyser coupled to a Micromass IsoPrime (Micromass, Manchester, UK) continuous flow isotope ratio mass

spectrometer. Isotope ratios are expressed conventionally using the δ notation in per mil (‰) relative to secondary standards of known relation to the international standard of Vienna Pee Dee Belemnite for carbon and atmospheric nitrogen:

$$\delta(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) * 1000$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. A precision of <0.2‰ for both carbon and nitrogen was achieved from repeated measurement of an internal standard inserted between every five samples.

1.2. Results

Abiotic factors and zooplankton

The day-time water temperature at the beginning of the experiment was 12°C, increasing throughout the experimental period to a maximum of 18°C at day 12, with a mean of 14.2°C. Oxygen concentrations varied between 11 and 13 mg per litre for all bags. At the start of the experiment, logarithmically scaled nominal copepod densities were successfully established, varying from 4 to 86 copepods per litre (Figure 1-4, panel A). *Daphnia* densities did not differ markedly between bags at day 1. Estimates ranged from 0.3 to 2.3, with one exception of 17 individuals per litre in one bag with highest *Daphnia* density. After eight days, increases in *Daphnia* density were apparent (Figure 1-4, panel B), resulting in a gradient from 1 to 23. In the copepod bags, abundances declined considerably during the first 10 days (up to 87% animals were lost from day 1 to 12) but remained relatively constant thereafter and a reduced density gradient was maintained for approximately 13 days (Figure 1-4, panel A). All analyses were conducted using both counted and nominal zooplankton stocking densities, but because there was negligible difference in results, nominal stocked densities have been used from here on.

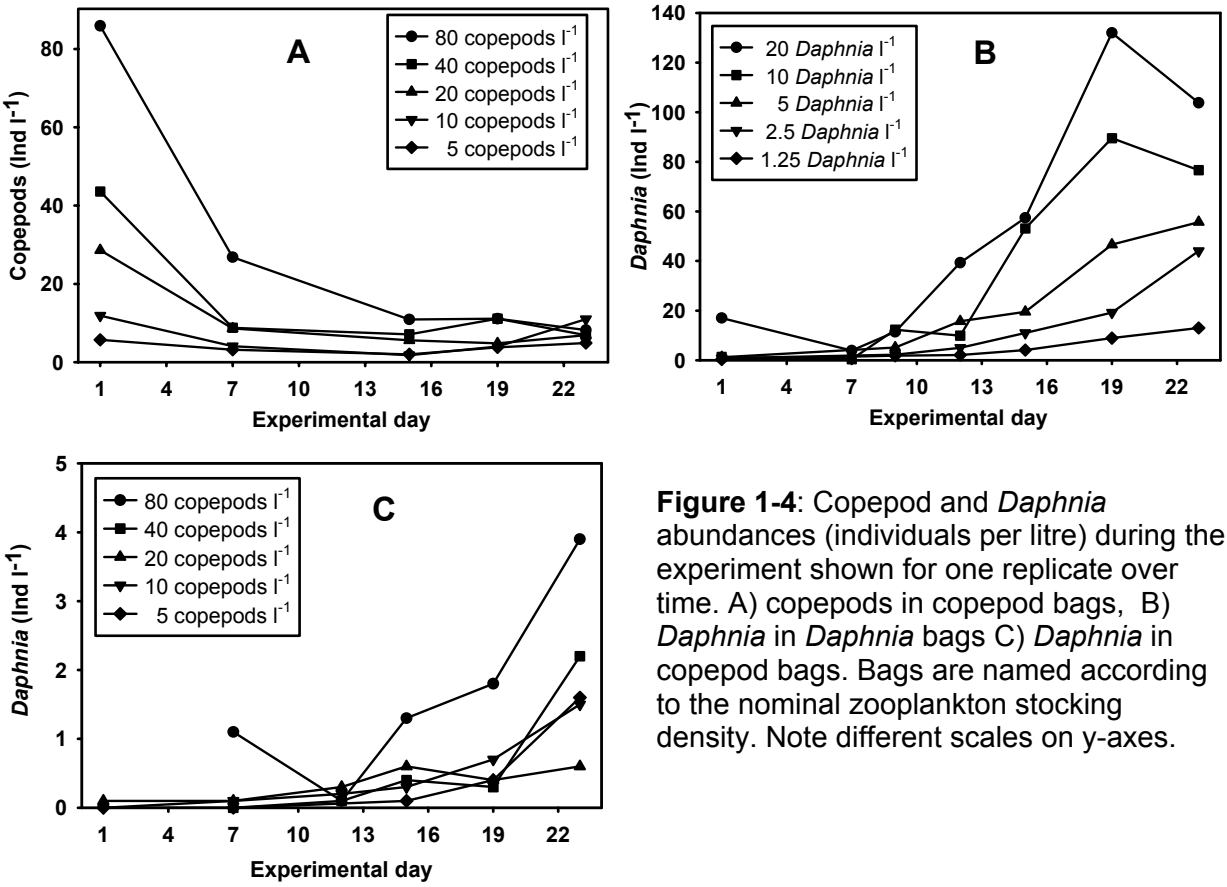


Figure 1-4: Copepod and *Daphnia* abundances (individuals per litre) during the experiment shown for one replicate over time. A) copepods in copepod bags, B) *Daphnia* in *Daphnia* bags C) *Daphnia* in copepod bags. Bags are named according to the nominal zooplankton stocking density. Note different scales on y-axes.

This decline was mainly caused by mortality of the calanoid copepods *Eudiaptomus gracilis* and *E. graciloides*. Initially, cyclopoids comprised around 50% of all copepods, yet they dominated towards the end of the experiment and increased in the highest density bags up to 100% (Figure 1-5). The higher the nominal stocking density, the higher the percentage of cyclopoid copepods, especially after day 15. The relative increase in cyclopoids coincided with an increase in *Daphnia* abundance within the copepod bags (Figure 1-4, panel C), starting at experimental day 12.

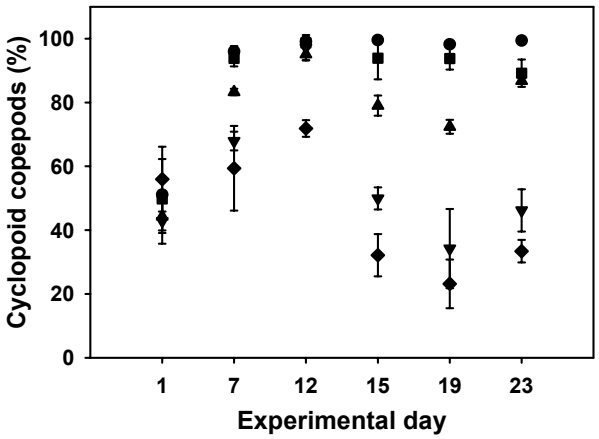


Figure 1-5: Mean percentage contribution (± SD) of cyclopoid copepods to total copepod abundance within the copepod bags shown over experimental time. For legend to symbols see Figure 1-4, panel A.

Phytoplankton

Chlorophyll *a*, an indicator of phytoplankton biomass, decreased over time in all bags. From all different treatments, *Daphnia* grazing caused greatest reductions (5.4 µg l^{-1} to 0.2 µg l^{-1}), whereas in copepod bags, a mean of 2 µg l^{-1} remained on day 22. Interestingly, *Daphnia* decreased algal biomass mostly independent of their own biomass (calculated according to mean individual dry mass of 17 µg reported by Santer (1990)) below 1 µg chl *a* l^{-1}. The higher initial *Daphnia* densities of 5, 10 and 20 individual per litre bags reduced chl *a* to around 0.2 µg l^{-1} (Figure 1-6), while lower stocked densities with 1.25 and 2.5 *Daphnia* per litre resulted in slightly higher algal biomass around 0.8 µg chl *a* l^{-1} at day 22.

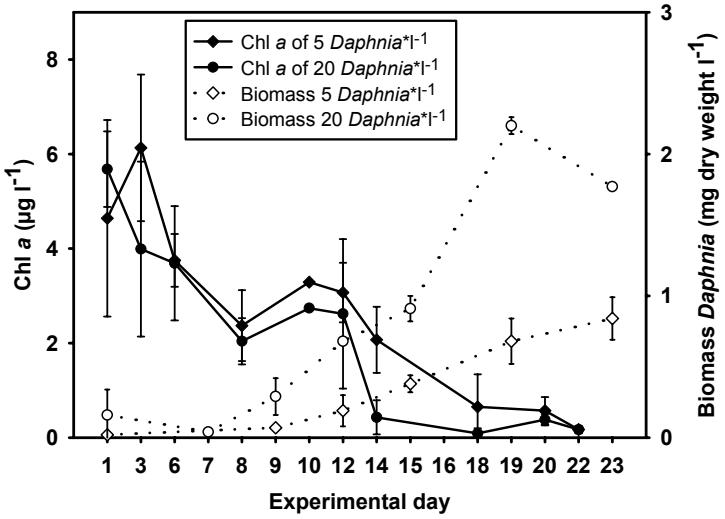


Figure 1-6: Means (± SD) of chlorophyll *a* (µg l^{-1}) and *Daphnia* biomass for two *Daphnia* density bags over the experimental period.

For a detailed investigation of the density-dependent zooplankton impact, slopes of the logarithmic regression lines are shown in Figure 1-7. A distinctly different pattern for copepods compared to cladocerans was found. While *Daphnia* exclusively decreased chl *a* over the experimental period dependent on *Daphnia* density, copepods had a significant, positive impact (R^2=0.6, p=0.05) until day 14. In later measurements, phytoplankton biomass in copepod bags decreased because *Daphnia* became abundant (see above) and thus the subsequent impact cannot be related solely to copepod feeding.

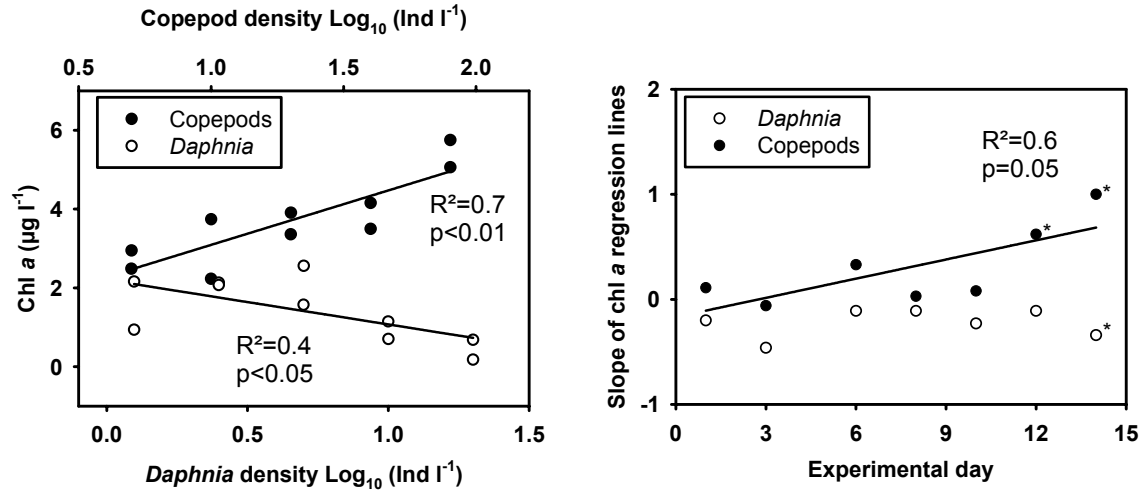


Figure 1-7: Left panel: Chlorophyll a values (µg l^{-1}) of copepod and *Daphnia* bags at day 14. Nominal copepod and *Daphnia* densities were log transformed and significant slopes of regression lines and statistical parameters are shown. Right panel: Slopes of regression lines of total chlorophyll *a* (µg l^{-1}) values plotted for different density gradients of copepods and *Daphnia* over the experimental time. Significant slopes (p<0.05) of regression lines are marked with *. Significant slopes of regression lines and statistical parameters are shown.

Phytoplankton consisted predominantly of diatom species (Table 1-4), typical for the spring bloom in lake Schöhsee (e.g. Fußmann, 1996), and *Dinobryon*, *Cryptomonas* and *Rhodomonas* also occurred. Some dinophyceae, cryptophyceae and chrysophyceae are able, besides photosynthesis, to ingest particulate matter. This mixotrophy was considered negligible for the determination of zooplankton feeding behaviour, and thus potentially mixotrophic species were included in the analyses as phytoplankton (also in chapter 2 and 3). There was one filamentous cyanobacterial species, and *Bitrichia chodatii* (chrysophyceae), which is rarely identified in freshwaters due to the invisibility of its appendages with bright-field microscopy; they are visible only with phase contrast illumination. Greatest algal abundances were found for the smallest size fraction (<100 µm³), comprising *Rhodomonas minuta, Cyclotella sp., Bitrichia chodatii, Stephanodiscus parvus,* an unidentified chlorophyte species, nanoflagellates, *Diatoma elongatum* and *Asterionella formosa*. The latter two diatom species can form colonies >1000 µm³, but in our experiment predominantly occurred as single cells, possibly due to the daily mixing regime.

Table 1-4: Phytoplankton species or taxa, order, calculated biovolume and exponent b values from regression analyses ($y=ax^b$) for *Daphnia* and copepod bags on day 7 and day 12. Significance is denoted by: *** when p<0.001; ** when p<0.01; and * when p<0.05.

Species/Taxa	Order	Bio-volume (µm³)	b value *Daphnia*		b value copepods	
			Day 7	Day 12	Day 7	Day 12
Dinobryon sociale	Dinophyceae	20400 (colony)	-0.21	-0.1	0.19	0.22
Dinobryon divergens	Dinophyceae	13600 (colony)	0.03	0.02	0.2*	0.1
Stephanodiscus alpinus	Bacillariophyceae	6470	-0.04	-0.12	0.08	0.02
Fragilaria crotonensis	Bacillariophyceae	6111 (colony)	0.01	-0.24	0.11	-0.2
Cryptomonas sp.	Cryptophyceae	2024	-0.14	-0.09	-0.05	-0.18
Aphanizomenon flos-aquae	Cyanobacteria	1552	-0.05	-0.33*	0.06	0.07
Diatoma elongatum	Bacillariophyceae	540	-0.21**	-0.33*	-0.05	-0.09
Asterionella formosa	Bacillariophyceae	440	-0.11	-0.33*	0.07	-0.01
Green algae, unidentified	Chlorophyceae	333	-0.23**	-0.18*	0.1	0.54***
Cyclotella sp.	Bacillariophyceae	244	-0.07	-0.25*	0.02	-0.03
Bitrichia chodatii	Crysophyceae	147	-0.23	0.19	0.17	-0.16
Rhodomonas minuta	Cryptophyceae	65	-0.22*	-0.12	0.34**	0.76*
Stephanodiscus parvus	Bacillariophyceae	59	-0.28***	-0.61***	0.01	-0.18***
Nanoflagellates		28	0.02	-0.16	0.1	0.11

Means from two replicate bags illustrate a contrasting impact of copepods and *Daphnia* on the phytoplankton abundance (Figure 1-8). In copepod bags, abundance of small sized phytoplankton (<100 µm³) tended to increase with copepod densities at day 7, consistent with the mainly positive slopes for chl *a* values (see Figure 1-7). In contrast, phytoplankton abundance tended to decrease with increasing *Daphnia* density. Highest copepod stocked bags and lowest *Daphnia* stocked bags do not follow this trend, yet the two replicate bags show highest differences for these densities. ANOVA analysis revealed no differences between the different treatments probably due to the relatively high standard deviations. However, decreasing trends of phytoplankton abundance with *Daphnia* density are concordant with negative chl *a* slopes (see Figure 1-7). Similar impacts of *Daphnia* on the phytoplankton abundances are illustrated for day 12 (Figure 1-8). In addition to day 7, phytoplankton abundances were also determined for control and mixed zooplankton bags at day 12. Since ANOVA revealed treatment means to be statistically different for total algal values ($F_{14,23} = 5.6$, p<0.01), the different density bags were tested against the controls via Dunnett's post-hoc test. There was no pattern evident for the different copepod densities

(Figure 1-8): total phytoplankton abundances in copepod bags showed no significant difference ($p>0.05$) to control bags, along with cells <100 µm³ and >1000 µm³. Algal cells of the size class 100-1000 µm³ appeared to be reduced by copepods, but a significant reduction compared to the control could only be found for the bags stocked with 40 copepods per litre ($F_{14,23} = 5.5$, $p<0.01$). *Daphnia* at the two highest densities induced a significant decline of total phytoplankton abundances ($\alpha < 0.05$), as well as of cells sized 100-1000 µm³ ($\alpha < 0.05$). The impact in mixed species bags was similar to the 5 *Daphnia* and 20 copepods per litre bag treatments.

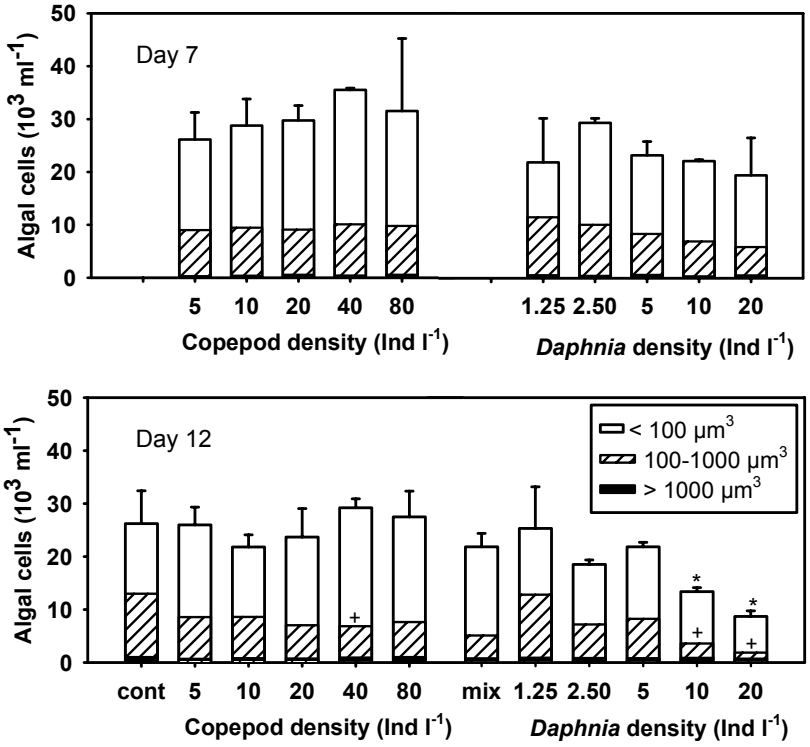


Figure 1-8: Mean phytoplankton or taxa abundance from two replicate bags on day 7 (upper panel) and day 12 (lower panel), classified into 3 biovolume size ranges for the nominal copepod and *Daphnia* density gradient, mix bags (20 copepods and 5 *Daphnia* per litre) and controls (no addition of zooplankton). Significant differences between treatments and controls of total cell abundances are marked with *, algae sized 100-1000 µm³ marked with +.

For phytoplankton species-specific impacts caused by zooplankton, regression lines were calculated for each algal species. In order to show the zooplankton impact not for all algal species separately, exponent b values (i.e. slopes) from regression lines (see Table 1-4) were determined. The b values were then plotted against algal biovolume for all phytoplankton species (Figure 1-9). Negative exponent b values mainly reflect grazing impacts of zooplankton, while positive b values can be caused by released grazing pressure or beneficial nutrient supply. The majority of phytoplankton taxa were reduced by *Daphnia* density dependently on day 7 and day 12, showing negative exponent b values. Except for

Dinobryon divergens, Fragilaria crotonensis and Nanoflagellates on day 7, and *Dinobryon divergens* on day 12, b values were positive, but never exceeded 0.03 (see Table 1-4). However, in *Daphnia* bags, one exceptionally high b value (0.19) for *Bitrichia chodatii* occurred on day 12. Predominantly positive b values were calculated for copepods, increasing algal species abundance with increasing nominal copepod abundance. Copepods showed distinctly higher b values than daphniids for algal species with low biovolume.

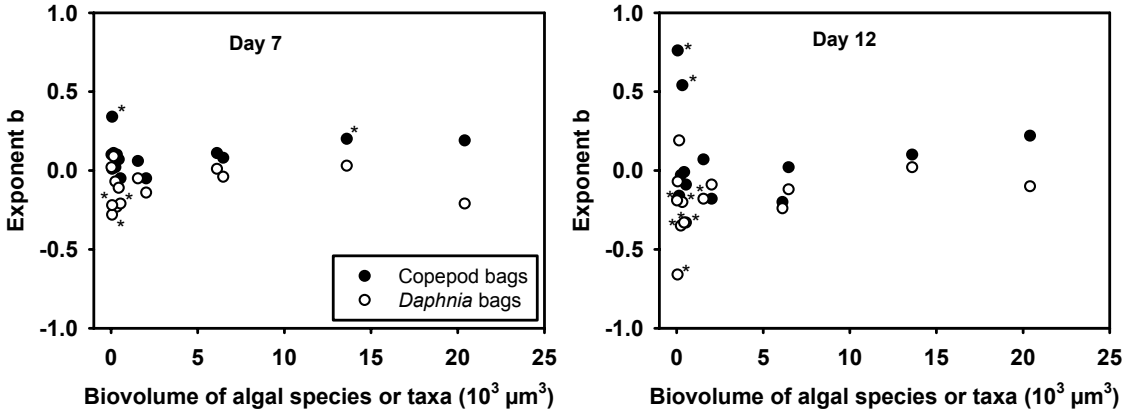


Figure 1-9: Species-specific regression analyses were performed using $y=ax^b$ for each algal species or taxa counted, and exponent b values plotted against biovolume. *Daphnia* and copepod bags for day 7 and day 12 are shown. Wherever regression analysis was significantly related to copepod or *Daphnia* nominal densities ($\alpha < 0.05$), values are marked with*.

Stoichiometry

Dissolved nutrients

Differing densities of copepods and *Daphnia* manipulated TDN and TDP concentrations in contrasting ways. While copepods significantly increased TDP concentrations relative to their initial density, daphniids reduced total dissolved phosphorus after 7 days. After 13 days, copepods and daphniids changed TDN concentrations in a similar manner as for TDP (Figure 1-10). All densities of Daphnia reduced nitrogen as well as phosphorus relative to the concentration of these nutrients at the start of the experiment (TDP=0.36±0.06 µmol l^{-1}, TDN=30.74±3.12 µmol l^{-1}, typical for Schöhsee in spring (Lampert & Sommer, 1997)). Yet, low copepod abundances reduced initial TDN and TDP concentrations while high copepod treatments enhanced them. However, for TDN:TDP ratios, the opposite pattern was found. TDN:TDP ratios were significantly reduced with nominal copepod density, and increased with *Daphnia* density (Figure 1-11).

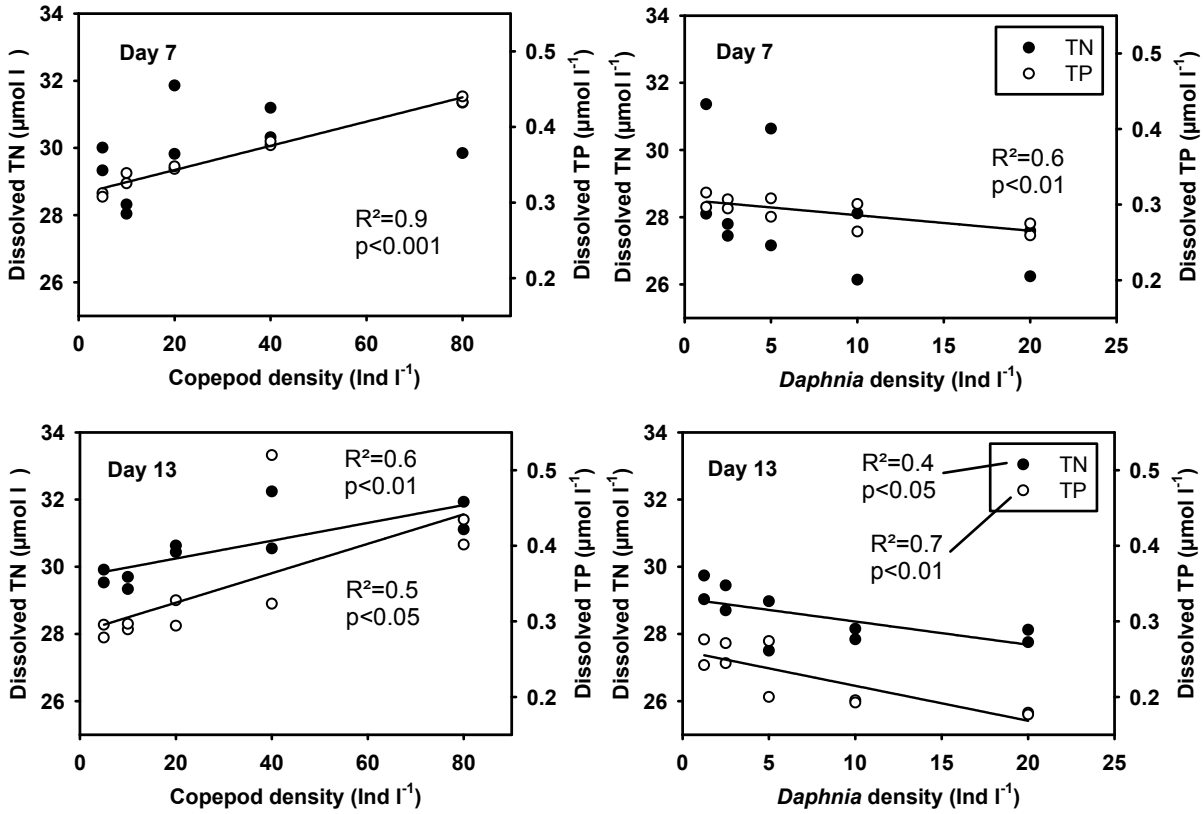


Figure 1-10: TDN and TDP in relation to nominal copepod (left panels) and *Daphnia* (right panels) density bags day 7 (upper panels) and day 13 (lower panels). Significant slopes of regression lines and statistical parameters are shown.

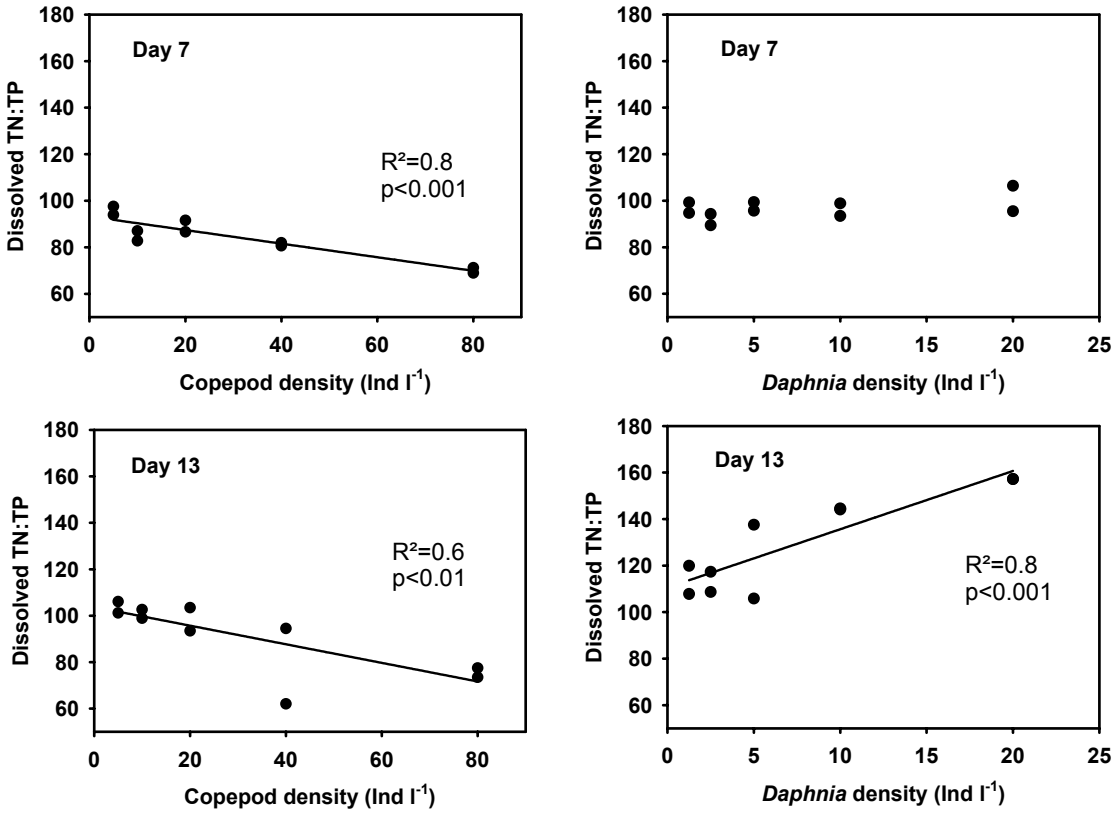


Figure 1-11: TDN:TDP ratios (molar) in relation to nominal copepod (left panels) and *Daphnia* (right panels) density bags on day 7 (upper panels) and day 13 (lower panels). Significant slopes of regression lines and statistical parameters are shown.

The second highest density was selected randomly to compare zooplankton impact on TDN:TDP ratios over time, because trends were similar for all densities. Despite a negative manipulation of TDN:TDP with copepod density, ratios within each copepod bag were approximately consistent until day 14 (bags stocked with 40 copepods per litre, randomly chosen are shown in Figure 1-12). In contrast, *Daphnia* significantly increased the TDN:TDP ratio linearly until day 14 (bags stocked with 10 *Daphnia* per litre, randomly chosen, shown in Figure 1-12).

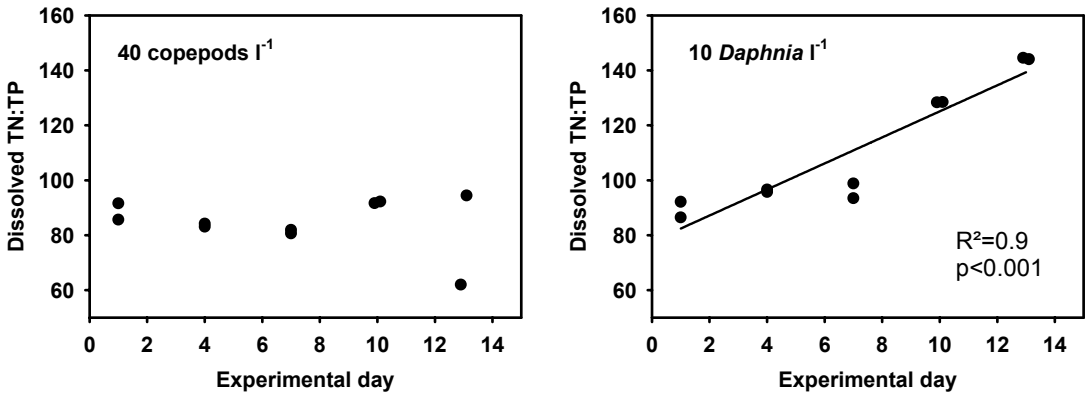


Figure 1-12: TDN:TDP ratios (molar) over experimental time of 14 days for randomly chosen treatments (40 copepods and 10 *Daphnia* per litre). Significant slopes of regression lines and statistical parameters are shown.

Sestonic nutrients

The C:N:P ratios of <100 µm particulate organic matter (POM) were compared to the Redfield ratio of 106:16:1 (Redfield, 1958) to determine possible limitations by essential elements. Low levels of phosphorus were available in food particles for zooplankton, relative to nitrogen and carbon, as indicated by a mean ratio of 240:28:1 at the start of the experiment in Schöhsee. The rather high N:P start value was further elevated to ~100 by *Daphnia* on day 7, as well as on day 13 (Figure 1-13). As copepods were more abundant on day 7 compared to day 13, an induced reduction of seston N:P ratios over the nominally stocked density was expected, showing a slightly negative trend.

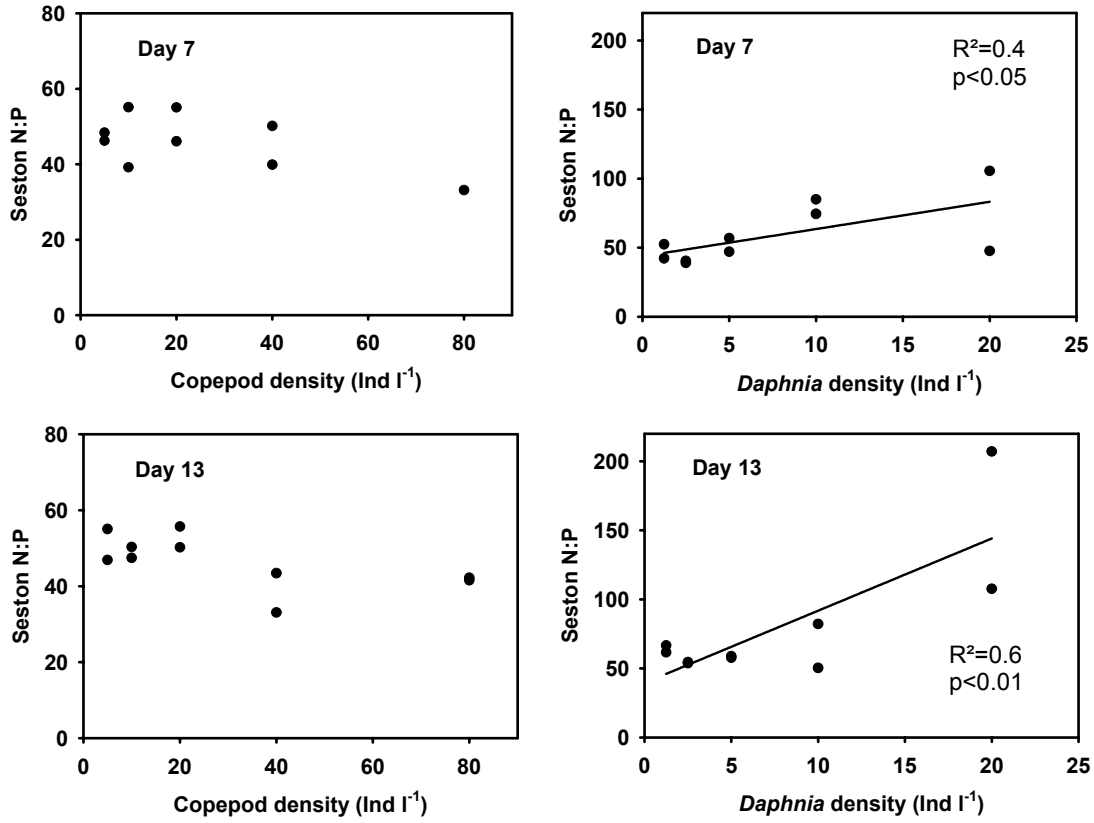


Figure 1-13: Seston N:P ratios (molar) in relation to nominal copepod and *Daphnia* density bags for day 7 (upper panels) and day 13 (lower panels). Significant slopes of linear regression lines and statistical parameters are shown. Note different scales on y-axes for *Daphnia* bags.

Daphnia increased the N:P ratio over time as the comparison between day 7 and day 13 in Figure 1-13 shows. This time dependent impact can be found for several *Daphnia* density bags. Here, the second highest density was chosen randomly for comparison of *Daphnia* to copepods (40 copepods and 10 daphniids per litre). *Daphnia* increased the sestonic N:P ratio continuously and linearly over 14 days. In contrast, copepods did not show a time-dependent impact: seston N:P ratios were similar to start values (Figure 1-14).

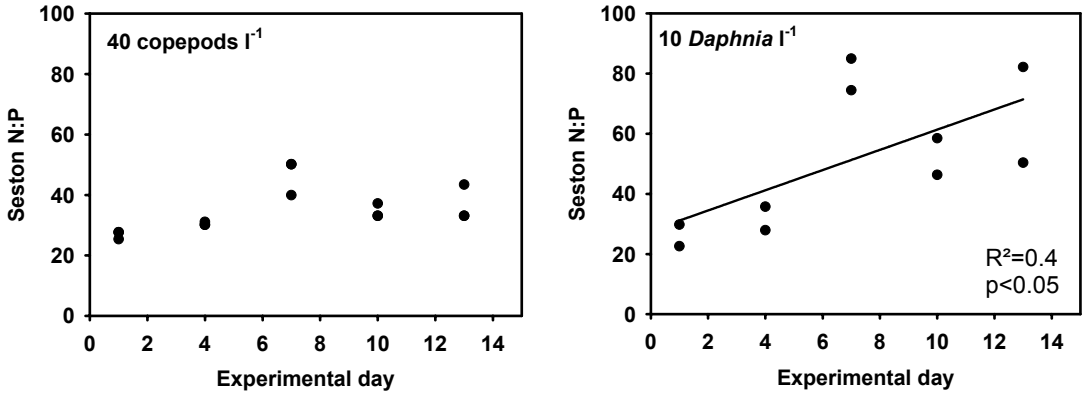


Figure 1-14: Seston N:P ratios (molar) over the experimental time of 14 days for randomly chosen treatments (40 copepods and 10 *Daphnia* per litre). Significant slopes of regression lines and statistical parameters are shown.

As food C:P ratios are often reported to limit growth above a ratio of ~300 (Elser *et al.*, 2000), this threshold is particularly important for *Daphnia*, which require relatively high amounts of phosphorus. *Daphnia* abundances considerably increased over the experimental period, hence, C:P ratios were investigated for *Daphnia*. The C:P ratios of POM manipulated by *Daphnia* increased in all *Daphnia* bags, up to extreme ratios of ~1200 (densities of 5 and 20 *Daphnia* l^{-1} were chosen as examples, shown in Figure 1-15).

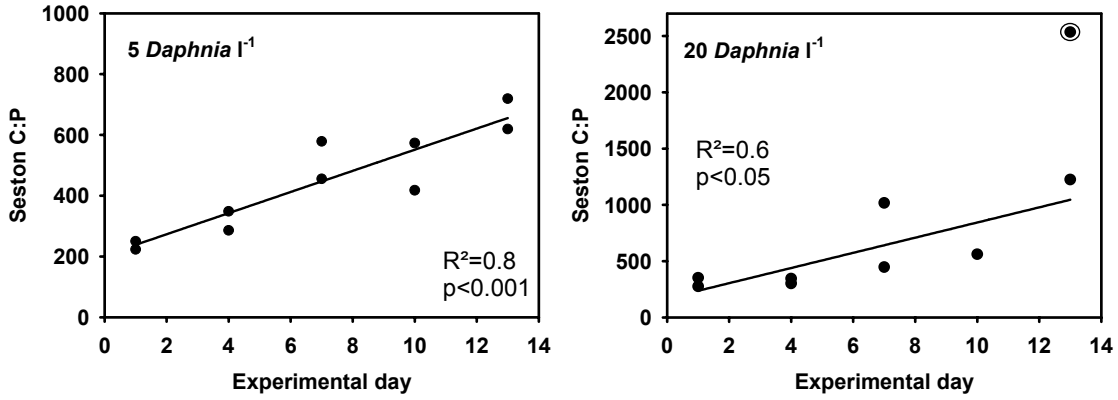


Figure 1-15: Changes in seston C:P ratios (molar) until day 14, in enclosure bags with two different nominal densities of 5 and 20 *Daphnia* per litre. Significant slopes of linear regression lines and statistical parameters are shown. The marked value in the right panel was treated as an outlier and not included in regression analysis. Note different scales on y-axes.

Sediment nutrients

Particulate organic matter which settled as sediment in the bags exhibited a different elemental ratio (within 12 hours) compared to suspended POM of the water column. Values for N:P in Figure 1-16 should scatter around the line of equality if N:P ratios were unaltered by sedimentation. In fact, the ratios clustered below the line of equality, suggesting sediment contained more phosphorus relative to nitrogen than suspended seston. Sediment N:P was significantly lower in copepod bags ($t=-2.7$, $p<0.05$) compared to in *Daphnia* bags.

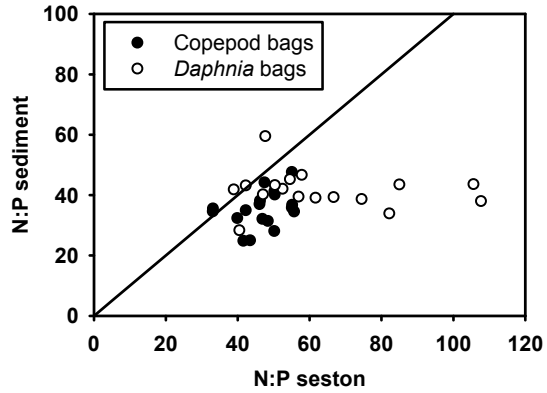


Figure 1-16: N:P ratios (molar) of suspended particulate matter and sediment. Data from day 7 and 13 for seston, and day 8 and 14 for sediment, were grouped. The line of equality is shown.

Zooplankton stoichiometry

Copepods and *Daphnia* from all different density bags were analysed for C:N ratios at the end of the experiment. However, there were no significant relationships between zooplankton C:N and density gradient, in contrast to the zooplankton induced manipulation of dissolved and seston nutrients. Each species maintained a rather constant C:N ratio, independent of their abundance. Thus, means of different taxa from copepod bags, as well as daphniids from *Daphnia* bags, were calculated to determine differences between taxa (Figure 1-17). Cyclopoid copepods had significantly higher C:N ratios compared to the other taxa, and *Daphnia* in *Daphnia* bags had significantly lower values than copepods and *Daphnia* from the copepod bags (ANOVA, $F_{3,29} = 3.95$, $p<0.05$ and Tukey-Kramer post hoc test). Marked differences in copepod and *Daphnia* body stoichiometry were found in N:P ratios. Copepod body N:P was significantly elevated compared to daphniids (t-test, $t=9.8$, $p<0.001$).

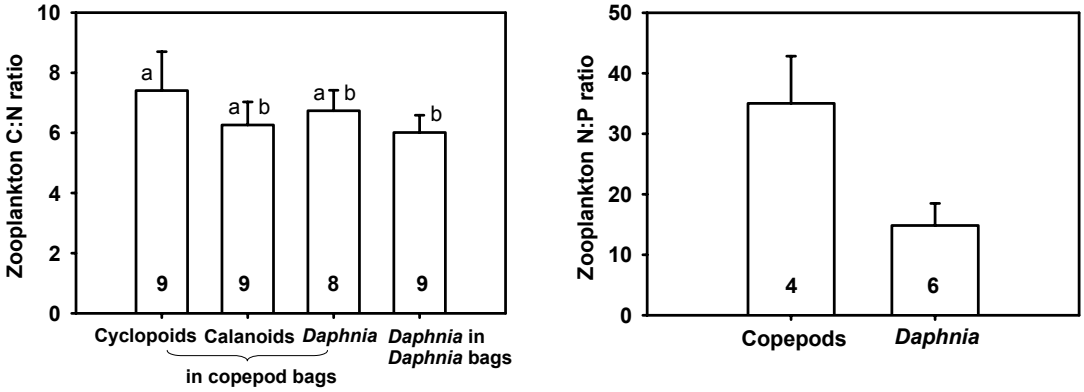


Figure 1-17: Mean (±SD) zooplankton C:N ratios of all different zooplankton taxa (left panel) and N:P ratios of copepod and *Daphnia* (right panel) at termination. Data from different density bags were pooled, numbers in bars denote n values, i.e. enclosure bags (up to 3 samples per bag). Bars not connected by the same letter are significantly different in left panel.

Zooplankton with low body N:P and high body N:P can affect the stoichiometry of the seston in contrasting ways, indicated in bi-plots of consumer and resource N:P ratios (Figure 1-18). Both copepods and daphniids retained more nitrogen than phosphorus available in POM. *Daphnia* showed similar body N:P (homeostasis), whereas copepods varied considerably in their elemental composition when fed a nearly constant resource N:P.

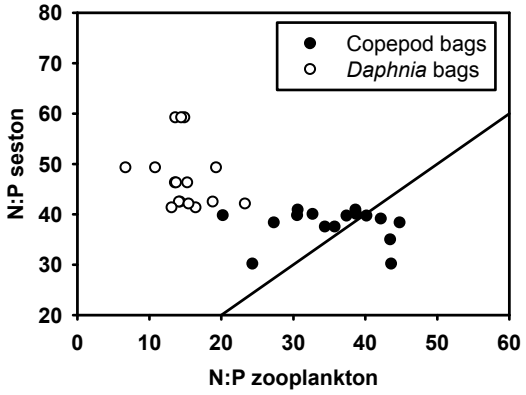


Figure 1-18: N:P ratios of copepods and *Daphnia* versus seston. Seston N:P data were calculated as mean from experimental period, N:P of zooplankton was measured at termination. The line of equality is shown.

Stable Isotope Analysis

Stable isotope analysis of cyclopoid copepods and *Daphnia* from within their respective bags revealed little variability between density treatments: cyclopoid copepod $\delta^{13}C$ values ranged from –27.2 to –28.4‰, and $\delta^{15}N$ from 8.1 to 10‰; *Daphnia* $\delta^{13}C$ ranged from –25.7 to –26.6‰ and $\delta^{15}N$ from 2.7 to 3.6‰ (Figure 1-19). Calanoid copepods showed more variable signatures compared to cyclopoids, $\delta^{13}C$ from –26.6 to –28.5‰, and $\delta^{15}N$ from 5.0 to 7.8‰. *Daphnia* harvested from the copepod bags exhibited a little more variability in both $\delta^{13}C$ (2‰) and $\delta^{15}N$ (2.3‰), but lower $\delta^{13}C$ values compared to their counterparts in the *Daphnia* only bags. There was no significant effect of mean *Daphnia* density on *Daphnia* isotopic signature in single bags (R^2=0.002, p>0.05 for $\delta^{13}C$, R^2=0.3, p>0.05 for $\delta^{15}N$), or food abundance and quality. However, there were significant correlations between mean copepod densities and both cyclopoid copepod $\delta^{13}C$ (R^2=0.7, p<0.01) and $\delta^{15}N$ (R^2=0.5, p<0.05), and also between mean densities of *Daphnia* in the copepod bags and cyclopoid copepod $\delta^{15}N$ (R^2=0.8, p<0.01 Figure 1-20).

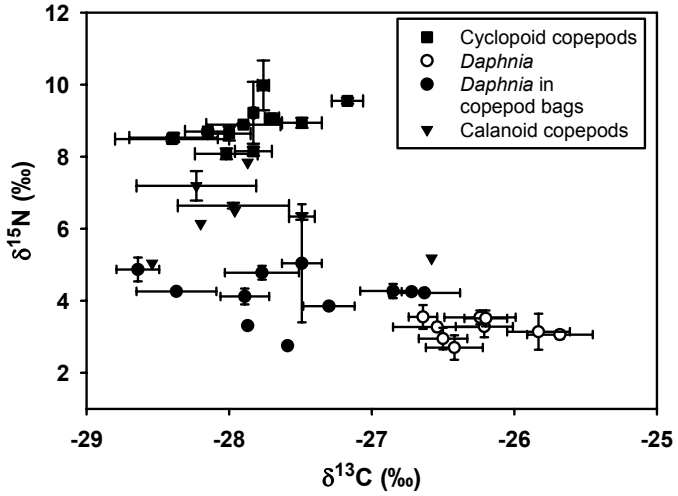


Figure 1-19: Mean (± SD, n = 3) $\delta^{13}C$ and $\delta^{15}N$ values of *Daphnia* from *Daphnia* bags, cyclopoid and calanoid copepods from copepod and mixed bags, and *Daphnia* from copepod and mixed bags.

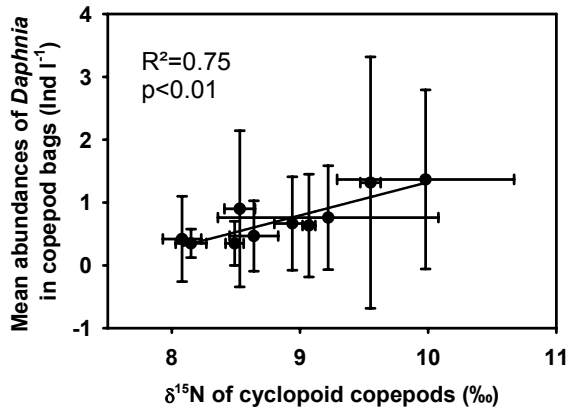


Figure 1-20: Mean (± SD, n = 3) $\delta^{15}N$ values of cyclopoid copepods from copepod and mixed bags vs. mean abundances of *Daphnia* in the same bags. Significant slopes of regression lines and parameters are shown.

1.3. Discussion

A variety of changes of phytoplankton abundance or nutrient stoichiometry induced directly or indirectly by zooplankton abundances were observed during the experiment. The procedure also allowed investigation of feedback mechanisms, resulting from zooplankton interactions, by stable isotope analysis or manipulated nutrient stoichiometry. The population decrease of calanoid copepods was probably a direct result of predation by cyclopoid copepods, especially the large and common *Cyclops abyssorum*. Advanced cyclopoid copepod stages can feed on larger prey items such as nauplii and fully grown cladocerans or calanoid copepods (Fryer, 1957; Confer, 1971; Kerfoot, 1977; Bosch & Santer, 1993). In the highest initial stocking densities, probability of an encounter with a cyclopoid copepod was high and probably resulted in decimation of the calanoid copepods (also see stable isotope results below). At lower initial stocking densities, calanoid copepods survived better because cyclopoid copepods were less abundant (Figure 1-5). Declines in copepod abundances were most marked within the first twelve experimental days, and I suggest the residual low copepod abundances eased competition and allowed growth of *Daphnia* in copepod bags (Figure 1-4). The low abundances of *Daphnia* in *Daphnia* bags at the beginning of the experiment was likely due to considerable mortality of cultured *Daphnia* by surface-film entrapment, despite careful introductions. The growth of *Daphnia* through the experiment at all density treatments in *Daphnia* as well as in copepod bags, reflected fast reproduction by parthenogenesis, low predator abundance, and the availability of suitable food sources.

Daphnia considerably decreased algal biomass (Figure 1-6 and 1-7), inducing a clear water phase (CWP) with high water transparency in all bags (Lampert, 1988; Lampert & Sommer, 1997). Surprisingly, this reduction occurred independent of *Daphnia* biomass. However, when *Daphnia* became abundant in some bags, chl *a* values were already quite low and the effect of different *Daphnia* abundances on chl *a* is probably not visible. It is generally believed that grazing by *Daphnia* is the main causative factor inducing freshwater CWP due to their fast metabolism and reproduction. In addition, the grazing impact could have been supported by complementary grazing of *Daphnia* and protozoa (ciliates and/or rotifers). Typically, in bags where there were less daphniids, then more ciliates were found (Zöllner, 2004), supplementing grazing on the predominantly small sized algae. Copepods were not able to cause a CWP, even when stocked in high densities. Of course, the lack of inducing a CWP can be caused by the decline of calanoid copepod abundances, but are there also other mechanisms involved?

Algal biomass increased with decreasing copepod abundances and favourable TDN:TDP ratios (relative to nominal copepod densities - Figure 1-11). This increase also ameliorated over time (14 days, Figure 1-7). Concordant with density-dependent positive slopes of chl *a*, the time dependent increase could result from the decreasing abundances of

calanoid copepods, i.e. allowing phytoplankton growth by reduced grazing pressure and beneficial nutrient conditions. Furthermore, copepods are known for their high negative impact on large phytoplankton along with compensatory growth of small species, as a previous mesocosm experiment (of the same design) during summer in Schöhsee showed (Sommer *et al.*, 2001). However, in my experiment in spring, large algae with a biovolume >1000 µm³ were rare (see Figure 1-8). While calanoid copepods declined in enclosure bags, cyclopoid copepods increased, known to feed raptorial. Wickham (1995) has previously demonstrated that *Cyclops* species can feed extensively on variously sized ciliate species. Similarly, in a summer mesocosm experiment in Schöhsee, Zöllner *et al.* (2003) revealed that copepods fed upon medium-sized ciliates in enclosure bags. Consequently, copepods can support algal growth by released grazing pressure on smaller particles, either directly, or indirectly via ciliates. Indeed, I found a copepod density-dependent positive impact on algal species smaller 100 µm³ on day 7. On day 12, copepod density was <20 individuals per litre in all bags, resulting in no significant differences in algal densities (Figure 1-8). In addition to reduced direct and/or indirect grazing pressure by decreasing ciliate or copepod abundances, the increase of chl *a* and small phytoplankton in copepod bags on day 7 can also be ascribed to bottom-up effects such as advantageous nutrient conditions. However, due to a rather constant seston N:P ratio over time (Figure 1-13), phytoplankton did not mirror favourable dissolved nutrient conditions.

In comparison to copepods, *Daphnia* abundances had a negative density-dependent impact on phytoplankton biomass (Figure 1-8 and 1-9). Their large impact reflects the suitability of the available photosynthetic prey. Indeed, the phytoplankton community in the bags consisted primarily of small and medium sized species (<1000 µm³), a suitable particle size for filtering by *Daphnia* (see Geller & Müller, 1981; Brendelberger & Geller, 1985; Rothhaupt, 1997; Sommer *et al.*, 2001). Phytoplankton abundances reflect a negative density-dependent impact of *Daphnia* on particles smaller than 1000 µm³, confirming the ability of *Daphnia* to prey on smaller sized food sources compared to copepods. *Daphnia* made a greatest impact on *Stephanodiscus parvus* (60 µm³ biovolume, Table 1-4, Figure 1-9). Prey morphology, as well as size, determines suitability of prey for *Daphnia* filtering apparatus (Geller & Müller, 1981). Spines might prevent *Daphnia* grazing, and thus, I expected *Bitrichia chodatii* to remain in the bags and benefit from the removal of algal competitors (for light and nutrients) by *Daphnia* because this alga has two ~30 µm long, oppositely situated needle shaped appendages. In fact, *B. chodatii* was the only species <1000 µm³ that increased in abundance with *Daphnia* density, but only for day 12. Large phytoplankton >1000 µm³ were less affected by *Daphnia* density compared to the smaller species. Abundances of the large algae *D. sociale, S. alpinus, Cryptomonas* sp.*, A. flos-aquae* and *F. crotonensis* did marginally decrease density dependently, but the decrease was not significantly different from controls at day 12. *D. sociale* occurred in colonies of

~20,000 µm³, an unsuitable size for daphniid filtering apparatus, but individually, the loricas or monads are actually much smaller. A negative impact was also found by Sommer *et al.* (2001), emphasising the rather fragile character of *Dinobryon* colonies, from which single cells can easily break loose and be grazed upon by *Daphnia*. The other large species, *S. alpinus*, *Cryptomonas* sp. and *F. crotonensis* could either marginally be grazed upon by *Daphnia* or reduced due to unfavourable nutrient conditions the more *Daphnia* present (Figure 1-11).

Copepods were expected to reduce the larger sized phytoplankton density dependently (Sommer *et al.*, 2001), but predominantly positive b values rather refute this argument. As stated above, this is caused by decreasing abundances of partly herbivorous calanoid copepods and beneficial nutrient conditions. However, not only prey size, but also motility can be an important factor when copepods select food particles (DeMott & Watson, 1991), often reported to preferentially feed on ciliates (Burns & Schallenberg, 1996; Adrian & Schneider-Olt, 1999; Ehret, 2000; Hansen, 2000). Accordingly, a negative impact on the motile species *Cryptomonas* sp. was found. However, b values for *Cryptomonas* on day 12 were in the same range as *S. parvus* and *B. chodatii* (both species <1000 µm³). *S. parvus* often occurred in colonies, and thus the combined size of many cells together made them vulnerable to predation by copepods. A significant, high exponent b was calculated for *R. minuta* and an unidentified chlorophyte species. They likely benefited from released grazing pressure due to their small size.

A negative impact by *Daphnia* on the filamentous cyanobacteria *Aphanizomenon flos-aquae* is most surprising, since cyanobacteria are assumed to be unsuitable food due to their large size (Brendelberger & Geller, 1985), chemical composition (Brett & Müller-Navarra, 1997) and potential toxicity (Lampert, 1981). Indeed, Ghadouani *et al.* (2003) reported a decline of *Daphnia* abundance caused by *A. flos-aquae* in an *in situ* experiment. However, *Daphnia pulex* was reported to prevent a *A. flos-aquae* bloom before the algae grows large colonies (Lynch, 1980), and *D. magna* is able to break and shorten blue-green filaments (Dawidowicz, 1990). Thus, the significant decrease of *A. flos-aquae* in my experiment could results from a direct grazing impact by *Daphnia*, but also from unfavourable nutrient conditions. Filamentous cyanobacteria are known for their ability to form blooms whenever sufficient phosphorus is available for growth (Paterson *et al.*, 2002). Since *Daphnia* decreased TDP concentrations (Figure 1-10), they could limit cyanobacteria growth indirectly, via *Daphnia* density.

At the beginning of the experiment, N:P and C:P ratios of 28 and 240 indicated low food quality for the zooplankton, exceeding Redfield ratio of 16 and 106, respectively, by far (Redfield, 1958). As copepods were expected to retain relatively more nitrogen (Elser & Hassett, 1994; Gismervik, 1997), they indeed significantly decreased TDN:TDP ratios (Figure 1-11 and 1-17). By repetition of this cycle in the closed mesocosms, deterioration of this

impact was assumed, but neither TDN:TDP nor seston N:P ratios changed time-dependent. Also, zooplankton N:P was similar to sestonic resource N:P, indicating no preferential elemental retention from food sources. Considering the variable body N:P ratio of copepods in relation to their food, stoichiometric theory of copepod homeostasis (Sterner & Hessen, 1994) cannot be confirmed. However, this can be caused by copepod mortality, since data from Figure 1-18 represent integrated values of sestonic stoichiometry from the enclosure experiment until termination. The copepod decrease should be mirrored in the sediment N:P, but ratios are even lower than for the seston (Figure 1-16) since zooplankton was removed from the filters before analysis.

As Schöhsee was limited in phosphorus at the beginning of May, investigations of *Daphnia* enclosure bags are particularly important, since Daphnia requires relatively high amounts of phosphorus compared to its food (Figure 1-17 and 1-18 and see Hessen & Lyche (1991) and Sterner & Elser (2002)). Indeed, *Daphnia* significantly increased TDN:TDP ratios by a density-dependent retention of phosphorus, but also over the course of the experiment, mainly due to growth (Figure 1-11 and 1-12). As a consequence, seston became severely phosphorus depleted as experimental time progressed, N:P ratios exceeding 100 (Figure 1-13 and 1-14). This large impact of *Daphnia* on seston N:P is also shown in Figure 1-16, however not passed on to sediment stoichiometry. Sediment N:P is approximately constant, believed to be influenced by moulting during *Daphnia* growth. Approximately 14 % of *Daphnia's* total phosphorus content are bound in their exoskeleton, resulting in a phosphorus drain to the sediment after moulting (Vrede, Andersen & Hessen, 1999). Concordant, when *Daphnia* became abundant in enclosure bags, sediment P concentrations increased with *Daphnia* density at day 14. During the experimental time of 25 days, *Daphnia* could moult around 7 to 8 times, calculated based on reported intermoult durations of *Daphnia magna* for 18°C (Hessen & Rukke, 2000). In contrast to my results, Sommer (2003b) found a negligible contribution of sedimentation of faecal pellets and carapaces to phosphorus dynamics in the summer enclosures in lake Schöhsee. By higher temperatures and permanent sediment traps in enclosure bags during summer, P bound in carapaces might be more rapidly lost or remineralised than in spring.

The low seston food quality in terms of phosphorus could have a negative feedback effect on *Daphnia*, presumably facing food phosphorus deficiency (Elser & Hassett, 1994). Since phosphorus is not only needed for skeletal tissue, ATP and phospholipids, but also for RNA and DNA production, growth of daphniids is probably restricted. Elser *et al.* (2000) stated, that *Daphnia magna* faces phosphorus deficiency when the C:P ratio of their food exceeds around 300 leading to a reduced gross growth efficiency. Egg production and population density of *Daphnia cucullata* were reported to decline with a seston C:P ratio of ~300-500 (DeMott, Gulati & Van Donk, 2001). Moreover, reduced somatic growth above an algal food threshold of C:P 350 was found for the clone of *Daphnia h. x g.* used in my

enclosure experiment by Becker and Boersma (2003). In my experiment, surprisingly, seston C:P ratios of all *Daphnia* density treatments exceeded the threshold of 350 by far. According to reported thresholds, *Daphnia* should not have been able to grow at all after around 7 days. Instead, cladocerans growth started after day 7 and thus C:P ratios increased even more over time, with values between 600 to 800 for all treatments and maximum values >1000 for 20 *Daphnia* per litre bags (see Figure 1-15 for 5 and 20 *Daphnia* per litre).

Surprisingly, *Daphnia* abundances in the enclosures increased for all densities until day 19 (see Figure 1-4, panel B). To my knowledge, *Daphnia* growth under these high C:P ratios was not reported before. As Elser (2000) summarised from the Norwegian Academy of Sciences in 2000, 'there is a need for investigations how grazers respond to high C:nutrient food ratios'. Juveniles accounting for abundances at day 19 represent nutritional conditions of their mothers at day 14, since mean egg development times were calculated at around 5 days (after Bottrell *et al.*, 1976). To judge, if *Daphnia* was limited in growth, specific growth rates between day 7 and 9, assumed to be least limited in food availability and nutrient stoichiometry, were calculated and used to calculate theoretical unlimited growth (Figure 1-21). Actual densities in enclosure bags were lower than theoretical abundances of after day 13, indicating a restriction of *Daphnia* growth. The limitation is likely caused by phosphorus, since food quantity was sufficient (seston carbon equalled 700 to 900 µg l^{-1}). Additionally, food quality could have restricted *Daphnia* growth, highly unsaturated fatty acids were considerably low at day 12 with a mean of 0.1µg µmol^{-1} C^{-1} (C. Becker, personal communication). Becker and Boersma (2003) found growth rates of 0.3 d^{-1} at a concentration of 5 µg phosphorus l^{-1}, similar to my enclosure experiment results with mean 6.3 µg P l^{-1} at growth rates of 0.22 d^{-1} for bags illustrated in Figure 1-15 until day 15. However, the C:P ratio of the food used by Becker and Boersma (2003) was ~350, much lower than in enclosure bags. This indicates, that *Daphnia* are very efficient in retaining phosphorus, independent of the given C:P ratio. Thus, my data suggest that it is more accurate to address nutrient thresholds for single (i.e. phosphorus) nutrients alone, instead of ratios. As in the enclosure experiment, extremely high carbon concentrations can likely occur simultaneous to low phosphorus concentrations in freshwater systems.

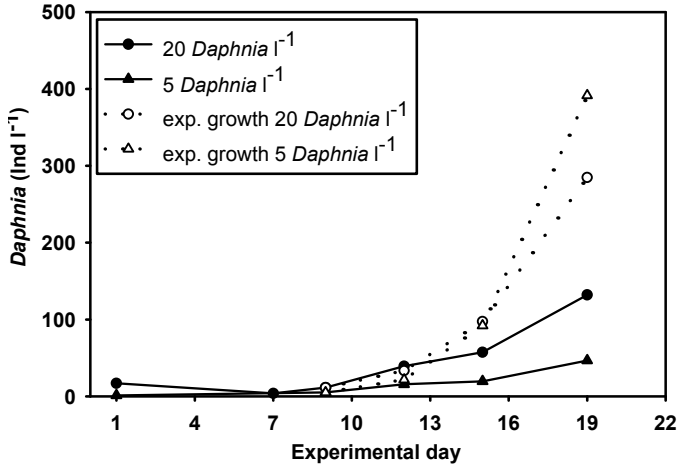


Figure 1-21: Theoretical unlimited growth rates calculated with specific growth rates (determined from growth between day 7 and 9).The unlimited growth and the actual densities are shown for two *Daphnia* densities: 5 and 20 daphniids per litre.

I can reasonably assume greater abundance of dead organisms, more 'sloppy feeding' (Lampert, W., 1978) and elevated defecation in bags containing higher initial copepod or *Daphnia* densities. Consequently, there was likely to be a density-dependent availability of dissolved organic matter in both zooplankton taxa bags. Carbon ingested by zooplankton is either assimilated (and further absorbed or lost by respiration or excretion) or lost in faecal pellets. Xu & Wang (2003) reported that over 50% of carbon metabolic loss from a marine copepod was in the form of dissolved organic carbon (DOC). DOC resources are utilized by heterotrophic bacteria, which preferentially assimilate newly produced dissolved organic carbon (Norrman *et al.*, 1995). The data from my experiment appear consistent with this. Bacterial growth in copepod bags significantly increased in proportion to the nominal copepod density (Figure 1-22). Elevated bacterial abundances may not only result from higher resource concentrations, but also from released grazing pressure from ciliates (see Zöllner *et al.*, 2003). Consequently resulting in a cascading effect of increasing bacterial biovolume, activity and production along a gradient of copepod density.

For *Daphnia* a more widespread effect throughout the lower trophic levels was shown by Zöllner *et al.* (2003): there was reduction of small-sized ciliate and nanoflagellate abundances, and of bacterial biovolume, activity and production with increasing *Daphnia* density. As described above, *Daphnia* can prey on smaller sized particles and are also able to ingest bacteria (e.g. Bern, 1987; Ojala *et al.*, 1995; Burns & Schallenberg, 2001). Thus, *Daphnia* grazing on bacteria compensated enhancement of bacterial growth via the increased supply of DOC at higher *Daphnia* densities, resulting in constant HNA bacterial abundances in the *Daphnia* bags (see Figure 1-22).

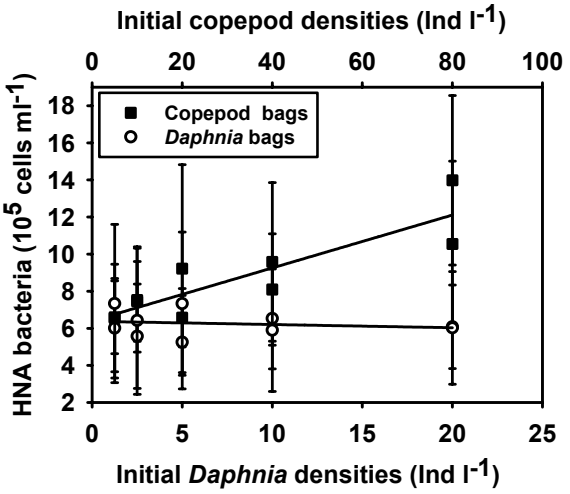


Figure 1-22: High nucleic acid (HNA) bacterial abundance in copepod and *Daphnia* bags. Means ± SD of HNA bacteria were calculated from measurements of every 3rd experimental day over 25 days. Slopes of regression lines of copepod compared to *Daphnia* bags are significantly different (ANCOVA, $F_{3,16} = 15.6$, $p < 0.01$). The increase, i.e. regression line of HNA bacteria with copepod abundance is significant ($R^2 = 0.77$, $p < 0.01$). Figure from Feuchtmayr *et al.* (2004).

Stable isotope signatures of cyclopoids, calanoids and *Daphnia* derived from my experiment are similar to those reported from meso- to eutrophic lakes (Grey, Jones & Sleep, 2000). The mean isotopic value for cyclopoids, compared to *Daphnia* from the *Daphnia* bags differed by 5.6‰ for $\delta^{15}N$ and 1.4‰ for $\delta^{13}C$, corresponding to more than one trophic level

(Fry & Sherr, 1984; Minagawa & Wada, 1984). This confirms exploitation of different food sources and supports the contention that cyclopoids were feeding on organisms at a higher trophic level compared to daphniids in the *Daphnia* bags. As stated above, predacious cyclopoids can also supplement their diet by calanoid copepods, with a 2.5‰ lower $\delta^{15}N$ signal than cyclopoid copepods.

However, we cannot exclude the possibility that the cyclopoid $\delta^{15}N$ values were elevated as a direct result of the daphniid contamination in the copepod bags, providing the copepods with *Daphnia* as an alternative food source. Indeed, cyclopoid $\delta^{15}N$ values were significantly correlated to the mean abundances of daphniids in the copepod bags (Figure 1-20). The copepods were also ^{15}N- and ^{13}C-enriched with increasing mean copepod densities in the bags, presumably resulting from predation of lower copepodite stages or calanoid copepods.

Abundances of *Eudiaptomus* spp. in high-density treatments at the end of the experiment were very low, restricting sample extraction mainly to low copepod density bags. Mean $\delta^{15}N$ of 6.4‰ were significantly higher than herbivorous, filter feeding *Daphnia*. Traditionally, *Eudiaptomus* was classified herbivorous, until Porter, Pace and Battey (1979) reported their ability to feed on susceptible ciliates. Recently, more evidence for their broader feeding behaviour was reported, able to ingest rotifers (e.g. *Keratella* sp.) and *Ceratium* sp., a hard-bodied dinoflagellate (Lair & Hilal, 1992; Santer, 1996) and even a preference and selective ingestion of ciliates (Ehret, 2000). More evidence that *Eudiaptomus* spp. are not solely restricted to herbivorous food sources is given by their higher stable nitrogen signatures compared to *Daphnia* in the enclosures. Moreover, by their ability to prey on phytoplankton as well as organisms of higher trophic levels, calanoids seem to be relatively flexible in their feeding behaviour, varying by 1.9‰ for carbon and 2.8‰ for nitrogen (Figure 1-19).

Daphnia growing in the copepod bags exhibited a similar $\delta^{15}N$ signature (range 2.9 to 5.0‰) to *Daphnia* from *Daphnia* bags (range 2.7 to 3.6‰), suggesting that they were feeding at approximately the same trophic level. There was no effect of *Daphnia* density on $\delta^{13}C$ of daphniids from *Daphnia* bags (across the density gradient $\delta^{13}C$ varied only about 1‰), suggesting constant food source. However, *Daphnia* in copepod bags were more ^{13}C-depleted compared to the *Daphnia* in *Daphnia* bags, and the carbon isotopic variability between bags of differing copepod density was >2‰, indicating that a different carbon food source was available to *Daphnia* in the copepod bags compared to those in the *Daphnia* bags.

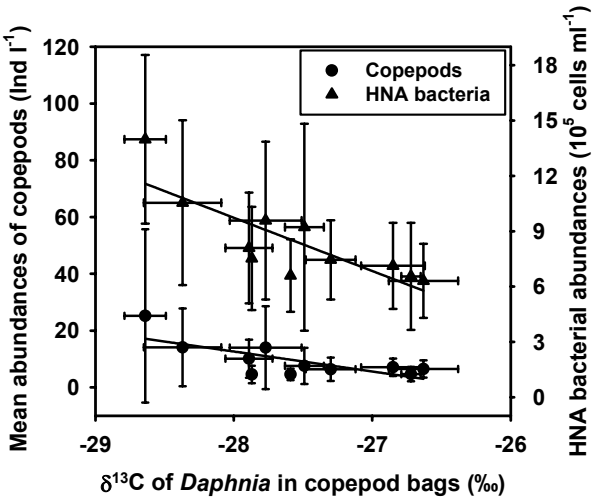


Figure 1-23: $\delta^{13}C$ of *Daphnia* from copepod bags in relation to mean abundance of copepods over the course of the experiment and HNA bacterial abundance. Means ± SD of HNA bacteria shown were calculated from measurements of every 3rd experimental day over 25 days, copepod abundances were counted every 3rd to 6th day. Slopes of regression lines are significant (R^2 = 0.54, $p < 0.05$ and R^2 = 0.67, $p < 0.01$ for copepod and HNA bacterial abundance, respectively). Figure from Feuchtmayr *et al.* (submitted).

Higher initial copepod abundances increased mean HNA bacterial abundances, which were also correlated with the carbon isotopic signature of *Daphnia* from the copepod bags (Figure 1-23). Differences in *Daphnia* $\delta^{13}C$ would be due either to increased bacterial grazing by *Daphnia*, or to grazing on phytoplankton with lower $\delta^{13}C$ caused by uptake of isotopically light carbon dioxide produced by bacterial respiration. A high abundance of active bacteria would result in increased respiration within the bag, suggesting that there was higher CO_2 production with increasing zooplankton density. Smaller algae have a more favourable surface to volume ratio, and we might expect them to exhibit a more ^{13}C-depleted signal compared to larger phytoplankton cells. Hence, the *Daphnia* $\delta^{13}C$ in our study may reflect HNA bacterial abundance indirectly via grazing on phytoplankton, and especially the smaller size fractions (see Figure 1-8 and 1-9). However, *Daphnia* can also ingest bacteria directly, since *Daphnia* is a filter-feeder and cannot discriminate against particles suitable for their filtering apparatus.

Saltwater enclosures

Chapter 2

2. Saltwater enclosures

2.1. Study site and methods

Site and experimental design

Marine mesocosms were exposed in Hopavågen, a small fjord situated around 120 km west of Trondheim at the Trondheimsfjord on the western coast of Norway (Figure 2-1). The small fjord (area: 27 ha, depth: 22-32 m) is favourable for mesocosm experiments since it is sheltered from wind and waves, and 14% of the water is exchanged by tides every day (http://www.ntnu.no/trondheim-marine-RI/). The experiment started on the 20th of April 2002 but had to be terminated on the 27th of April due to stormy weather conditions. Afterwards, a second experiment was run from the 2nd to the 8th of May.

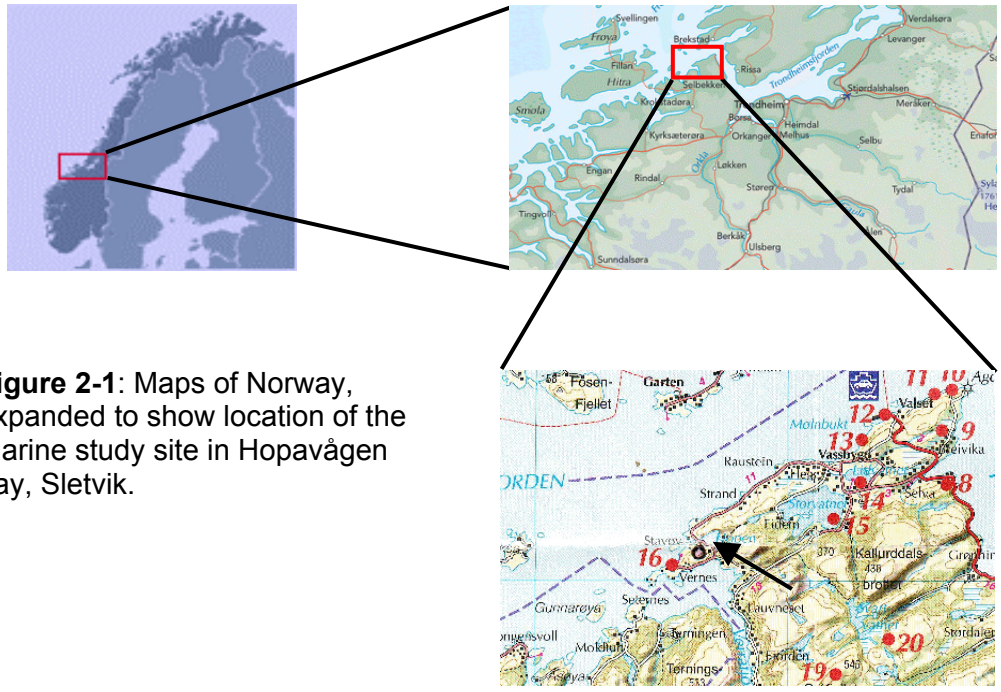
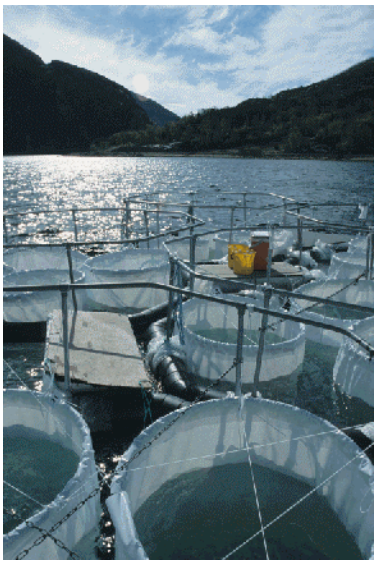


Figure 2-1: Maps of Norway, expanded to show location of the marine study site in Hopavågen bay, Sletvik.

A similar enclosure set up compared to Schöhsee (24 polyethylene bags, around 2 m deep and 1 m in diameter) was chosen (see picture 2-1). Bags were filled in Hopavågen and zooplankton was removed from the bags by plankton net tows (10 tows with 250 µm mesh size net and 10 tows with 150 µm net). Remaining seston consisted of phytoplankton, protozoa and bacteria smaller than 150 µm, passing through the net.



Picture 2-1: Enclosure bag set-up in Hopavågen fjord.

The copepod community in Hopavågen consisted of six calanoid species (*Calanus finmarchicus* (Gunnerus)*, Temora longicornis* (Müller), *Acartia clausi* (Giesbrecht), *Centropages hamatus* (Lilljeborg), *Centropages typicus* (Krøyer), *Pseudocalanus elongates* (Boeck)), and one cyclopoid (*Oithona* spp.). Since body size of *C. finmarchicus* (~3.5 mm in length) differed considerably from the other species (which spanned ~1 to 1.5 mm; (Todd, Laverack & Boxshall, 1996), copepods were divided in two different size classes for density-dependent comparison: copepods >500 µm, termed 'large copepods', and small copepods sized 250 to 500 µm accordingly. Large copepods were collected with a 500 µm net, small copepods with a 250 µm net. In the latter, zooplankton >500 µm was removed by screening through a 500 µm gauze. Copepods of each size class were placed in 250 litre containers, and bubbled with air for 6 h to remove cladocerans by surface entrapment prior to enclosure inoculation. Marine cladocerans are difficult to rear under laboratory conditions (Turner, 1984b) because they are extremely fragile and susceptible to surface entrapment, and so they could not be collected in Hopavågen for enclosure addition. Herbivorous appendicularians (*Oikopleura dioica* or *Frittillaria* sp.) which are similar in their non-selective feeding behaviour to cladocerans were substituted instead. In a similarly designed enclosure experiment in summer, Sommer (2003a) showed that when released from the grazing pressure of copepods, small appendicularians in the bags survived and grew. Thus, six enclosure bags were left without addition of copepods. Eight bags were stocked with large copepods in four logarithmically scaled densities, another eight enclosures with small copepods in the same manner, and two bags served as control (by regular removal of zooplankton larger 150 µm by net tows). In the first experiment, large copepod bags were stocked with zooplankton to achieve 0.3, 0.9, 2.7 to 8.1 copepods per litre, and small copepod bags were stocked to achieve 1, 3, 9 and 27 copepods per litre. In the second experiment, 1.3, 2.5, 5 and 10 large copepods per litre and 5, 10, 20, 40 small copepods per litre were inoculated (for an overview see Table 2-1). Different densities between the large-

and small-fractions were chosen to achieve a similar copepod biomass. However, measurements conducted after the experiment finished revealed that large copepods contained around 9 times more carbon (mean: 107 µg C copepod^{-1}) than small ones (mean: 12 µg C copepod^{-1}) (Saage, 2003).

Table 2-1: General overview of the experiment in Hopavågen.

	Hopavågen	
	Experiment 1	**Experiment 2**
Date and (duration) of experiment	20th – 27th April 2002 (7 days)	2nd – 8th May 2002 (6 days)
Treatments (no. of bags) and zooplankton abundances per litre, 2 replicates each	Copepods: large (8): 0.3, 0.9, 2.7, 8.1 small (8): 1, 3, 9, 27 Appendicularians (6): no growth Control (2): 0	Copepods: large (8): 1.3, 2.5, 5, 10 small (8): 5, 10, 20, 40
Copepod species	*Calanus finmarchicus* *Temora longicornis* *Acartia clausi* *Centropages hamatus* *Centropages typicus* *Pseudocalanus elongatus* *Oithona spp.*	As experiment 1
Ambient temperature	ø 7.2°C in April	ø 12.5°C in May
Water temperature	~ 8 – 9°C	~ 7.5 – 9.5°C
Salinity in enclosures	31.7 PSU	33.1 PSU

In accordance with the freshwater experiment (see chapter 1.1), 10 litres of water were sampled every day after the mesocosms were mixed with a Secchi disc. Chlorophyll *a*, temperature and nutrients were measured, and phytoplankton and zooplankton sampled every few days (Figure 2-2).

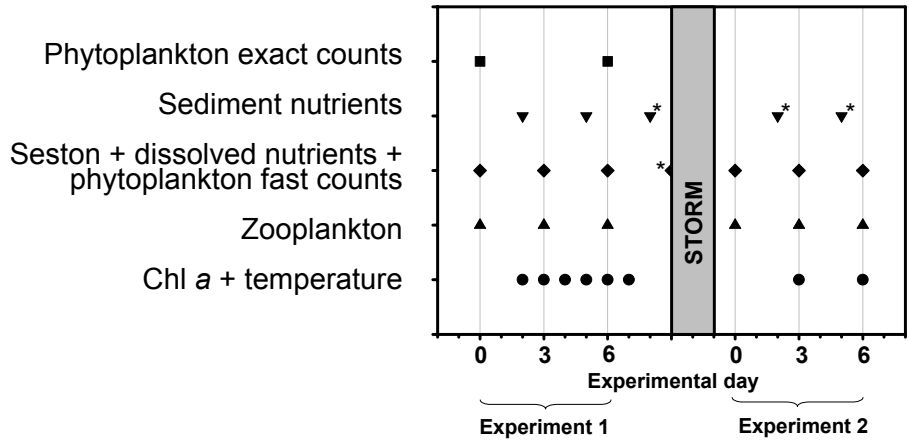


Figure 2-2: Time schedule of sampling, measurements and analyses. * denotes samples taken for control and appendicularian bags only.

Zooplankton

Zooplankton abundance was monitored by taking two net hauls every 3rd day (Figure 2-2), and calculating population density as described in chapter 1.1. The only differences to the freshwater experimental protocol were firstly the fixation method used (Lugol's solution ~2% final concentration) for a better detection of appendicularians in samples used for immediate counts, and secondly, the additional counting of naupliar abundance.

Phytoplankton

Chlorophyll *a* and temperature were measured *in situ* on a daily basis using a Fluoroprobe (BBE Moldaenke, Germany) (Beutler *et al.*, 2002), until technical problems with the probe prevented further measurements on day 7 of experiment one. Chlorophyll *a* was recorded in the enclosure bags during the experiment to follow changes in the phytoplankton biomass. In order to determine phytoplankton biomass of experiment 2, pre-filtered (<100 µm) samples from day 3 and day 6 were collected onto pre-combusted GF/F glass fibre filters and stored frozen until analysis of chl *a* by high performance liquid chromatography (HPLC) according to Wiltshire (1998). In order to compare impacts of large and small copepods, and their impacts over time, slopes of linear regression lines were calculated after logarithmical transformation of copepod densities.

Phytoplankton samples of both experiments were taken every 3rd day and counted in the same manner as described in chapter 1.1. Detailed phytoplankton counts were performed for distinct treatments at day 0 and 6. Algal biovolume was determined, calculated (Hillebrand *et al.*, 1999) and grouped according to the three different biovolume classes. To examine species-specific zooplankton impacts, a regression analysis was performed using the model $y=ax^b$ for each algal species counted (see chapter 1.1).

Stoichiometry

Dissolved, sestonic and sediment C, N and P (molar) as well as copepod C:N ratios (molar) were analysed as described in chapter 1.1. Sediment traps consisted of three 250 ml bottles exposed in all bags at maximum depth for 24 h every 3rd day of experiment 1, and days 2 and 5 of experiment 2. After careful removal of the traps from the enclosure bags, 300 ml of a mixture of the three sediment bottles were filtered onto pre-combusted Whatman GF/F filters for C and N analyses. For phosphorus analyses, another 300 ml were filtered onto acid washed Whatman GF/F filters. Before drying overnight at 60°C and analysis according to seston samples, filters were carefully examined microscopically and mesoplankton removed. Copepods were sorted into species for inter- and intraspecific analyses of copepod C:N ratios of *Calanus finmarchicus*, *Centropages* spp., *Temora longicornis*, and *Acartia clausi* for large and small bags.

Stable Isotope Analyses

Zooplankton was sampled from the storage containers filled with tows from Hopavågen (see above) at the beginning of the experiment (day 0), concentrated, shock frozen in liquid nitrogen and stored at –20°C (see appendix Feuchtmayr & Grey, 2003). At termination (day 7) of the first experiment, copepods from the large- and small-fraction bags, as well as from Hopavågen, were sampled by 250 µm and 100 µm net hauls. Zooplankton was maintained alive to gut evacuate (4 to 6 h) and treated as described for Schöhsee (see chapter 1.1). Thawed samples were sorted manually into taxa: *C. finmarchicus*, *Centropages* spp., *T. longicornis*, *Semibalanus balanoides*, *Evadne sp.* and *A. clausi*. Animals were concentrated onto pre-combusted Whatman GF/F filters after rinsing 3 times with artificial saltwater to avoid chemical reactions due to osmotic changes. Filters were oven dried at 60°C (~6 h) and stored in a desiccator. Between 2 and 5 *Calanus* spp., and up to 150 dried individuals for other species, were pooled per replicate into a tin cup for subsequent analysis of stable carbon and nitrogen isotopes. Three replicates were analysed when sufficient animals were available. In addition to copepod samples, particulate organic matter <50 µm from enclosure bags at termination was filtered onto pre-combusted Whatman GF/F filters and folded into tin caps. Stable isotopes were measured according to the procedure described in chapter 1.1. A precision of <0.1‰ for carbon and <0.2‰ for nitrogen was achieved by repeated measurement of an internal standard. Means of species-specific $\delta^{13}C$ and $\delta^{15}N$ zooplankton removed from enclosure bags at termination were calculated from large and small density bags.

2.2. Results

Unfortunately, despite repeated removal of zooplankton by net tows, copepod abundances in control bags could not be reduced below 2 individuals per litre, similar to the density of the lowest stocked bags. Strictly, these bags could not be used as controls and were not included in further analyses. Development within appendicularian bags initially seemed promising as *Frittillaria* sp. was detected at day 3, increasing to ~2 individuals per litre until day 6. However, further growth was inhibited by the storm, which damaged three appendicularian bags and which introduced copepods into the remaining three treatments via wave action. The first experiment was chosen for detailed analysis due to lower copepod mortality and/or more successful stocking of copepod densities compared to the second experiment (see below). Thus, most data presented for phytoplankton, stoichiometry and stable isotope analysis will focus on experiment one.

Abiotic factors and zooplankton

Mean copepod abundance in the fjord during the experimental period (20th of April to 8th of May) was 2.3 individuals per litre. This value is probably unrepresentative due to vertical migration. While sampling top 5m of the water column, copepods and cladocerans likely accumulated in deeper waters during daylight to avoid visual detection by predators or UV radiation (Bollens & Frost, 1989; Williamson, 1995). Indeed, copepod abundances increased with depth, up to 27 copepods per litre were found in 10-13 m depth of Hopavågen (Saage, 2003). Naturally occurring maximum densities in Hopavågen in April/May 1997 to 1999 were reported at ~10 individuals per litre, and increased with warmer temperatures to around 100 individuals per litre in June/July (N. Tokle, unpublished data). As ambient temperatures during the experiment were exceptionally high (mean values for Trondheim were reported at 7.2°C and 12.5°C in April and May in comparison with yearly means of 3.5 and 8°C, respectively), we felt that it was justified to inoculate higher densities in small copepod treatments (http://met.no/english/index.html, www.dwd.de/de/WundK/Klimadaten/index.html).

At the start of **experiment 1**, the zooplankton community in Hopavågen was dominated by copepod species, accounting for more than 70% copepods in all bags except in the two highest large copepod treatments, where only ~60% of the zooplankters were copepods. *Semibalanus balanoides* nauplii were most abundant within the remaining zooplankton, with a mean of 38% in the two highest stocked copepod densities. These nauplii developed into cyprids over the experimental time (from 41% on day 3 to 89% on day 6). Besides *Balanus*, low abundances (mean 3%) of zoea larvae, polychaetes, pluteus, bipinnaria, veliger and cyphonaute larvae, appendicularians, rotifers, small jellyfish and cladocerans were found in all bags. *Calanus finmarchicus* was the prevailing copepod species, followed by *Centropages*

spp. and *Oithona* spp. at start of experiment 1 (Figure 2-3). Mean species composition was similar in both size fractions, the only difference given by developmental stages: large copepods consisted of adults and advanced copepodite stages, small copepods of young copepodite stages. Temperature and salinity were similar in all bags.

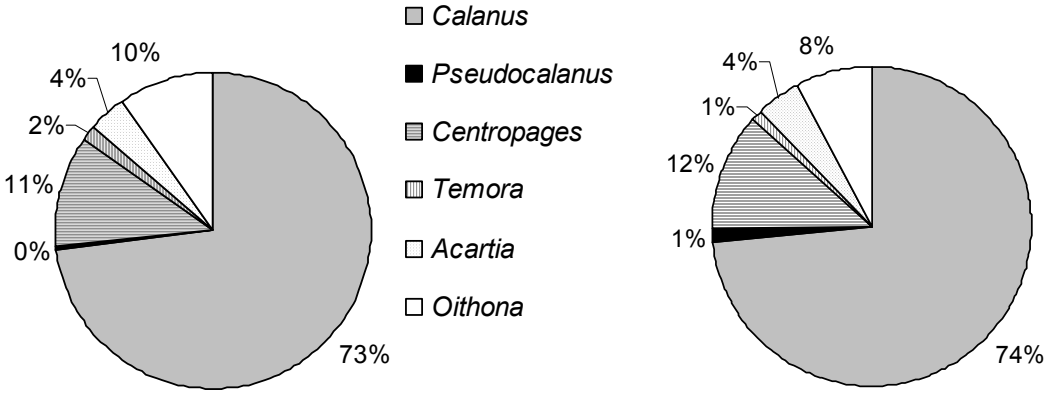


Figure 2-3: Mean copepod species composition at the start of experiment 1 in all large (left panel) and small (right panel) fraction bags (after Saage, 2003).

At the beginning of experiment 1, a density gradient in both large and small copepod treatments was successfully established, and it was largely upheld for the whole experimental period (Figure 2-4). Stocked abundances of copepods slightly exceeded desired values at the beginning, but densities decreased considerably over time, especially in highest treatments. The loss resulted mainly from decreasing *Calanus* numbers: from 73 % and 74 % of the total copepod assemblage on day 0 to 63 % and 65 % on day 6 for large and small copepod density gradients respectively.

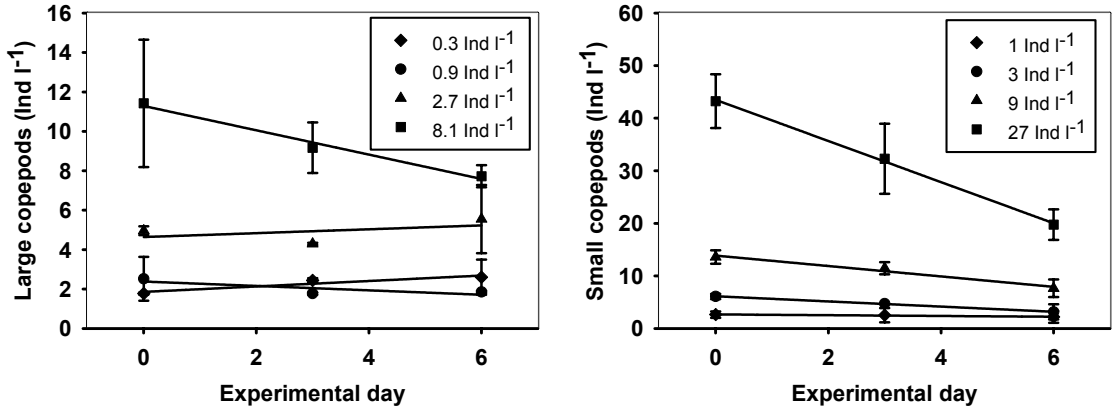


Figure 2-4: Large (left panel) and small (right panel) copepod abundance (individuals per litre) during experiment 1 shown as mean (±SD) of two replicate bags over time. Bags are named according to the nominal zooplankton stocking density. Note different scales on y-axis.

Zooplankton at the start of **experiment 2** consisted of more than 88% copepods in large, and 77% in small zooplankton bags (except for one small-fraction bag with 66% copepods, increasing to 98% at day 3). Copepod species recorded were similar to experiment 1, but proportions differed. *Calanus finmarchicus* again was the dominant species of the large copepods (86%), but only 26% *Calanus* was present at day 0 in small zooplankton bags (Figure 2-5). Here, *Oithona*, *Pseudocalanus* and *Temora* contributed considerably to the copepod biomass.

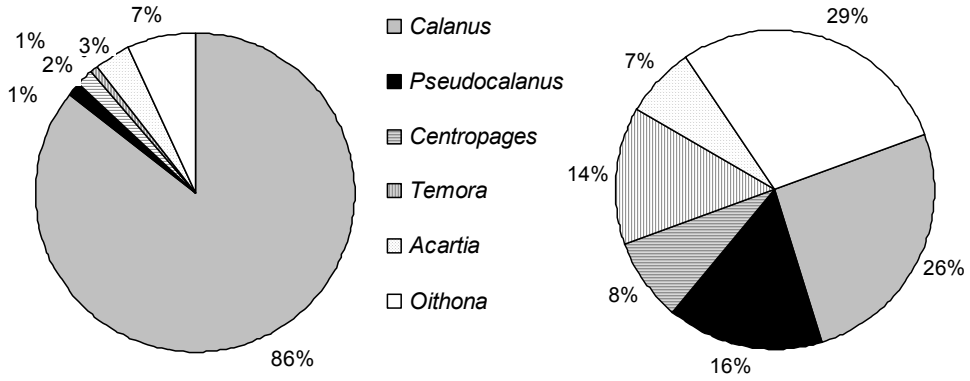


Figure 2-5: Mean copepod species composition at the start of experiment 2 in all large (left panel) and small (right panel) fraction bags.

As in experiment one, copepod densities decreased over time. *Calanus* declined over 6 days on average by 13% and 11% from inoculated abundances, for large and small copepods respectively. Overall, 55% (large) and 50% (small) of the initially stocked copepods were lost in high-density bags (Figure 2-6), and losses were more marked than observed in experiment 1 (32% in large, and 53% in small zooplankton bags).

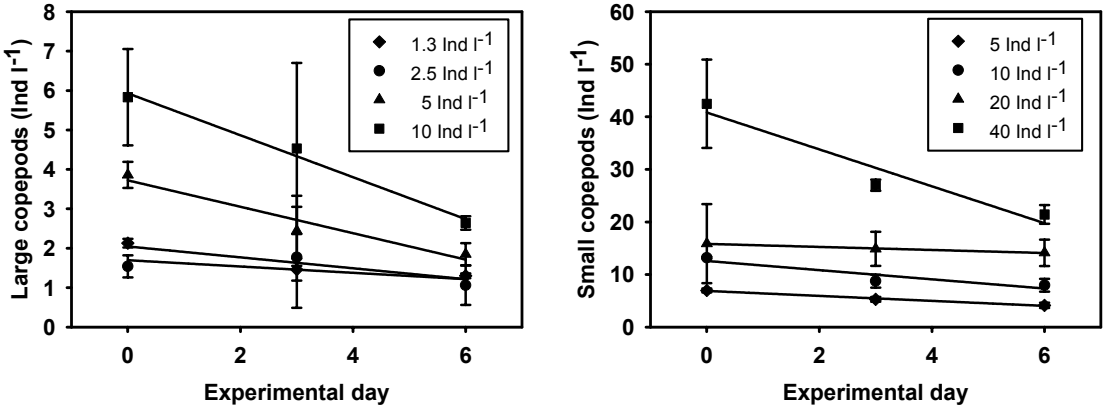


Figure 2-6: Large (left panel) and small (right panel) copepod abundance (individuals per litre) during experiment 2 shown as mean (±SD) of two replicated bags over time. Bags are named according to the nominal zooplankton stocking density. Note different scales on y-axis.

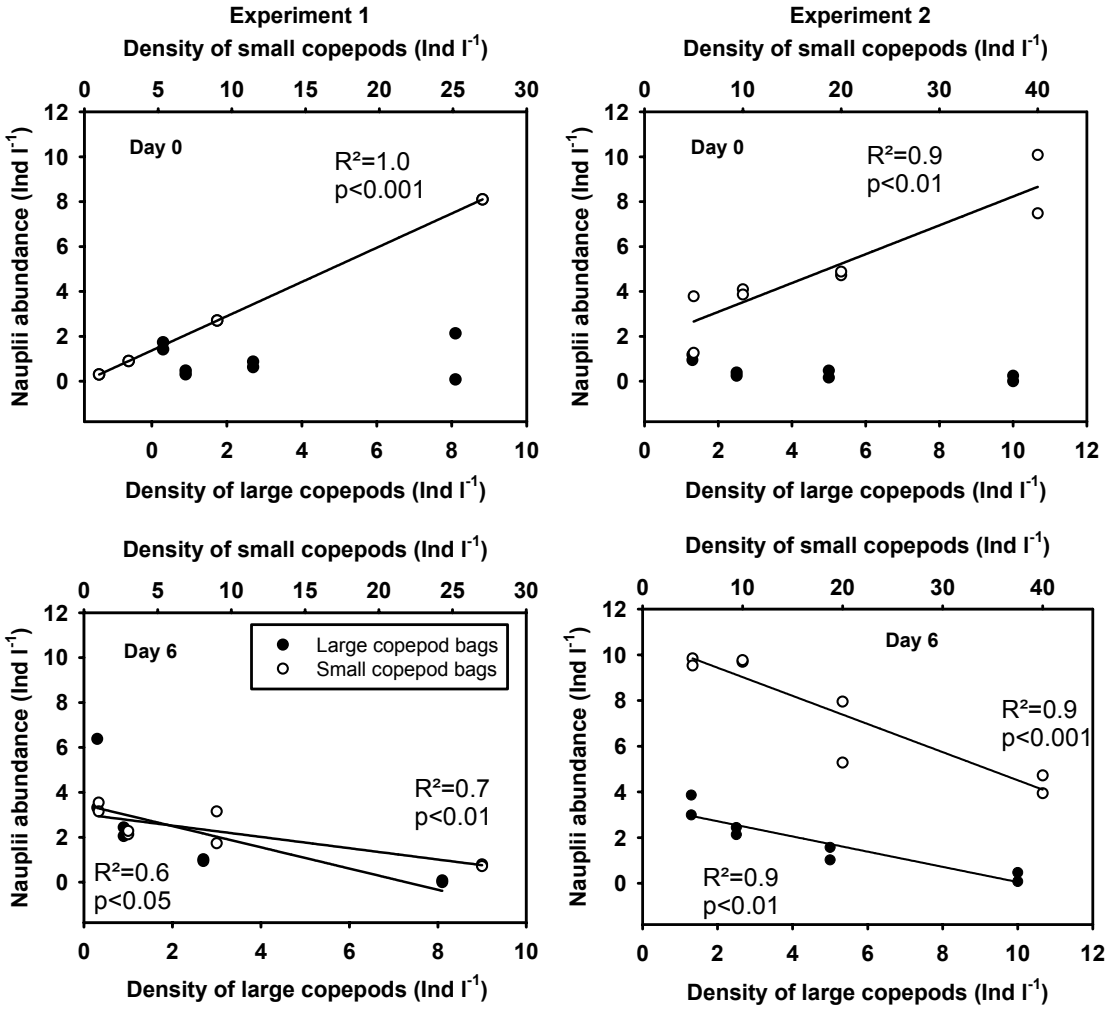


Figure 2-7: Copepod nauplii versus nominal copepod abundance for large (filled circles) and small (open circles) zooplankton density gradients at day 0 (upper panels) and day 6 (lower panels) of experiment 1 (left panel) and experiment 2 (right panel) (after Saage, 2003). Significant slopes of linear regression lines are shown.

Nauplii were added to the bags along with initial copepod inoculation. For small copepods, this 'nauplii enrichment' effect was more pronounced than for large treatments (Figure 2-7). Yet, positive relationships of nauplii and small copepod abundances were restricted to day 0 sampling. At the end of both experiments (day 6), nauplii showed the reverse trend, significantly declining with increasing copepod densities for all experimental gradients (Figure 2-7).

Phytoplankton

Chlorophyll *a* content at day 2 of the first experiment ranged from 1.8 to 2.9 µg chl *a* l^{-1} (mean: 2.2 µg chl *a* l^{-1}). While these concentrations and thus phytoplankton biomass remained relatively constant in high-density large-copepod bags, there was a noticeable decrease in bags stocked with low abundances of large copepods (see Figure 2-8 left panel for day 6). As a result, large copepods had a positive density-dependent impact, increasing chl *a* concentrations and algal biomass over time (Figure 2-8). Small copepod abundances also positively affected phytoplankton biomass (Figure 2-8, right panel) but not to the same extent as large copepods (compare slope parameters in Figure 2-8, right panel).

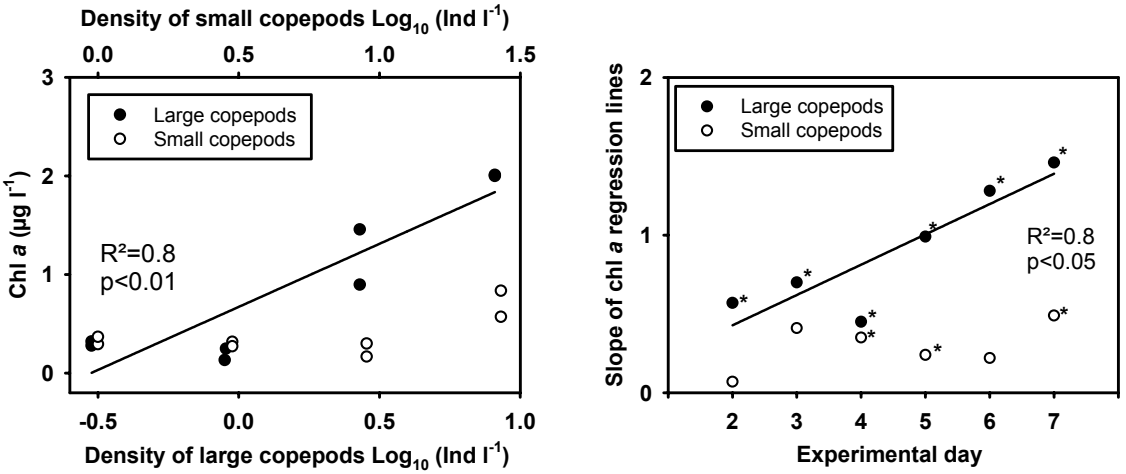


Figure 2-8: Left panel: Chlorophyll *a* values (µg l^{-1}) from large and small copepod bags at day 6 of experiment 1. Nominal copepod densities were log transformed. Right panel: Slopes of regression lines of total chlorophyll *a* (µg l^{-1}) values plotted for the different density gradients of large and small zooplankton over the experimental time for experiment 1. Significant slopes ($p<0.05$) of regression lines are marked with *. Significant slopes of regression lines and statistical parameters are shown on both panels.

The large copepod bags of experiment 1 were chosen for detailed phytoplankton analyses based on lowest copepod declines. Twelve species of Bacillariophyceae, Dinophyceae, Cryptophyceae and flagellates were distinguished for the first experiment (Table 2-2). A broad range of species biomass from 17 to ~ 6000 µm³ was available for zooplankton grazing in Hopavågen at the beginning of experiment 1. Maintenance of a

classification into three size classes: <100, 100-1000 and >1000 µm³ according to their biovolume is justified for comparison to fresh- and brackish water results. Yet, in Hopavågen, no species between ~180 and 1000 µm³ occurred, and the mid-size class only consists of species between ~100-180 µm³ (Table 2-2).

Table 2-2: Phytoplankton species or taxa, order, calculated biovolume and exponent b values from regression analysis ($y=ax^b$) for large copepod bags on day 6 of experiment 1. Significance is denoted by: *** when p<0.001; ** when p<0.01; and * when p<0.05.

Species/Taxa	Order	Biovolume (µm³)	b value large copepods Day 6, exp.1
Gyrodinium sp.	Dinophyceae	5810	-0.75***
Gymnodinium sp.	Dinophyceae	4945	-0.59**
Thekate dinoflagellates	Dinophyceae	3360	-0.52**
Peridinium sp.	Dinophyceae	2882	-0.5**
Naked dinoflagellates	Dinophyceae	1288	-0.56**
Cryptomonas sp.	Cryptophyceae	1006	-0.18
Skeletonema costatum	Bacillariophyceae	172 (single cell)	-2.13***
Heterocapsa rotundata	Dinophyceae	155	0.11
Nanoflagellates ~8 µm		134	0.22
Teleaulax acuta	Cryptophyceae	124	1.13***
Pseudonitzschia sp.	Bacillariophyceae	106 (single cell)	-2.5***
Nanoflagellates ~3 µm		17	0.42

Abundance of phytoplankton species and taxa <1000 µm³ by far exceeded that of species >1000 µm³ at day 0 and day 6. Thus, biovolume of species/taxa in addition to species abundances is shown in Figure 2-9 (in contrast to freshwater results, chapter 1.2) in order to analyse the impact of copepods on all three algal size classes. On day 0 of experiment 1, algal size distributions were nearly equal within all differently stocked large copepod bags (Figure 2-9). Overall total phytoplankton biovolume was slightly lower for bags with 2.7 or 8.1, compared to 0.3 and 0.9 copepods per litre, but this was not significant (ANOVA, $F_{3,4}$ = 3,04, p>0.05). After 6 days, mean algal biovolume was heavily reduced, from 690*10³ µm³ ml^{-1} on day 0, to 220*10³ µm³ ml^{-1} on day 6, across all different density treatments. Still, biovolume of cells >1000 µm³ increased for lowest copepod abundances over 6 days, but decreased with increasing copepod density. ANOVA ($F_{3,4}$ = 79,2, p<0.05) and a post-hoc Tukey-Kramer test revealed a significant difference between the two highest and the two lowest copepod density bags. Cells of size 100-1000 µm³ were impacted most heavily over the 6 days, declining from a mean algal biovolume of 550*10³ to 40*10³ µm³ ml^{-1} in the two lowest copepod density treatments. The impact was less marked (down to ~200*10³ µm³ ml^{-1}) at higher copepod densities. The reduction observed in the two lowest copepod densities was significantly different to the highest stocked copepod density

(ANOVA; $F_{3,4}$ = 35,7, p<0.01 and post-hoc Tukey-Kramer). Yet, small nanoflagellates <100 µm³ increased over the 6 days for the two highest copepod treatments, ending up significantly different from the two lower densities (ANOVA; $F_{3,4}$ = 77,4, p<0.01 and post-hoc Tukey-Kramer).

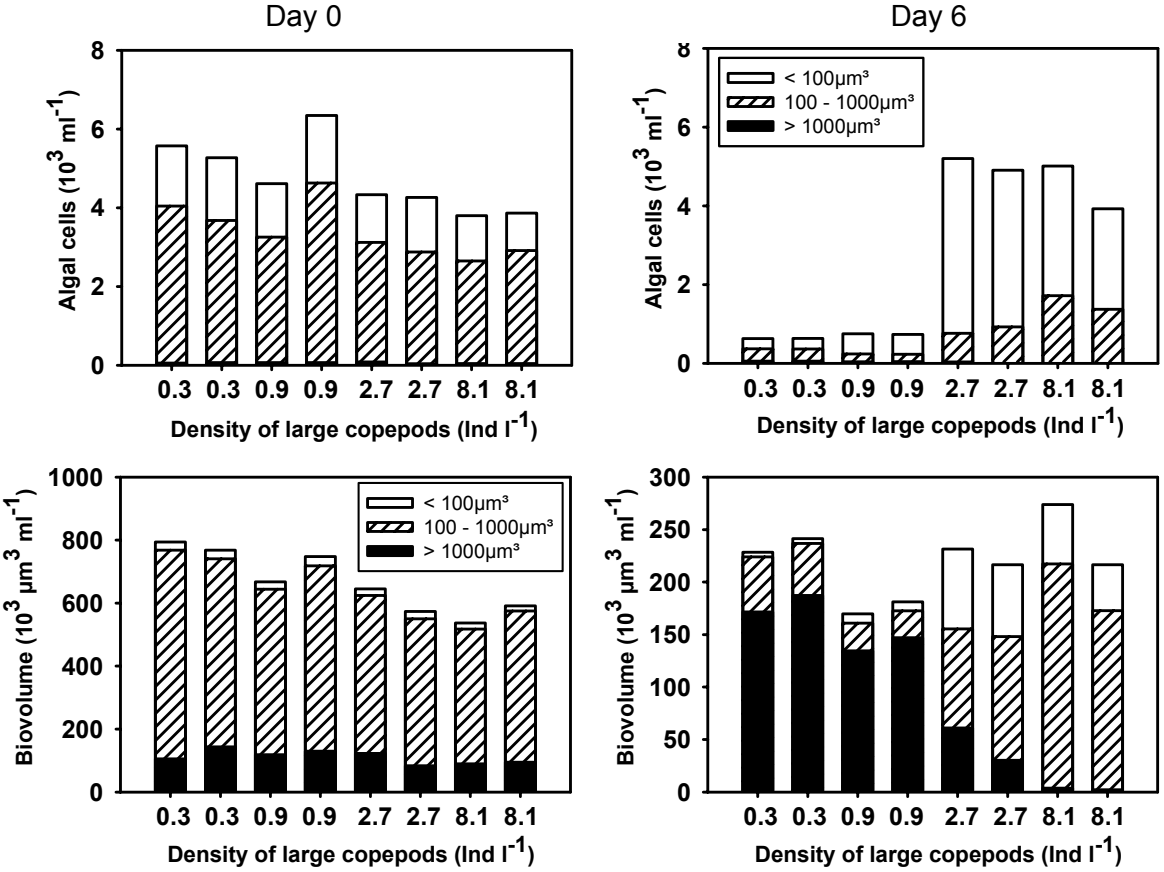


Figure 2-9: Phytoplankton or taxa abundance (upper panels) and biovolume (lower panels) on day 0 (left panels) and day 6 (right panels), experiment 1, classified into 3 biovolume size ranges for the large copepod density gradient. Nominal zooplankton densities are shown. Note different scales on y-axis.

Copepods showed density-dependent impacts on algal abundance for the different phytoplankton species, with b values ranging from –3.1 for the diatom *Pseudonitzschia* sp. to ~1 for *Teleaulax acuta* at day 6 (Figure 2-10, compare Table 2-2). The pronounced decline of the *Skeletonema* bloom (slope of the negative copepod density-dependent impact: –2.1) is primarily responsible for the extreme reduction of algal biovolume sized 100-1000 µm³. Density-dependent impacts by copepods were much lower on non-diatom species. Concordant with Figure 2-9, small sized species benefited from increasing copepod abundances, especially *T. acuta* (Figure 2-10, Table 2-2). *Pseudonitzschia* sp. and *Skeletonema costatum* are treated here as single cells, but prevailed as colonies. However, colony length was extremely variable, justifying their classification as single cells for biovolume determination. In addition, storage and transport of the Lugol-fixed samples from

Norway might have caused breakage of algal chains restricting detection of their naturally occurring length.

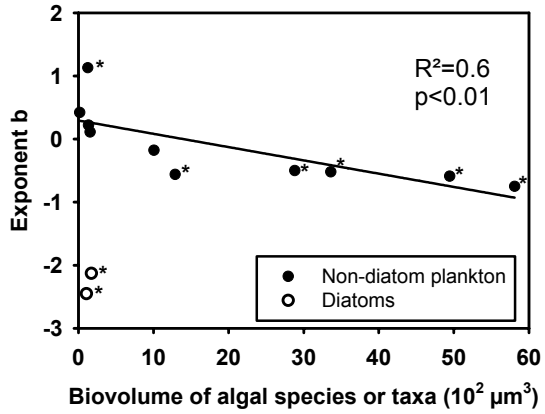


Figure 2-10: Species-specific regression analyses were performed using $y=ax^b$ for each algal species or taxa counted from large copepod bags of day 6, experiment 1, and exponent b values plotted against biovolume. Wherever regression analysis was significant ($\alpha < 0.05$), values are marked with*. Significant slope of regression line and statistical parameters are shown.

Stoichiometry

Dissolved nutrients

Mean total dissolved phosphorus (TDP) concentration at the start of the experiment was 0.4 µmol l^{-1}, and mean total dissolved nitrogen (TDN) concentration was 12 µmol l^{-1}. After 3 days (of experiment 1), neither large nor small copepods had induced a density-dependent impact, either on the single nutrients or their ratios (Figure 2-11). After 6 days however, large copepods mediated TDN and TDP ratios positively, while small copepods showed no significant relation (Figure 2-12). The increase of TDN and TDP with large copepod density resulted in a significant decrease of TDN:TDP. Small copepods did not change TDN and TDP concentrations density-dependent, resulting in a constant TDN:TDP ratio.

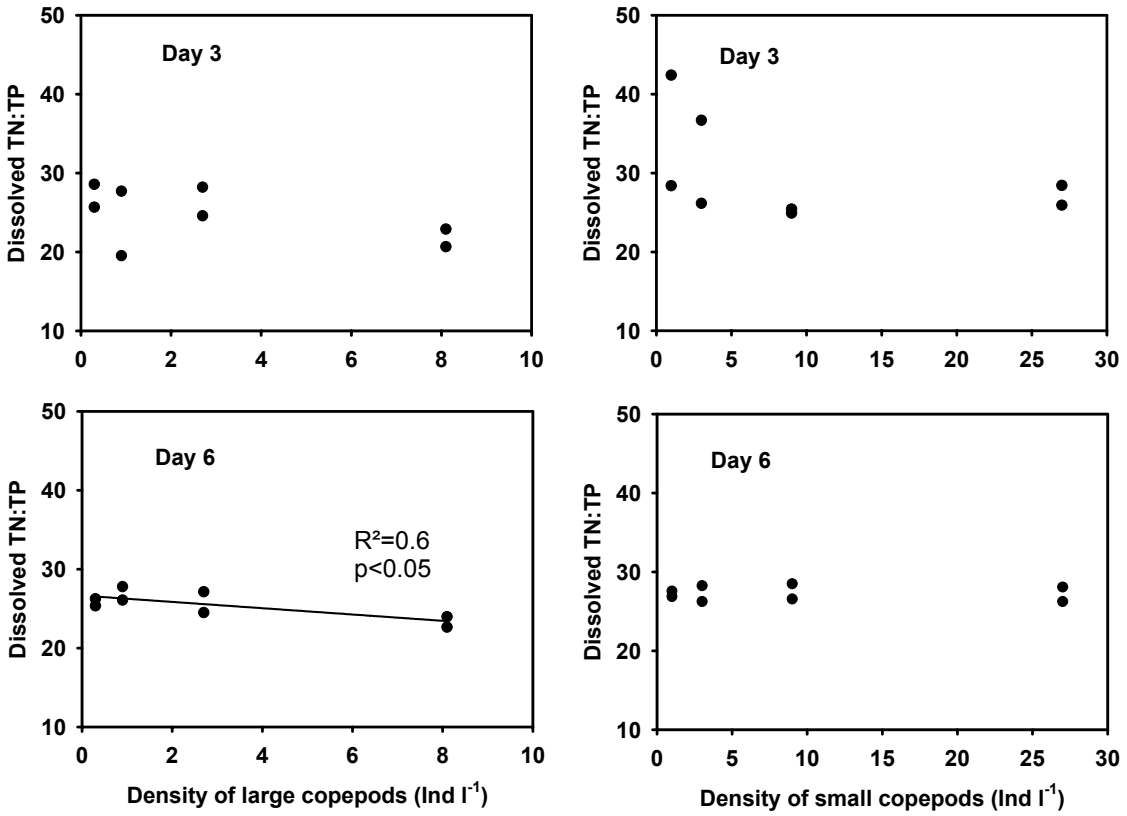


Figure 2-11: TDN:TDP ratios (molar) in relation to nominal copepod densities for treatments with large (left panels) and small (right panels) copepods at experimental day 3 (upper panels) and 6 (lower panels). Significant slopes of regression lines and statistical parameters are shown.

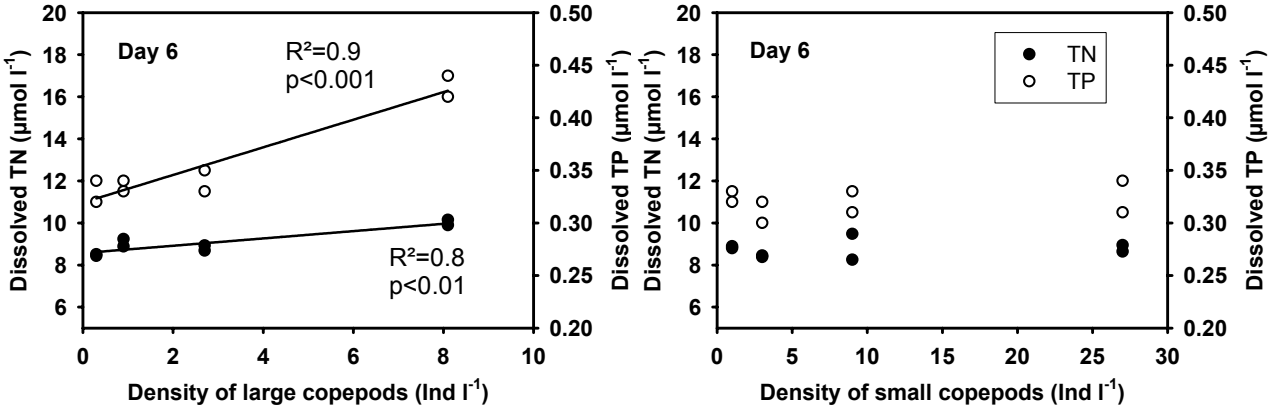


Figure 2-12: TDN and TDP in relation to nominal copepod densities for treatments with large (left panel) and small (right panel) copepod treatments at experimental day 6. Significant slopes of regression lines and statistical parameters are shown.

Since the chain-forming diatom *Skeletonema* was highly abundant at the beginning of the first experiment and because the main constituent of diatom frustules is silicate, we measured total dissolved silicate (TDSi) in order to track changes in concentration. A relation between copepod abundance and TDSi was evident (Figure 2-13); total silicate significantly decreased with an increasing number of large and small copepods (except after 3 days in the

large-copepod bags). Silicate concentrations also decreased in all bags over time, starting with 1 µmol l^{-1} at day 0, to mean 0.6 µmol l^{-1} at day 6.

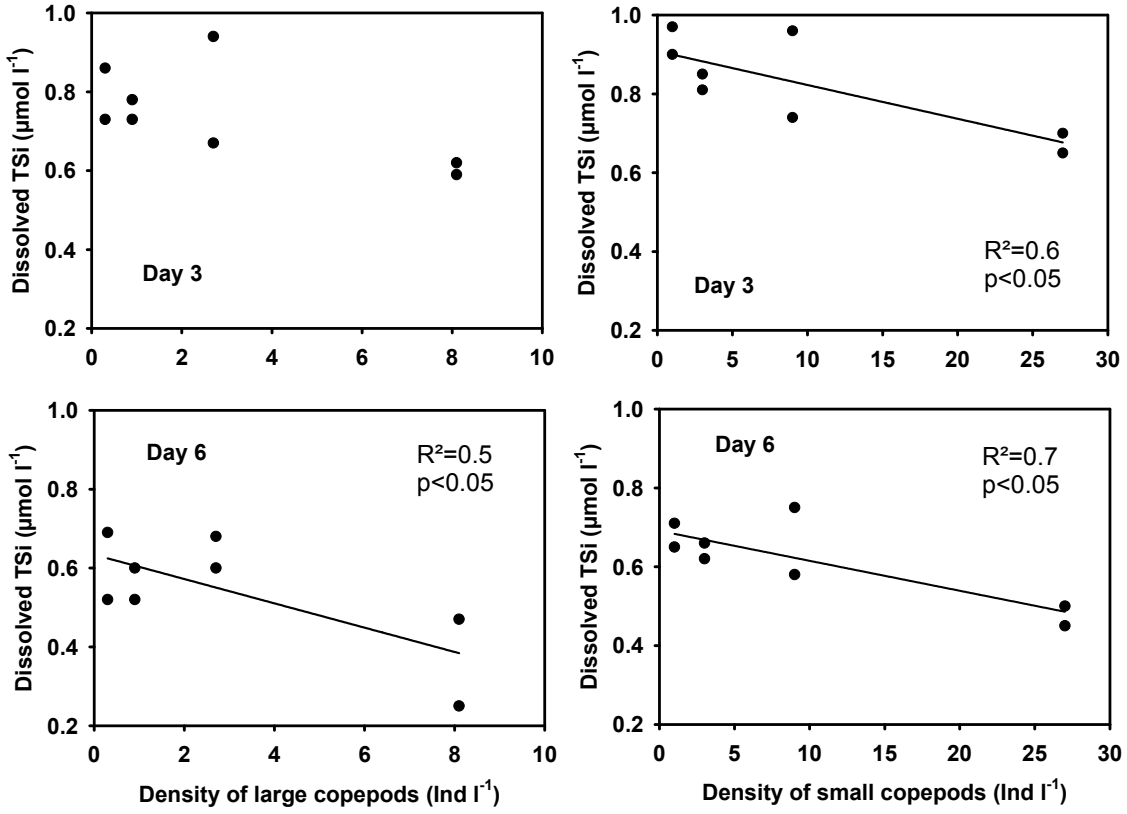


Figure 2-13: TDSi in relation to nominal copepod densities for treatments with large (left panels) and small (right panels) copepods at experimental day 3 (upper panels) and 6 (lower panels). Significant slopes of regression lines and statistical parameters are shown.

Sestonic nutrients

Starting concentrations of C and N were rather high (mean 37.4 ± 4.2 µmol C l^{-1} and 6.2 ± 0.6 µmol N l^{-1}) compared to low particulate phosphorus (mean 0.13 ± 0.07 µmol l^{-1}). Phosphorus was difficult to measure and varied quite considerably (SD ~ mean/2) due to low concentrations, not only at the beginning of the experiment, but also between replicates, over time, and between copepod densities. Thus, no copepod-induced changes of seston N:P or C:P ratios could be found. The N:P and C:P ratios varied from 30-200, and 160-600 respectively, in copepod bags at experimental start. Both small and large copepods made significant, density-dependent, negative impacts on seston C:N after 3 and 6 days (Figure 2-14). This could arise from an increase of particulate nitrogen or a decrease of carbon, and so seston N and C are shown separately in Figure 2-15. No increase of sestonic nitrogen with copepod density could be found, but there was a significant negative impact after 3 days for large copepods. Rather, a negative impact on particulate carbon was found for small copepods, and at day 3 for large copepods.

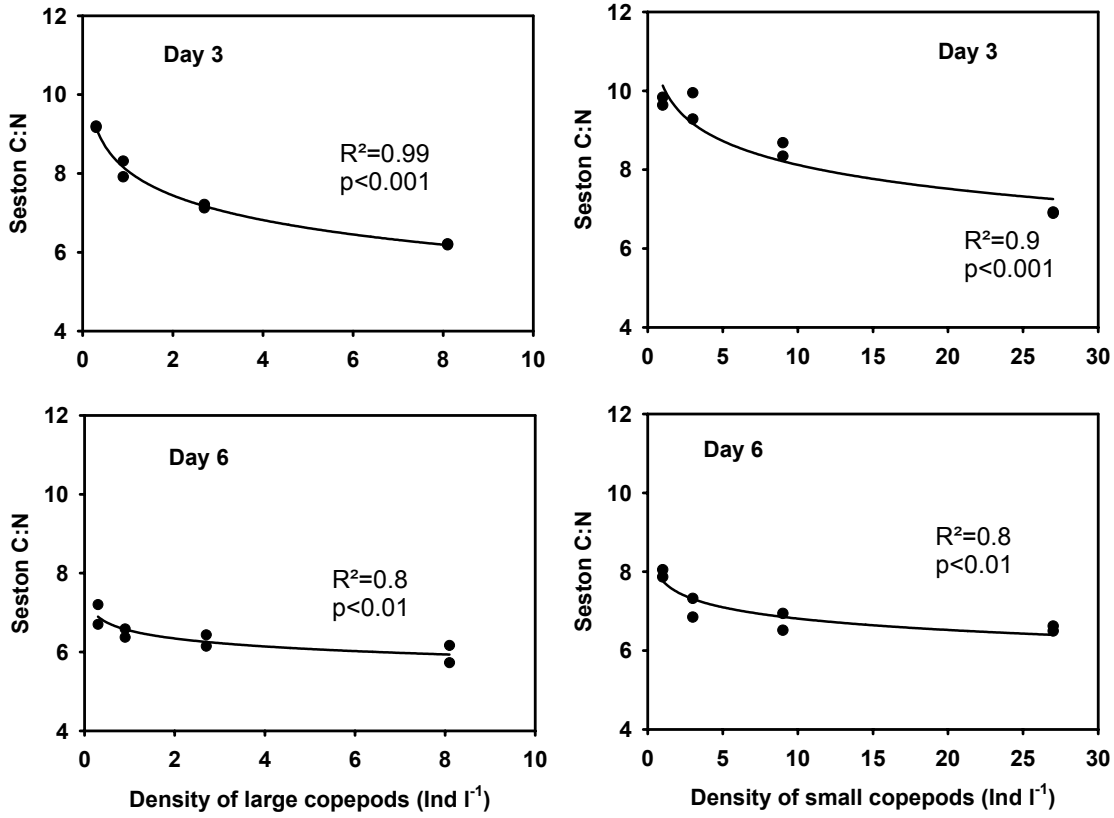


Figure 2-14: Seston C:N (molar) in relation to nominal copepod densities for treatments with large (left panels) and small (right panels) copepods at experimental day 3 (upper panels) and 6 (lower panels). Significant slopes of logarithmic regression lines and statistical parameters are shown.

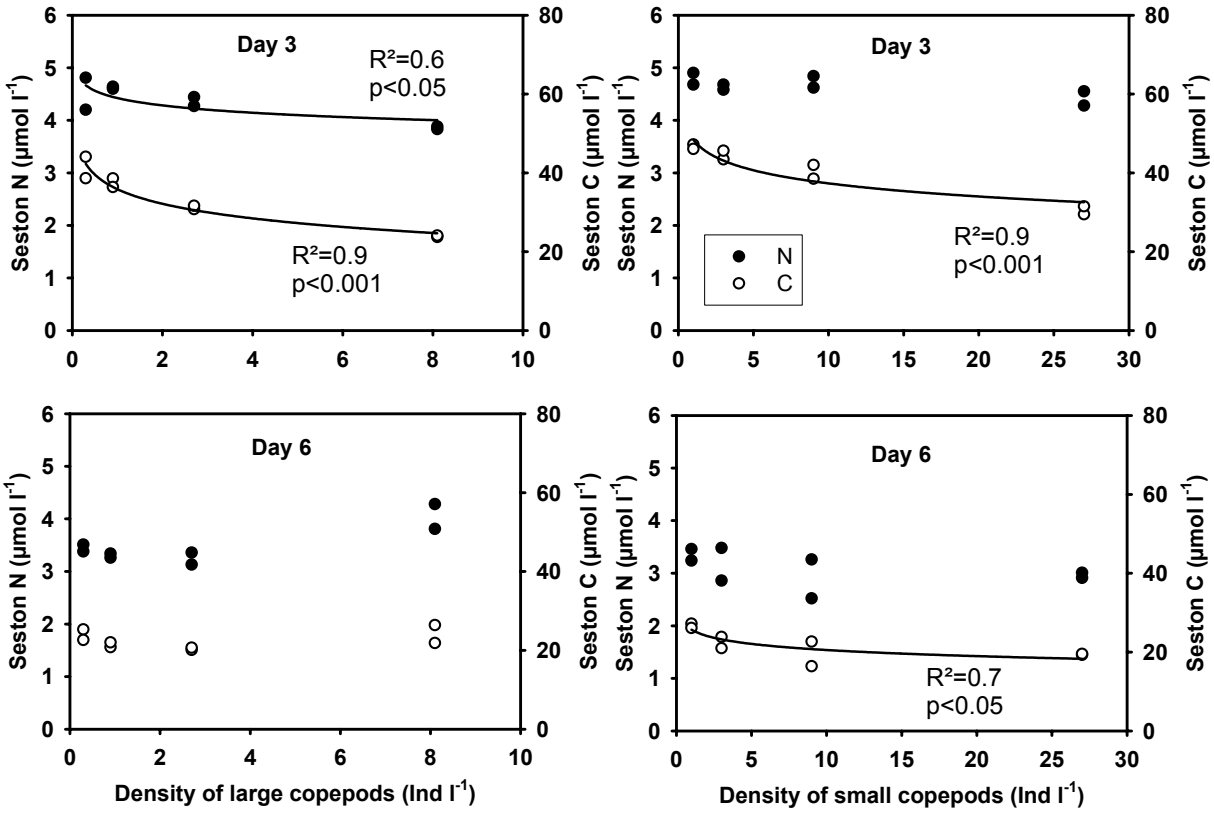


Figure 2-15: Seston N and C (µmol l^{-1}) in relation to nominal copepod densities for treatments with large (left panels) and small (right panels) copepods at experimental day 3 (upper panels) and 6 (lower panels). Significant slopes of logarithmic regression lines and statistical parameters are shown.

Sediment nutrients

Particles settling as sediment in the bags within 24 hours showed a different N:P ratio compared to suspended particles from the water column (Figure 2-16). Sediment was richer in nitrogen relative to phosphorus in most bags, compared to seston N:P ratios. No difference was found between large and small copepod bags.

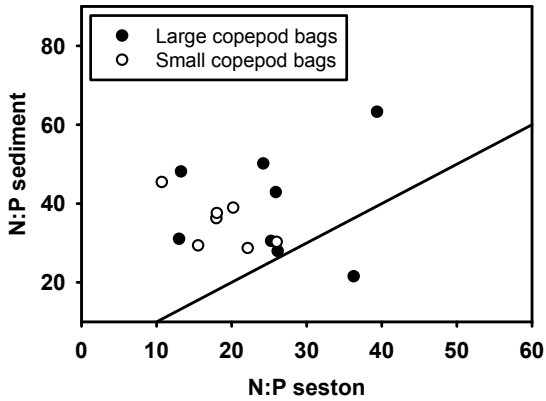


Figure 2-16: N:P ratios (molar) of suspended particulate matter and sediment. Data from day 6 for seston, and day 5 for sediment of experiment 1. The line of equality is shown.

Zooplankton stoichiometry

At the end of the experiment, *C. finmarchicus, Centropages* spp., *T. longicornis, S. balanoides* and *A. clausi* were sampled for C and N body stoichiometry. *A. clausi* was sampled from small copepod bags, but was insufficiently abundant in large copepod bags, and the opposite was found for *S.balanoides*. Individual species showed no significant intraspecific differences, mean C:N ratios were similar between large and small copepod bags (Figure 2-17). However, there were significant interspecific differences between *Calanus* and the other zooplankton species (ANOVA, $F_{6,41}$=21,2, p<0.001 and Tukey-Kramer post-hoc test, α=0.05). Comparing C:N of zooplankton to particulate seston ratios, values should scatter around the line of equality if zooplankton contain similar amounts of carbon in relation to nitrogen as their resources (Figure 2-18). For *C. finmarchicus* and *S. balanoides* C:N ratios are below, while ratios for *Centropages* spp., *T. longicornis* and *A. clausi* are above the line of equality. Thus, *Calanus* and *Semibalanus* contain a higher amount of carbon in relation to nitrogen than sestonic particles, while the other copepod species contain more nitrogen than carbon compared to their food particles.

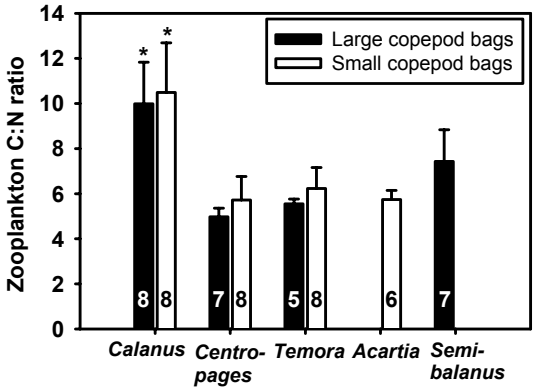


Figure 2-17: Mean zooplankton carbon to nitrogen ratios (molar) of *Calanus finmarchicus, Centropages* spp., *Temora longicornis* and *Acartia clausi* for small and large copepod bags at termination. Numbers in bars denote n values. Significant differences between species are marked with *.

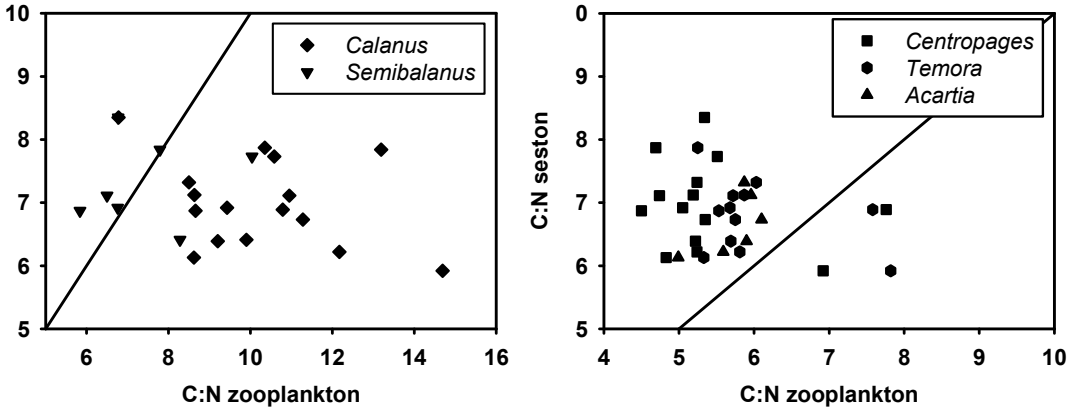


Figure 2-18: C:N ratios (molar) of *C. finmarchicus* and *S. balanoides* (left panel) and *Centropages* spp., *T. longicornis* and *A. clausi* (right panel) in relation to seston C:N (molar). Seston data were calculated as mean of the experimental period, whereas zooplankton was measured at day 6. Lines of equality are shown.

Stable Isotopes in Hopavågen and enclosures

In a similar manner to the copepod C:N ratios, *C. finmarchicus* stable isotope (SI) signatures differed markedly from the other species, exhibiting highest $\delta^{15}N$ (8.6 to 9.7‰) both in Hopavågen and in enclosures (Figure 2-19). The cladoceran *Evadne* occupied the lowest trophic position (relative to $\delta^{15}N$) with 4.4‰ at the start of the experiment. However, this value was derived from one sample, because *Evadne* are small and thus a large number are required to provide sufficient material for analysis. Zooplankton from Hopavågen at the start of the experiment exhibited a stepwise enrichment in both ^{13}C and ^{15}N. Changes in stable isotope signatures were noticeable after six days. *Calanus finmarchicus* was ^{13}C-depleted by 1.5‰ in Hopavågen. The other species in Hopavågen were only slightly ^{13}C-depleted, whereas copepods (except *C. finmarchicus*) maintained in the enclosures became more enriched in both ^{13}C and ^{15}N. As species showed negligible differences in $\delta^{13}C$ or $\delta^{15}N$ values between large and small copepod size groupings, each gradient was amalgamated and shown as a mean (± SD ‰, n= number given in Figure 2-17). No relationship between SI and copepod density was evident. SI signatures of seston <50 µm varied from –22.7 to –25.0‰ for $\delta^{13}C$ and 3.4 to 5.4‰ for $\delta^{15}N$. The POM $\delta^{15}N$ was significantly higher in enclosure bags compared to Hopavågen at the end of the experiment (t-test, t=3.3, p<0.05).

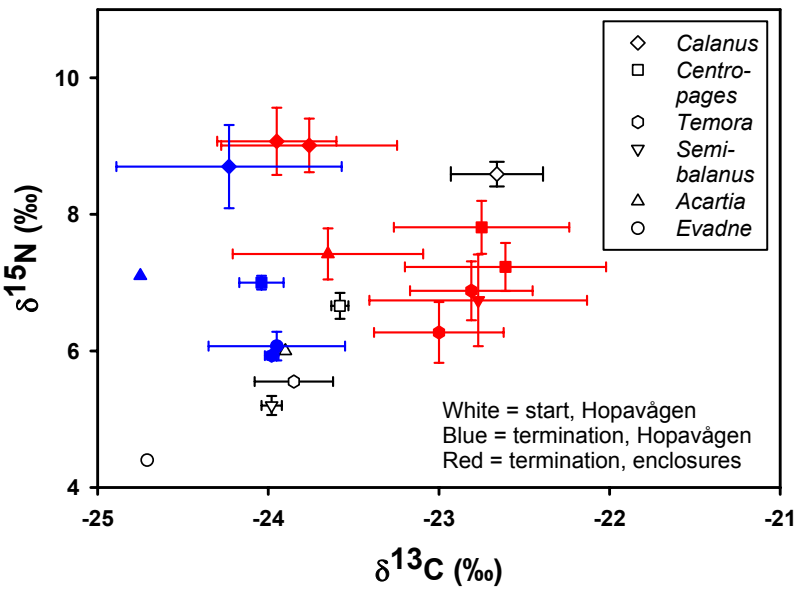


Figure 2-19: Mean (± 1SD, n = 3) $\delta^{13}C$ and $\delta^{15}N$ values of dominant Hopavågen zooplankton at start and end of experiment 1. Means of stable isotope signatures of the different density treatments of copepods and *Semibalanus* from the enclosures at termination were calculated for large and small copepod treatments.

2.3. Discussion

As copepods represented more than 70% of the zooplankton community in most bags at experimental set up (Figure 2-3), I am confident that copepods caused the main observed impacts on phytoplankton and nutrient stoichiometry. Differences in the proportion of copepods were found between treatments but the reasons for this are unclear. Wherever copepods comprised <70% (namely in high densities of large copepod bags), the majority of the remaining fraction consisted of *Semibalanus* nauplii and these nauplii developed into cyprids during the experiment. Cyprids characteristically do not feed (Todd *et al.*, 1996), and so I consider the experimental results as effects mainly caused by the different copepod species inoculated in enclosure bags.

The copepod species composition present in Hopavågen and added to enclosure bags (Figure 2-3 and 2-5) was typical for the Trondheimsfjord (Strømgren, 1973). *Calanus finmarchicus*, the dominant and one of the largest coastal species sampled (up to 3.5 mm in length), is the most abundant in the North Sea and Norwegian fjords (e.g. Tande, 1982; Aksnes & Magnesen, 1983), and is able to accumulate lipids to bridge seasonal food scarcity (Miller *et al.*, 1998). It emerges in early spring from overwintering in deep water (Heath, 1999), and reproduction starts around 40-50 days before the phytoplankton spring bloom (Niehoff *et al.*, 1999; Richardson *et al.*, 1999). Favourable food conditions in spring allow fast development of the offspring. At the start of experiment 2, juveniles had probably already developed into larger stages, driving the *C. finmarchicus* fraction up to 86% in the large copepod bags, whereas only 26% small *C. finmarchicus* stages were inoculated into the small copepod bags (Figure 2-5). Due to this life-cycle (reviewed in Hirche, 1996), advanced copepodite stages accumulate lipids when preparing for overwintering, but also adults of *C. finmarchicus* are lipid-rich (Kattner & Krause, 1987).

Naupliar enrichment of enclosure bags was higher in bags stocked with small copepods than in large copepod bags. Small copepods were netted in Hopavågen with a 250 µm net, and large *Calanus* nauplii are likely to be caught together with copepodites. Large copepods were sampled with a 500 µm net, through which nauplii can pass more easily. Thus, copepod nauplii were introduced in bags stocked with small copepods at day 0. This inoculation occurred copepod density-dependent, as more water from the containers was added to enclosure bags of high copepod abundances (Figure 2-7). The opposite was found after 6 days, when nauplii had declined with increasing densities of large and small copepods. Predation on naupliar stages is not described for *C. finmarchicus*, but inter- and intraspecific naupliar predation is reported for *Acartia tonsa* (Lonsdale, Heinle & Siegfried, 1979), *Temora longicornis* (Daan, Gonzalez & Breteler, 1988) and *Centropages hamatus* (Conley & Turner, 1985). Cannibalism is generally believed to occur in periods of poor food conditions in order

to ensure survival and reduce food competition (e.g. Landry, 1981; Daan *et al.*, 1988). Accordingly, in my enclosure experiments, the decrease of nauplii coincided with decreasing availability of suitable food particles with copepod abundances (see below). In addition to cannibalism, it is also possible that nauplii died of starvation, since the decrease of food particles was copepod density-dependent. However, the food spectra of nauplii differs from copepodites and adults. Nauplii are known to feed on smaller particles (Zankai, 1991; Roff *et al.*, 1995), and obtained higher growth rates at low food levels compared to older life stages when maintained in *in situ* mesocosms (Hygum, Rey & Hansen, 2000).

Copepods considerably reduced 100-1000 µm³ sized phytoplankton, and in particular *S. costatum*. This diatom contributed 71% to total biovolume at experimental start, but was reduced to 0.01% and 20.3% in highest and lowest large copepod densities after 6 days, respectively. To a certain degree, this observed decrease could result from sedimentation, although the classical view identifies feeding by zooplankton (here: *Calanus*) as the major component of spring bloom declines (Marshall & Orr, 1955; Meyer-Harms *et al.*, 1999; Hygum *et al.*, 2000). If sedimentation was important, the robust diatom frustules should have been visible during microscopic counting of phytoplankton since samples were taken immediately after mixing of enclosure bags when sediment was re-suspended. However, empty frustules were rarely detected in the samples. Thus, I assume that feeding by copepods caused the main impact on diatom reduction. Copepods reduced diatoms to a greater extent than all other potential food, including large dinoflagellates (Figure 2-10). As silicate frustules of *S. costatum* and other diatom species are often reported to pass nearly undamaged through the copepod gut, silicate is believed to be locked up in faecal pellets (e.g. see Turner, 1984a, and photos within). Thus, it is somewhat unclear why dissolved silicate decreased with copepod abundance (Figure 2-13) along with diatom removal.

It was expected that copepods should feed on phytoplankton and thus reduce its biomass, but both copepod size classes positively affected chl *a* concentrations. Large copepods increased phytoplankton biomass more than small copepods, even though a faster and greater reduction should be caused by large species due to their higher stocked biomass. This increase in chl *a* with copepod density, and over time, is consistent with findings for Schöhsee in spring (see chapter 1.2) and summer (Sommer *et al.*, 2001), which was ascribed to an increase of small and middle sized algal species <160 µm³ (Table 2-2, Figure 2-9). The greatest positive copepod impact was found upon the small cryptophyceae, *T. acuta* , which managed to increase considerably in abundance (Table 2-2). Similar to the freshwater experiment, the positive effect of copepods on small algae could reflect either a reduction of predation by removal of grazers (e.g. ciliates, (see Wiadnyana & Rassoulzadegan, 1989; Jonsson & Tiselius, 1990) or favourable resource conditions due to removal of nutrient competitors and/or beneficial nutrient stoichiometry. Although the dissolved TN:TP ratio decreased for large copepod bags on day 6 (Figure 2-11), both TDN

and TDP concentrations increased with copepod abundance (Figure 2-12). Thus, higher absolute amounts of nutrients were available to the phytoplankton. Besides, Zöllner (2004) showed that copepods decreased ciliate abundances in the same experiment in Hopavågen. Thus I suppose that both increased nutrient availability ('bottom-up') and decreased predation pressure ('top-down') promoted growth of small algae. Small copepods impacted chl *a* to a lesser degree than large copepods. Smaller copepods had a slightly lower feeding impact on ciliates compared to large copepods (Zöllner, 2004) and TDN and TDP concentrations were constant in small copepod bags (Figure 2-12). Thus, small phytoplankton could supposedly not increase in abundance as in large copepod bags due to higher grazer abundance and constant nutrient conditions.

There was also a copepod density-dependent impact on large dinoflagellates, consistent with studies showing feeding of *C. finmarchicus* on diatoms, ciliates and dinoflagellates (Nejstgaard *et al.*, 1994). Large dinoflagellates increased in abundance only in low copepod density bags (Figure 2-9). This result is rather surprising because selective feeding on dinoflagellates is reported (Meyer-Harms *et al.*, 1999) and cultured dinoflagellates contain more protein, carbohydrates and lipids than cultured diatoms (Mauchline, 1998). Several authors have reported diatoms to cause reduction in fecundity and/or egg hatching success in copepods (e.g. Chaudron *et al.*, 1996; Turner *et al.*, 2002; Ianora, Poulet & Miralto, 2003). Yet, copepods stocked in low densities in the enclosure bags had a higher impact on diatoms despite the availability of potentially higher quality food sources, suggesting that abundance of potential prey, coupled to energetic costs of active particle catching is of major importance: at experimental start, diatoms made up 72% of total biomass, dinoflagellates only 11%. Food abundance per copepod was lower in high copepod density bags, and after 6 days hardly any large food species were left. The decline in suitable food sources could have caused the decrease in copepods by food limitation in the highest copepod treatments (Figure 2-4). Abundance also may have declined due to a crowding effect, as has been described for *Daphnia* in freshwater (Burns, 2000). However, this was more likely to occur within the small copepod bags because large copepod abundances were similar to mean densities in Hopavågen (~12 copepods per litre (Saage, 2003)).

By these direct feeding impacts on phytoplankton, copepods are supposed to mediate dissolved nutrients by sloppy feeding, defecation or differential assimilation. At the start of the experiment, seston was assumed to be limited in phosphorus (C:N:P ratio in enclosure bags was 288:48:1, compared to the Redfield ratio of 106:16:1, (Redfield, 1958). Large copepods negatively affected the TDN:TDP ratio at day 6 (Figure 2-11). Copepods are known to retain nitrogen during food processing in their body relative to other nutrients taken up by feeding, and thus excrete relative more P compared to N (Sterner, 1990). The relatively low availability of TDN in the enclosure bags was expected to be passed on to POM because seston lacks the possibility to actively retain certain nutrients. Yet, copepods

did not induce an observed decrease in sestonic nitrogen except for a significant negative impact of large copepod densities after 3 days (Figure 2-15). Instead of causing an expected increase of particulate C:N ratios, there was a significant copepod density-dependent decrease in seston C:N after 3 as well as 6 days (Figure 2-14), indicating a more pronounced impact of copepods on particulate carbon than on other elements.

Although stoichiometric theory predicts lower N:P ratios in sediment rather than in suspended particles due to nitrogen retention by copepods, I actually found higher sediment N:P ratios (Figure 2-16). Copepods themselves are richer in nitrogen than in phosphorus (Sterner, 1990). When copepods grow, they increase their size by moulting, and their exuviae consist of chitin, which is composed of C, N and exoenzymes (Kirchner, 1995). In addition, as seen in Figure 2-4 and 2-6, copepod abundances declined in the highest copepods bags and part of this loss is likely to contribute to the sediment fraction. Both factors result in sedimentation of fragments of copepods and can be responsible for the relatively high nitrogen content in the sediment.

Stoichiometric theory and the retention of nitrogen by copepods relative to food carbon is indeed reflected by the C:N ratios of *Centropages* spp., *T. longicornis* and *A. clausi* (Figure 2-18). Their C:N ratios were lower (mean 5.6) than seston (mean 6.7). The only two species with higher C:N values compared to food sources were *Calanus finmarchicus* and the barnacle *Semibalanus balanoides;* values scatter below the line of equality in Figure 2-18. At the end of the experiment, *Semibalanus* was predominantly found in the non-feeding cyprid stage when it subsists on stored resources. Thus, carbon storage might have caused their high C:N ratio. Compared to the other species, *C. finmarchicus* showed significantly higher C:N ratios in bags stocked with large as well as small copepods (Figure 2-17). Gismervik (1997) observed that C:N ratios of calanoid copepods were generally below the Redfield ratio of 6.6, except for the fifth copepod stage of *Calanus*. Stage 5 is the overwintering stage of *C. finmarchicus*, characterised by high lipid storage (Hirche, 1996). Microscope examination of *Calanus* from Hopavågen in spring revealed a large amount of lipid droplets compared to the other copepod species present. Lipids contain ~70% carbon (Sterner & Elser, 2002) and lipid composition varies considerably in marine calanoid copepods, but can comprise up to 70% of their body mass (Båmstedt, 1986). Hence, elevated *Calanus* C:N ratios were likely to be caused by their lipid content. Further supportive evidence is presented by Walve (1999) who found higher C:N values in fat-rich species like *Pseudocalanus minutus elongatus* from the Baltic Sea.

Calanus finmarchicus exhibited markedly higher δ^{15}N signatures compared to the other zooplankton species (Figure 2-19). Nitrogen SI values from the northern hemisphere are generally reported ~8 to 9‰ (e.g. Hobson *et al.*, 2002), and thus comparable to Hopavågen values derived from this study (see Figure 2-19). However, there may have been some alteration of δ^{15}N values caused by preservation under liquid nitrogen, as reported for

freshwater species (see appendix Feuchtmayr & Grey, 2003), so direct comparison must take this potential affect into account. For the purposes of the current study, I preserved all species using the same method and can thus safely discuss the relation of different copepod species based on stable isotope results. *Calanus* is large compared to the other zooplankton species, and so the number of animals necessary per analytical sample was only 2 to 5 compared to around 50 to 80 individuals for smaller species. Thus, intraspecific isotopic variability contributed to the high standard deviations calculated for *Calanus* in Hopavågen; variable lipid droplet storage might have caused the variability of $\delta^{13}C$.

The *Calanus* $\delta^{15}N$ of ~8.7‰ in Hopavågen is surprisingly high compared to the other species when considering that typically a diet of phytoplankton and protozoa is reported for *C. finmarchicus* (Nejstgaard *et al.*, 1994; Meyer-Harms *et al.*, 1999). Unexpectedly, *Calanus* was ^{15}N-enriched by 2‰ compared to *Centropages*, which is known to be predatory and includes copepod nauplii in the diet (Kleppel, 1993; Titelman, 2001). Considering an enrichment factor of ~1‰ for carbon and 3.4‰ for nitrogen per trophic level (DeNiro & Epstein, 1978; Minagawa & Wada, 1984), the SI values suggest that *Calanus* in the fjord potentially fed upon the other zooplankton available, such as *Evadne*, *Semibalanus* nauplii or *T. longicornis* (Figure 2-19). Further, predation on copepod nauplii might have caused the high nitrogen signature, although the ability of *C. finmarchicus* to feed on nauplii or copepodites is still unknown. To my knowledge, there are no studies which have investigated whether marine cladocerans are a potential source of prey for copepod species. The $\delta^{13}C$ of *C. finmarchicus* decreased by 1.5‰ in Hopavågen within seven days, suggesting that the copepod can respond quickly to changing food isotopic composition or accumulate lipids during that short period.

Evadne was relatively ^{15}N- and ^{13}C-depleted at day 0. Gut content analyses of *E. nordmanni* showed grazing on diatoms, especially *Skeletonema costatum* (Kim, Onbe & Yoon, 1989), but these cladocerans can also ingest motile cells like dinoflagellates (Poggensee & Lenz, 1981; Kim *et al.*, 1989) and *Ceratium* (Nielsen, 1991). Compared to the trophic level inferred from the $\delta^{15}N$ of the copepod community, the low $\delta^{15}N$ of *Evadne* at day 0 indicates a primarily herbivorous diet, probably derived from the concurrent *S. costatum* bloom. However, at the end of the experiment, *Evadne* $\delta^{15}N$ was similar to that of *T. longicornis*, and had thus changed (2.6‰ and 0.8‰ in nitrogen and carbon, respectively) within 6 days, presumably reflecting a diet switch.

The *C. finmarchicus* $\delta^{15}N$ was consistently elevated during the experiment. Values even increased up to 9.9‰ when the copepods were exposed to decreasing food availability in the enclosure bags. This highlights the ability of *Calanus* to feed on the higher trophic levels of the Hopavågen copepod community. *Centropages* spp., *T. longicornis*, and *Semibalanus* nauplii all exhibited similar stable isotope signatures indicating that they probably assimilate

similar food sources in the enclosure bags. Generally, zooplankton carbon and nitrogen SI values in the experimental bags became heavier compared to start values recorded in Hopavågen, while in Hopavågen itself, the SI values tended to become relatively lighter over the same period. Thus, maintenance of *Centropages* spp., *T. longicornis*, and *Semibalanus* nauplii in the enclosures resulted in isotopic change. As discussed previously, suitable food sources (large phytoplankton, nauplii and ciliates (Zöllner, 2004)) were reduced by copepods over time. Nitrogen uptake by grazers is very low when food resources are limited, but losses continue. Losses of chemical compounds consist mainly of the lighter ^{14}N isotope, animals preferably retaining the heavier ^{15}N. Hobson *et al.* (1993) showed that unfed quails and geese recycle existing nitrogen, and by excretion of ^{14}N, increase their $\delta^{15}N$. An increase in $\delta^{15}N$ of *Daphnia magna* was also observed after 2-3 days of starvation (Adams & Sterner, 2000). However, there was no significant relationship found between copepod $\delta^{15}N$ and phytoplankton abundance, or biomass, or seston carbon and nitrogen content. Instead, I found that POM (<50 µm) $\delta^{15}N$ was elevated in enclosure bags compared to Hopavågen. Thus, the higher zooplankton nitrogen isotopic signature of food sources within enclosure bags supposedly caused the SI 'enclosure effect' of zooplankton. For *Semibalanus* another possible effect could result in the high differences of start and experimental SI signatures: *Semibalanus* was present as nauplii at the beginning of the experiment and as cyprids at the end of the experiment. I suppose the elevated isotopic values are caused by morphological and metabolic changes of the cyprid stage, associated with the use of stored resources (e.g. lipids) accreted during the naupliar stages.

Brackish water enclosures

Chapter 3

3. Brackish water enclosures

3.1. Study site and methods

Mesocosms used in the brackish water experiment were exposed in Kiel Bight, a fjord at the westernmost part of the Baltic Sea (see Figure 3-1), from 3rd until 16th of April 2003. Racks were attached to the sheltered side of a pier from the "Wasser- und Schiffahrtsamt Kiel – Holtenau" for protection from wind and waves, and covered with transparent plastic sheets to avoid fertilization from bird defecation (Picture 3-1).

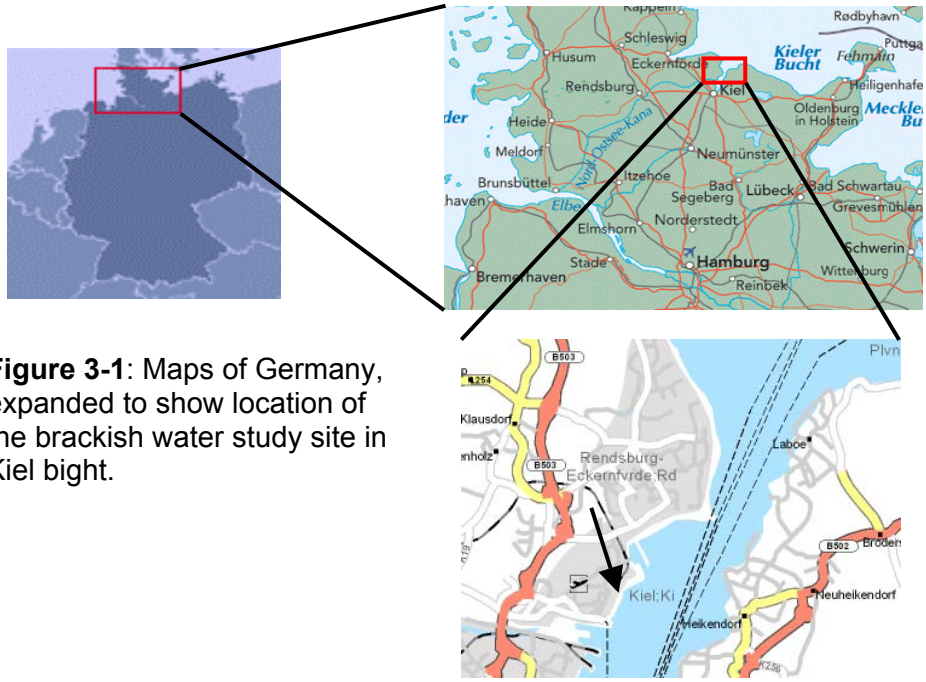


Figure 3-1: Maps of Germany, expanded to show location of the brackish water study site in Kiel bight.

For comparison with the other two experiments, a similar design of 24 enclosure bags was used and each bag was filled with 63 µm filtered water to prevent zooplankton inoculation. This mesh size allowed all phytoplankton to pass through, determined by microscopic examination three days before the start of the experiment. Copepods for inoculation were netted in Kiel Bight with a 250 µm net. The copepod community consisted mainly of *Centropages hamatus* (Lilljeborg) and *Acartia clausi* (Giesbrecht), with small quantities of *Temora longicornis* (Müller), *Oithona* sp. and *Pseudocalanus* sp. (Table 3-1). Jellyfish (small *Aurelia sp.*) also were abundant, and separated from copepods by screening the water through 500 µm gauze. Zooplankton was stored for around 6 hours in 250 litre containers before addition to enclosure bags. Different amounts of copepods were added to the bags in order to achieve a logarithmically scaled gradient: 5, 10, 20, 40 and 80 individuals per litre, replicated twice. The highest copepod abundances chosen are comparable to mean interim values for April-June (Albjerg *et al.*, 1996). It was unnecessary

to bubble containers with air because virtually no cladocerans were present. Another four bags received two different densities of *Aurelia* sp., gently added to enclosure bags at 6 and 12 individuals per litre. In a further eight bags, we supported appendicularian growth by removing zooplankton in a similar manner to the marine experiments in Hopavågen (see chapter 2.1). Two bags served as controls with regular removal of zooplankton by net tows.

Picture 3-1: Enclosure bag set-up of the Kiel bight experiment.

Table 3-1: General overview of the experiment in Kiel Bight.

Kiel Bight	
Date and (duration) of experiment	3rd – 16th April 2003 (14 days)
Treatments (no. of bags) and zooplankton abundance per litre, 2 replicates each	Copepods (10): 5, 10, 20, 40, 80 Jellyfish (4): 6, 12 Appendicularians (8): - Controls (2): 0
Copepod species	*Centropages hamatus* *Acartia clausi* *Temora longicornis* *Oithona* sp. *Pseudocalanus* sp.
Ambient temperature	ø 8.7°C
Water temperature	4 – 6°C
Salinity	14.4 PSU

After mixing the enclosures with a Secchi disc, 10 litres were sampled every third day. In contrast to Schöhsee and Hopavågen fjord, chlorophyll was not measured via the Fluoroprobe, but relative fluorescence was determined from sub-samples (similar to chlorophyll measurements) every 3rd to 4th day. Phytoplankton, zooplankton, dissolved and seston nutrients were sampled at regular intervals according to Figure 3-2.

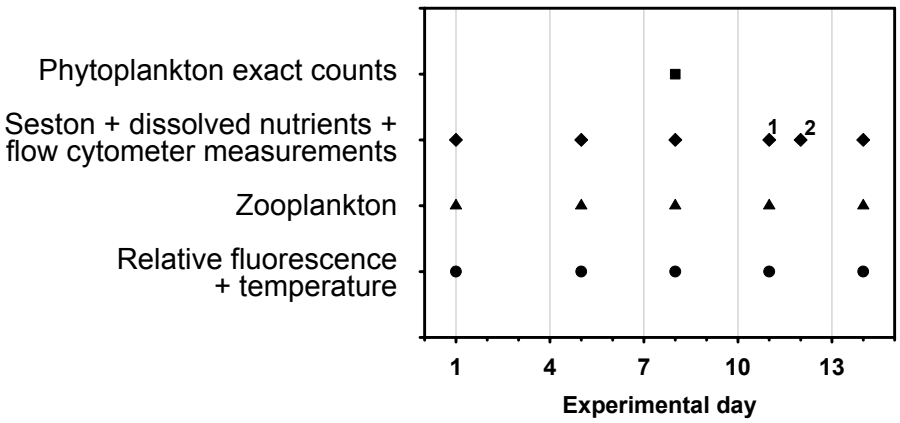


Figure 3-2: Time schedule of sampling, measurements and analyses. [1] denotes samples taken for seston nutrients only, [2] denotes no seston for nutrient analyses was sampled.

Zooplankton and phytoplankton abundances were monitored in the same manner as described for the saltwater experiment in Hopavågen (see chapter 2.1). Exact phytoplankton counts were performed at day 8, when 25-100 ml (depending upon species) were allowed to sediment and examined as described in chapter 2.1. Ciliate abundance from three different size ranges (diameter: <25 µm, 25-50 µm and >50 µm) was counted and biovolume calculated according to comparable algal shapes reported by Hillebrandt *et al.* (1999), and also, samples were analysed by flow cytometry to enumerate the smaller particles, such as picophytoplankton or bacteria. Undiluted samples were measured with a FACSCalibur flow cytometer (Becton Dickinson) at 488 nm and 633 nm. Phytoplankton samples were pre-filtered (64 µm), bacteria samples were fixed with formaldehyde and DNA stained with SYBR Green I before measurement. Dissolved inorganic nutrients (nitrate, nitrite, orthophosphate and silicate) were measured as described in chapter 2.1. Sestonic nutrient analyses were accomplished as described for Schöhsee and Hopavågen, with 0.5 litres of water collected on filters for seston C, N and P determination.

3.2. Results

Zooplankton

A copepod gradient was again successfully established, ranging from around 7 to 90 copepods per litre. However, copepod abundance declined over time in the two highest density treatments (Figure 3-3). Naupliar abundance increased with time, but was unrelated to copepod density. Exceptionally high nauplii numbers were found in bags with 20 copepods per litre (Figure 3-3).

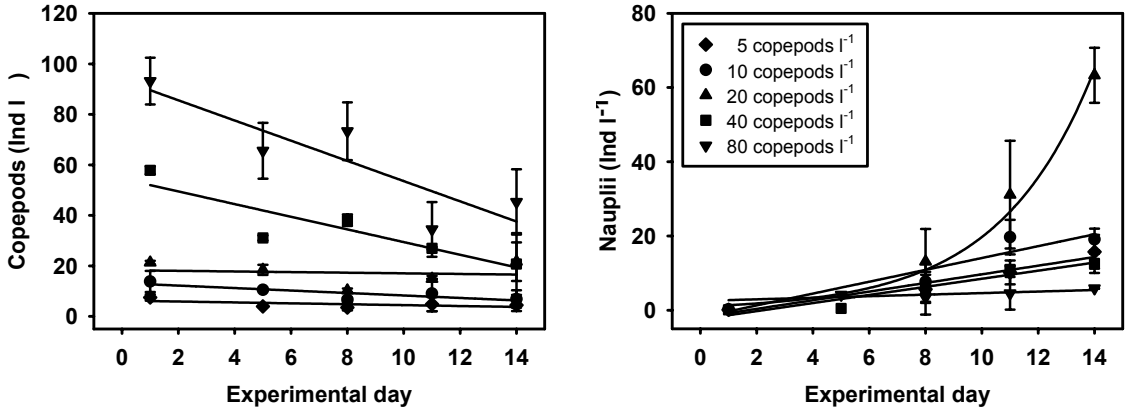


Figure 3-3: Copepod (left panel) and nauplii (right panel) abundance (individuals per litre) during the experimental period shown as mean (±SD) of two replicated bags. Bags are named according to the nominal zooplankton stocking density. Regression lines are shown. Note different scales on y-axis.

In three of the four *Aurelia* bags, copepod abundance was reduced (52%, 64%, 71% decline) to a greater extent than in copepod bags containing a comparable density of 5 and 10 copepods per litre (41, 50% decline). Simultaneously, nauplii increased in abundance (Figure 3-4) at a similar rate to the naupliar increase in the comparable copepod density bags.

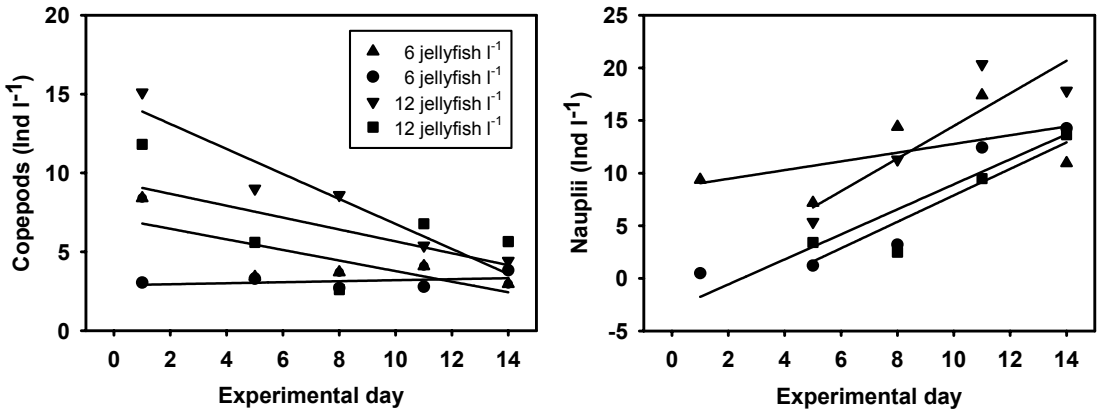


Figure 3-4: Copepod (left panel) and nauplii (right panel) abundance (individuals per litre) in bags stocked with different densities of *Aurelia*, during the experimental period. Bags are named according to the nominal jellyfish stocking density. Regression lines are shown. Note different scales on y-axis.

Unfortunately, as in the saltwater experiment, appendicularians could not be detected over the course of the experiment. Low temperatures during the experiment may have prevented growth. Appendicularians are reported to be very sensitive to temperature and generally occur from mid to late summer in the Kiel Bight, and off the British coast (Acuna *et al.*, 1995; Behrends & Schneider, 1995).

Phytoplankton

Relative fluorescence increased with copepod density after 3 days, indicating increasing phytoplankton biomass, and further increased until day 11 (for slope values see Figure 3-5). Afterwards, the density impact eased (day 14).

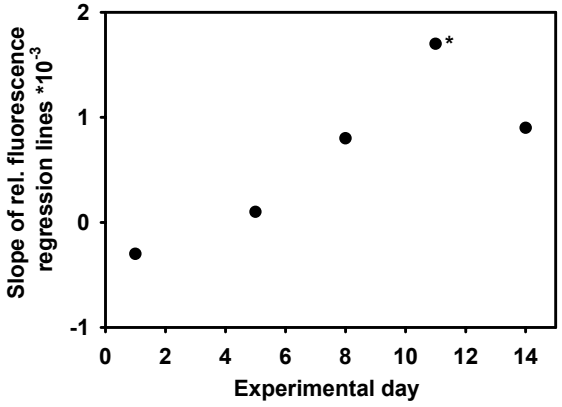


Figure 3-5: Slopes of logarithmic regression lines of relative fluorescence plotted for the density gradient over the experimental period. Significant slopes ($p<0.05$) of regression lines are marked with *.

Phytoplankton consisted of dinoflagellates and cryptophytes, common in Kiel Bight (Hydrographie, 2000). Within the ciliate community, *Mesodinium* sp. was most abundant and counted separately. Phytoplankton and ciliates showed a large variability in size from around 33,000 µm³ to 25 µm³ (Table 3-2). No species were found between 210 and ~2800 µm³. The copepod density-dependent impact on each species or taxa is shown as b values, negative impacts were found for all particles larger ~2800 µm³, the size category >1000 µm³ while small species (<210 µm³) increased with copepod density (Table 3-2).

Table 3-2: Phytoplankton species or taxa, order, calculated biovolume and exponent b values from regression analysis ($y=ax^b$) for copepod bags on day 8. Significance is denoted by: *** when p<0.001 and * when p<0.05.

Species/Taxa	Order	Biovolume (µm³)	b value copepods Day 8
Gyrodinium sp.	Dinophyceae	33115	-0.52*
Ciliates > 50 µm		25980	-0.72***
Ciliates 25-50 µm		20538	-0.26*
Gymnodinium sp.	Dinophyceae	9017	-0.35*
Ciliates < 25 µm		7238	-0.19
small *Gyrodinium*	Dinophyceae	5387	-0.27*
Micracanthodinium claytonie	Dinophyceae	4611	-0.14
Mesodinium rubrum	Ciliate	2845	-0.32
Teleaulax acuta	Cryptophyceae	210	0.26
Heterocapsa rotundata	Dinophyceae	184	0.08
Plagioselmis prolonga	Cryptophyceae	25	0.28*

After 8 days, dinoflagellates and ciliates larger1000 µm³ were rare, but high abundances were found for the three small species *T. acuta, H. rotundata* and *P. prolonga* (Figure 3-6). Classification in size ranges showed a significant difference between highest copepod densities and control bags for cells >1000 µm³ (ANOVA; $F_{5,6} = 5.8$, p<0.05 and post-hoc Dunnett's test). For smaller species, in contrast to the other enclosure experiments, abundances were not significantly different (ANOVA; $F_{5,6} = 3.6$, p>0.05 for 100-1000 µm³, $F_{5,6} = 1.9$, p>0.05 for <100 µm³ algae).

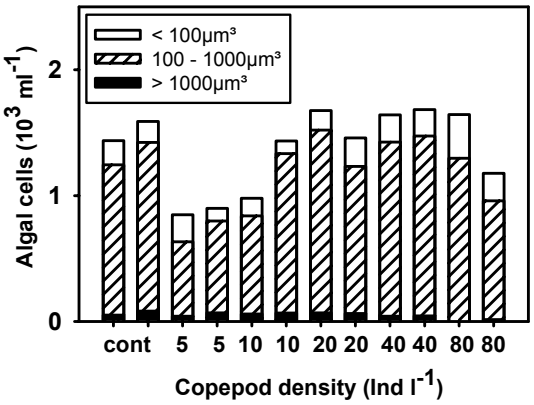


Figure 3-6: Phytoplankton and ciliate abundance on day 8, classified into 3 biovolume size ranges for the nominal copepod density gradient.

While negative impacts of copepod densities were found for all species >1000 µm³, small algal cells were positively affected, benefiting from increasing copepod abundances (see above and Figure 3-7). This change of algal community composition by copepods was linearly related to algal biovolume, i.e. the more copepods present, the higher the shift towards a phytoplankton community dominated by small sized species (Figure 3-7).

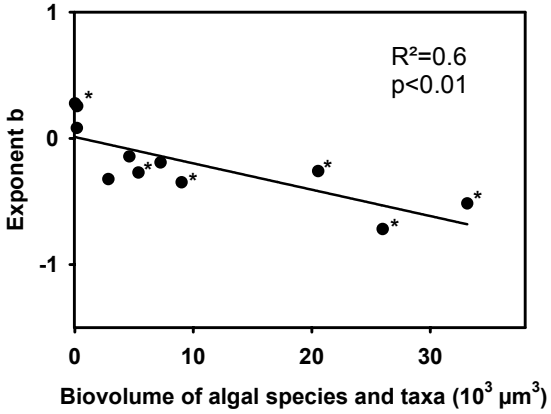


Figure 3-7: Species-specific regression analyses were performed using $y=ax^b$ for each algal species or taxa counted, and exponent b values plotted against biovolume for day 8. Wherever regression analysis was significantly related (α <0.05), exponent b values are marked with *. Significant slope of regression line and statistical parameters are shown.

Flow cytometry (FCM) provided an insight into the abundance of particles, which were too small to be examined using inverted microscopy, and also separated bacteria from autotrophic plankton. However, in the higher size range of the flow cytometer, an overlap between microscopically counted and FCM measured species occurred. By using size differentiation, and allocation of pigments to single phytoplankton taxa, species such as *T. acuta, P. prolonga* (dinoflagellates contain phycoerithrin (Beutler, 2003)) and *H. rotundata* could be distinguished via FCM. The combination of microscopic counts and FCM analysis revealed that ciliate abundance affected picophytoplankton smaller than ~4 µm³ and bacteria abundance negatively. However, increasing copepod density stimulated bacterial and picophytoplanktonic growth significantly (Figure 3-8).

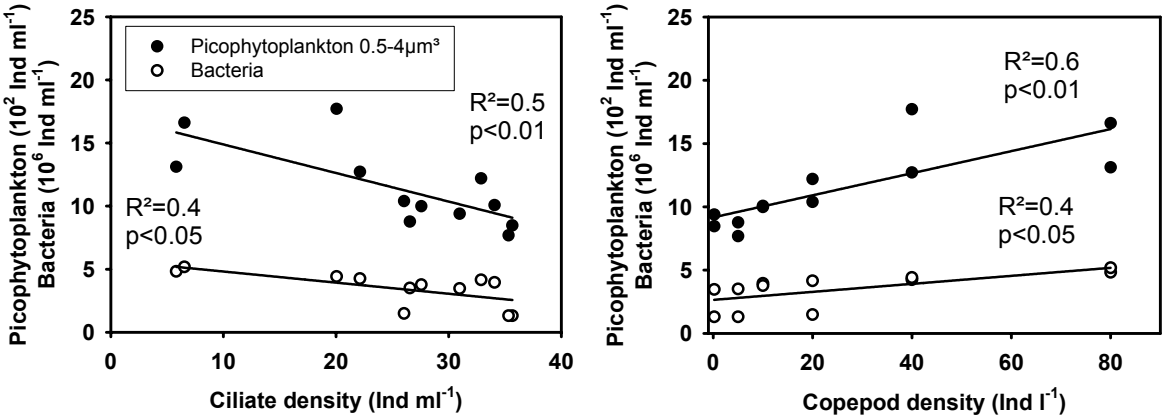


Figure 3-8: Picophytoplankton and bacteria abundance versus ciliate (left panel) and copepod density (right panel) at day 8. Significant slopes of regression lines and statistical parameters are shown.

Stoichiometry

Dissolved nutrients

Dissolved inorganic N:P ratios varied considerably between replicates at the beginning of the experiment, but were not copepod density-dependent. No significant impact was induced by the copepods after 8 days. However, at day 14, copepods increased DIN, but not as strong as DIP, resulting in a decreased DIN:DIP ratio (Figure 3-9).

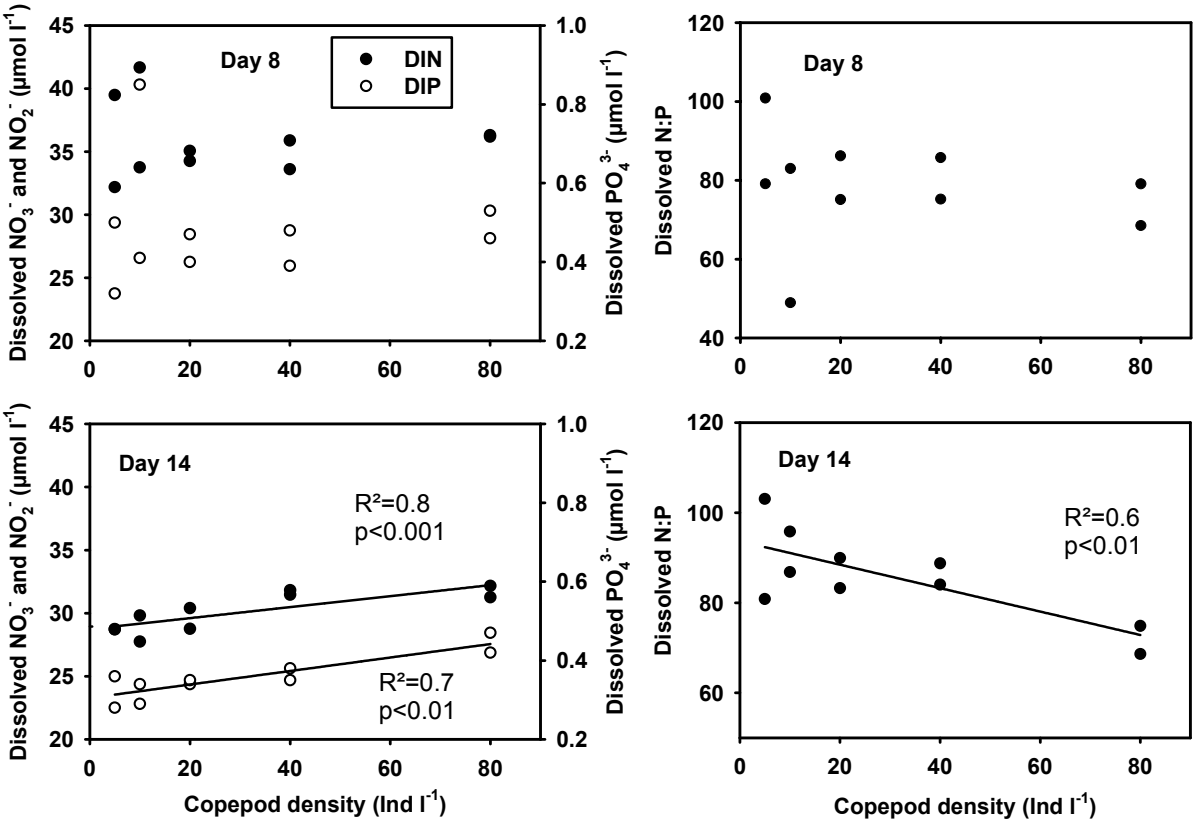


Figure 3-9: Dissolved nitrate, nitrite and orthophosphate (left panel) and their ratio (right panel) in relation to nominal copepod density bags for experimental day 8 (upper panels) and 14 (lower panels). Significant slopes of regression lines and statistical parameters are shown.

During the course of the experiment, copepods did not impact DIN or DIP time-dependently ($p>0.05$). Neither single nutrients (data not shown), nor the DIN:DIP ratio changed over time, shown here for two randomly chosen treatments: bags stocked with 20 and 80 copepods per litre (Figure 3-10). Silicate data cannot be presented due to analytical problems.

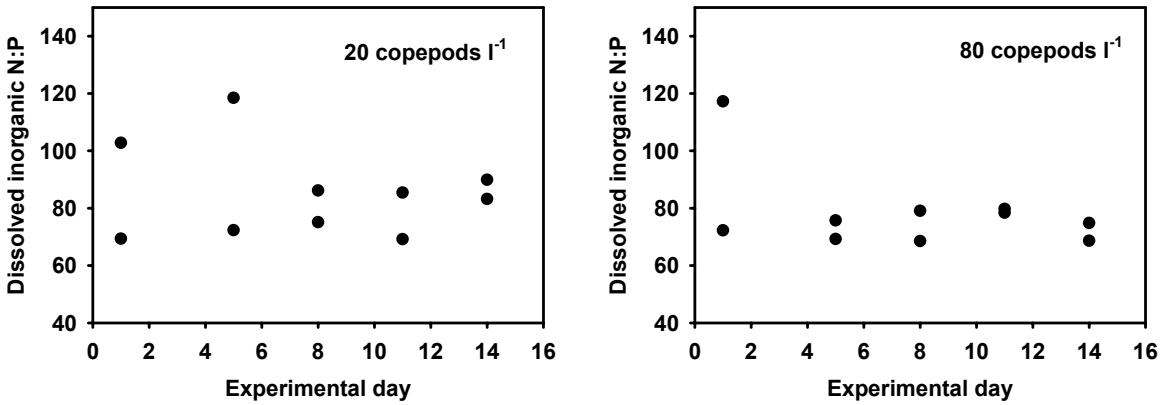


Figure 3-10: DIN:DIP ratios (molar) over experimental time of 14 days for randomly chosen treatments (20 copepods per litre - left panel, and 80 copepods per litre - right panel).

Sestonic nutrients

C:P ratios of seston at day 0 corresponded to the Redfield ratio of 106:1 (Redfield, 1958) but nitrogen was available in excess (N:P of 24:1). Elemental seston N and P concentrations were not altered with copepod density (data not shown) unlike the pattern found for DIN and DIP at day 14. Further, the decrease of the DIN:DIP ratio with copepod density was not passed on to the seston N:P ratios: no significant relationship was found after either day 8 or 14 (Figure 3-11). Generally, both phosphorus and nitrogen were positively affected by copepod density, levelling out in a rather constant N:P ratio.

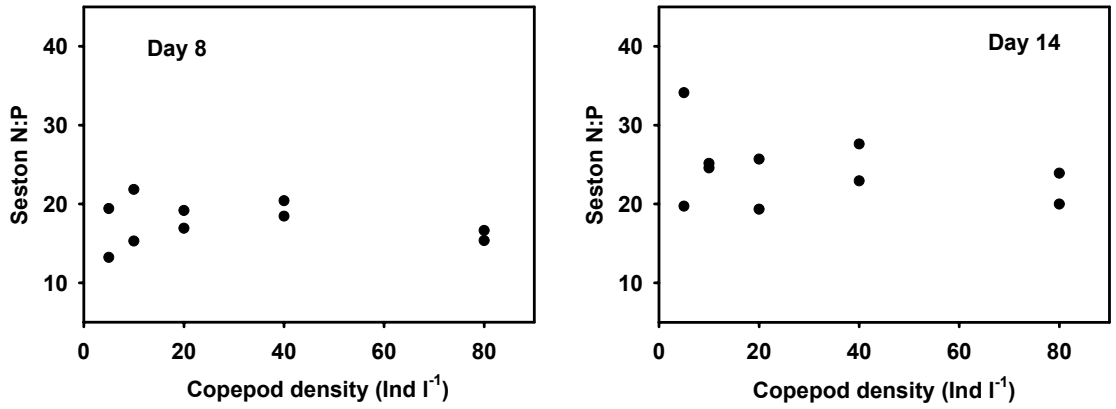


Figure 3-11: Seston N:P ratios (molar) in relation to nominal copepod density bags for day 8 (left panel) and day 14 (right panel).

I found no copepod induced change in seston N:P over the experimental period (data not shown), consistent with dissolved inorganic nutrients. However, all copepod densities had a significant negative impact on seston C:N ratios during the experiment (Figure 3-12), in contrast to seston N:P and C:P ratios (data not shown).

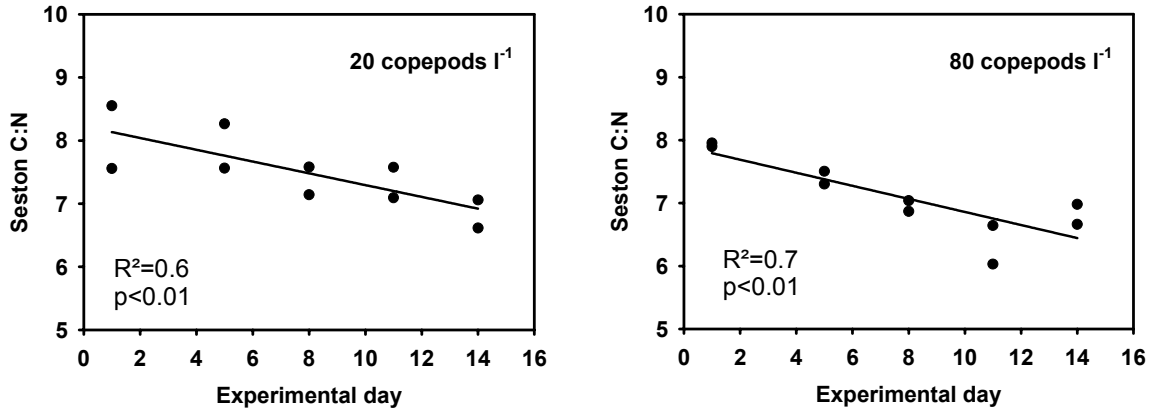


Figure 3-12: Seston C:N ratios (molar) over the experimental time of 14 days for randomly chosen treatments (20 copepods per litre - left panel) and 80 copepods per litre - right panel). Significant slopes of regression lines and statistical parameters are shown.

3.3. Discussion

Zooplankton abundance in the Kiel Bight typically peaks in early spring, followed by a tremendous reduction caused by jellyfish such as *Aurelia aurita* (Schneider, 1989), and the experiment was planned to coincide with this period. However, in 2003, an exceptionally early phytoplankton bloom was recorded in the Belt Sea, especially around the Kiel Bight and Fehmarn Belt, starting in mid February (Ærtebjerg, 2003). By the beginning of April, this bloom was reduced, presumably by a high abundance of copepods, which are in turn susceptible to jellyfish predation. Indeed, at the time of zooplankton netting in the Bight for the experiment, considerable amounts of small jellyfish were caught in the plankton nets along with mainly calanoid copepods, allowing *Aurelia* treatments in addition to the copepod bags.

Copepods induced changes on the lower trophic levels and stoichiometry within 14 days (as in the previous experiment) despite the low temperatures of 4-6°C which are known to slow copepod metabolism and prolong developmental time (Mauchline, 1998; Peterson, 2001). There was also evidence of a feedback effect from the lower trophic levels on copepods, potentially by food availability. Apart from natural mortality and copepod death as result of the handling procedure, the decline of copepods in the highest treatments (80 copepods per litre) may have been caused by copepod densities stocked too high above natural abundances (Figure 3-3). In high-density bags, copepods suffered either from a crowding effect and/or from limited food availability. Mean natural mesozooplankton abundance of ~30 individuals per litre were reported in Kiel Bight in April between 1985-1993, and between 20-60 copepods per litre have been reported between April and June (Albjerg *et al.*, 1996). During the experiment, 3-10 copepods per litre were counted from net tows of the upper ~5 m of the Kiel Bight water column. However, abundances are likely underestimated due to vertical migration of zooplankton and accumulation in depths during the day, as was suggested for Hopavågen. Certainly the particulate carbon concentration at the start of the experiment was ~0.5 mg l^{-1}, which is regarded as a high food concentration for *C. finmarchicus* (e.g. Wagner *et al.*, 2001), although carbon concentration as a sum parameter of all seston food resources is probably not the best way to determine food limitation. Rather, aspects of food suitability (see below) are of greater importance to selective particle catchers such as copepods. Copepods showed a negative impact on large sized prey in spring enclosure studies in Schöhsee (chapter 1.2), Hopavågen (chapter 2.2) and also for summer mesocosms (e.g. Sommer *et al.*, 2001). The particulate carbon content of large particles (>1000 µm³) based on proportional biomass calculations was calculated to be 0.06 mg C l^{-1}, a concentration limiting to copepod growth and likely to enhance mortality. *Calanus* species and *Centropages typicus* are reported to be limited in growth below ~0.15 mg C l^{-1} (e.g. Vidal, 1980; Davis & Alatalo, 1992). In bags stocked with *Aurelia,* predation by

the jellyfish probably contributed to the higher mortality observed (Figure 3-4), a phenomenon reported by Matsakis and Conover (1991).

Clearly, copepods negatively impacted upon all large sized (>2800 µm³) prey available (Figure 3-7). A preference for ciliates and dinoflagellates can be explained by their motility. *Acartia*, one of the most abundant copepods in the enclosure bags, is known to be highly selective for motile prey (Bollens & Penry, 2003) and this has been shown for various other copepods (Atkinson, 1995). Furthermore, food quality aspects can cause copepods to select specific species (DeMott, 1988a; Mayzaud *et al.*, 1998), because protozoa have a similar stoichiometry to crustaceans (Stoecker & Capuzzo, 1990). Dinoflagellates are known to contain rather high amounts of decosahexaenoic acid (an essential fatty acid), and total ω3 fatty acids compared to other phytoplankton taxa (Olsen, 1998). However, copepods had a positive impact on the small dinoflagellate *H. rotundata* (Table 3-2), but the impact was not as strong as for the other two small cryptophyte species. Thus, quality and/or motility may additionally account for the observed impact of copepods, but play a minor role compared to particle size.

All small phytoplankton species (<210 µm³; *T. acuta*, *H. rotundata* and *P. prolonga*), were positively affected by copepod abundance (Table 3-2, Figure 3-7), and contributed to increasing relative fluorescence (Figure 3-5). This increase is likely caused by released grazing pressure from copepods, which probably cannot effectively catch particles of such size. Bollens and Penry (2003) found that *Acartia* does not consume cells <10 µm in diameter, and the largest cells of *T. acuta*, *H. rotundata* or *P. prolonga* measured were ~8 µm in diameter. Further, bottom up factors could have contributed to growth of small species, as DIP increased relative to DIN and seston contained sufficient nitrogen. Small species can exploit these resources better than larger species due to their favourable surface to volume ratio. Not surprisingly then, even the smallest cell group of picophytoplankton and bacteria abundance increased with dependence upon copepod density (Figure 3-8, right panel). This increase was not due to a direct copepod impact, since copepods cannot catch and feed on these small particles. Besides advantageous nutrient conditions, I believe the main reason is a released grazing pressure from ciliates, and thus, an indirect impact of copepods. This trophic cascade concept was also suggested by Zöllner et al. (2003). Ciliates are well known to graze on bacteria and small algal cells (e.g. Albright *et al.*, 1987; Sherr, Sherr & McDaniel, 1991) and in my enclosure experiment, ciliate abundances decreased bacteria and picophytoplankton significantly (Figure 3-8, left panel). Thus, copepods are believed to induce a trophic cascade down to the microbial trophic level by feeding on ciliates and reduction of grazing pressure on small species. It is likely that these trophic interactions caused the decrease in relative fluorescence after 14 days. As copepod abundances declined in high stocked density bags, they released grazing pressure on ciliates (Figure 3-3, 3-5).

Naupliar abundance increased in copepod and jellyfish bags, but not in relation to the copepod density. The greatest numbers of nauplii were found in bags with 20 copepods per litre (Figure 3-3). Nauplii were assumed not to be limited in food abundance because they are known to feed on smaller particles relative to the copepodite and adult stages (Zankai, 1991; Roff *et al.*, 1995). However, because the most highly stocked copepods (40 and 80 copepods per litre) might have been limited in food resources (see above), they may have resorted to predation of their own eggs or nauplii (as discussed for Hopavågen) and thus, reduced the naupliar numbers to below those found in 20 copepod bags. Alternatively, a shortage of suitable food could also limit the resources that copepods can apportion to reproduction and egg production. This has been reported for *Centropages typicus* after periods of starvation (Dagg, 1977). In marine systems, copepods typically release their eggs into the water column and the eggs become a part of the seston upon which all the species feed. Predation on nauplii is reported for *Acartia* and *Centropages*, the two dominating species (Lonsdale *et al.*, 1979; Conley & Turner, 1985). For lower copepod densities (5 and 10 copepods per litre), resources per capita were higher and encounter probability of eggs or nauplii lower, thus higher nauplii abundances would be expected. However, it is likely that the abundance of adult females was insufficient to support higher reproduction. This theory was supported by the naupliar abundance in *Aurelia* treatments, where comparable amounts of copepods and nauplii were present (Figure 3-4). Nauplii reductions due to ingestion by *Aurelia* are assumed to be negligible, as inefficient capturing of copepod nauplii is reported (Sullivan, Suchman & Costello, 1997). In my experiment, bags stocked with 20 copepods per litre appeared to promote the most stable community with a rather constant copepod density and high nauplii recruitment. It is important to note that experimental impacts on naupliar abundance in the enclosure bags over 14 days mirror impacts on the first nauplii generation. Naupliar durations of around 13 days at 15°C are reported for experimental species (Hart, 1990), which I expect would be extended under the low temperatures of ~5°C in my enclosure experiment.

Copepods not only induced changes in naupliar, ciliate and phytoplankton abundance, but also changed nutrient stoichiometry. In fact, copepods negatively affected dissolved inorganic N:P at day 14 (Figure 3-9) and seston C:N ratios over time (Figure 3-12). As copepods are expected to retain relatively more nitrogen (Sterner, 1990), they are known to release a lower N:P ratio than ingested with food (Carrillo, Reche & CruzPizarro, 1996). Despite a conformant decrease in DIN:DIP with copepod density, individually both nutrients increased. This result is rather surprising because the loss of copepods should release relatively more nitrogen than phosphorus, and during the decomposition of copepod exuviae most nutrients are reported to be released within the first six days (Lee & Fisher, 1992).

Nitrogen was available in excess in the seston particles (C:N:P of 106:24:1), compared to the Redfield ratio of 106:16:6 (Redfield, 1958). The lack of clear top-down effects of

copepods on seston N:P and C:P ratios was probably caused by the relatively variable phosphorus concentrations. The impact of the copepods on the C:N ratio did not lower the ratio below Redfield (6.6), and thus not limiting to phytoplankton growth. Copepods were expected to cause a seston C:N increase by preferential retention of nitrogen within their bodies (Elser & Hassett, 1994; Gismervik, 1997). Indeed, reported copepod body C:N ratios from the Kiel Bight show means of 5.8. (Pertola, Koski & Viitasalo, 2002) and 6.6 (Walve & Larsson, 1999), i.e. copepods have to retain nitrogen from the higher C:N seston ratios in order to fulfil their stoichiometric demands. Decreasing copepod numbers, especially in treatments stocked with high densities (80 copepods per litre), could account for the decreasing seston C:N ratios, but that explanation is inconsistent with bags stocked with 20 copepods per litre, where copepod densities remained stable. Therefore, seston carbon concentrations available in the Kiel Bight in April 2003 seem to be the most important factor for copepod growth. Although food quantity for copepods revealed consistency in bags stocked with 80 copepods per litre over the experimental period (0,5 mg C l^{-1}), particles suitable for copepods declined. A low abundance of cells >1000 µm³ was left at day 8, around $3*10^4$, compared to $20*10^4$ µm³ ml^{-1} biovolume in 20 copepod per litre bags (Figure 3-6).

Comparison

4. Comparison between fresh-, brackish- and saltwater experiments

The similar experimental design of the three enclosure experiments provide the unique opportunity to compare the three habitats concerning the impact of copepods and cladocerans on lower trophic food webs. Nevertheless, this is challenging, especially due to different copepod and phytoplankton communities in each habitat, different development times at different temperatures and different stoichiometric ratios. However, the experimental set up allows the comparison of density dependent effects.

In all three enclosure experiments, copepods declined when stocked in high densities, while freshwater cladocerans (*Daphnia*) increased in abundance. Apart from freshwater copepods, which supposedly declined due to predation of cyclopoid copepods, these trends are rather surprising since crowding effects on growth or reproduction are reported for *Daphnia* (e.g. Burns, 2000; Lürling *et al.*, 2003), but not for copepods. Besides crowding effects, the decline of marine copepod abundances may result from a shortage in food availability. Highest food biomass in terms of carbon concentrations at experimental start was found in Schöhsee enclosures, followed by Kiel Bight and Hopavågen (Figure 4-1). Compared to concentrations after around one week, seston C remained approximately constant in enclosures stocked with copepods in Kiel Bight, decreased in Hopavågen and increased in Schöhsee (Figure 4-1).

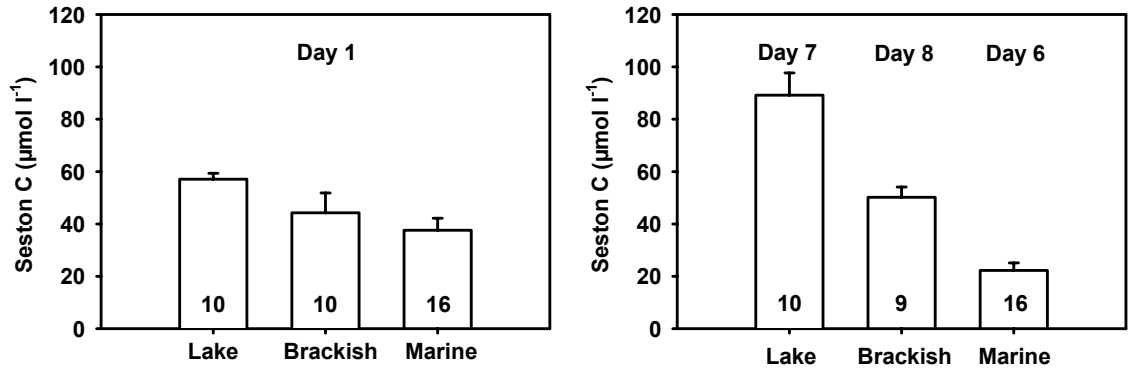


Figure 4-1: Mean (±SD) seston C (µmol l^{-1}) concentrations at experimental start (left panel) and after around one week (right panel) of copepod bags from lake (Schöhsee), brackish (Kiel Bight) and marine (Hopavågen fjord) system. Numbers in bars denote n values.

The contrasting patterns between the experimental sites evolved from differences of the food particle composition and zooplankton dynamics between the three systems. In Hopavågen, the decrease of particulate carbon after 6 days resulted from a high negative impact of calanoid copepods on large chains of diatoms (see Figure 2-11). In Kiel Bight, similar carbon concentrations at day 1 and day 8 might indicate that copepods had no impact on seston C, but these data are misleading. The copepods from brackish water had a contrasting impact on different size classes of their food particles, they decreased large cells,

mainly consisting of diatoms, dinoflagellates and ciliates, and increased small cells (Figure 3-7), as also shown in Hopavågen (Table 4-1). With decreasing abundances of large food particles including ciliates (Table 3-2; for Hopavågen see Zöllner (2004)), copepods supported small phytoplankton species, picophytoplankton and nanoflagellates growth (Table 2-2, Table 3-2, Figure 3-8 and Zöllner (2004)). These findings are similar to patterns found for summer enclosures in Kiel Bight, Hopavågen and also in Schöhsee (Sommer, 2003; Sommer, U. *et al.*, 2003). Only the freshwater copepods in spring caused an increase in seston carbon concentrations, likely to be caused by the released grazing pressure on all phytoplankton size classes (Table 4-1) by a more predatory feeding mode of cyclopoid copepods in the bags and on the associated high decline of copepod and ciliate abundances in general. In addition, small fragments of dead copepods could also have contributed to the high seston C concentration. In summer freshwater enclosures, when large phytoplankton was more abundant, copepods negatively impacted large phytoplankton species (Sommer, 2003). Cyclopoids inoculated in enclosure bags in summer were much smaller than in spring (personal communication B. Santer) and the larger size difference between cyclopoids and calanoids in spring probably resulted in predation of cyclopoids on calanoids.

Table 4-1: Food particles and chl *a* increase (↑) or decrease (↓) with zooplankton density after around one week. * denotes a predominant impact, however not shown for all species. Expected (exp.) and observed (obs.) results are abbreviated.

	Freshwater *Daphnia*		**Freshwater copepods**		**Brackish water copepods**		**Marine copepods**	
	Exp.	**Obs.**	**Exp.**	**Obs.**	**Exp.**	**Obs.**	**Exp.**	**Obs.**
Large food particles (>1000 µm³)	↑	↓	↓	↑	↓	↓	↓	↓
Small food particles (<1000µm³)	↓	↓ *	↑	↑ *	↑	↑	↑	↑
Chl *a*	↓	↓	↓	↑	↓	↑	↓	↑

Copepods had a positive density-dependent impact on the phytoplankton biomass measured as chl *a* after 3 experimental days in all three experiments. The increase in chl *a* or relative fluorescence with copepod density (Table 4-1) was supposedly caused by an increase of small phytoplankton in Kiel Bight and Hopavågen, and by an increase of all phytoplankton species and flagellates in Schöhsee. As copepods negatively impacted ciliates (Table 3-2 and Zöllner (2004)) and nauplii (in Hopavågen and potentially Kiel Bight, Figure 2-7 and 3-3), copepods eased the grazing pressure on phytoplankton. In contrast, freshwater cladocerans decreased chl *a* values considerably (Figure 1-6, Table 4-1). Their high impact on the phytoplankton spring bloom is considered as inducement of the spring clear water

phase in Schöhsee. By high growth rates, *Daphnia* managed to increase considerably in abundance and caused a clear water phase in all enclosure bags, independently of their stocked density. In summer, when mainly large phytoplankton species prevailed in Lake Schöhsee, *Daphnia* was not able to decrease phytoplankton biomass as strong as in spring. Thus, both zooplankton guilds, copepods and cladocerans, were needed to decrease the phytoplankton in summer (Sommer, U. *et al.*, 2003). In the sea, calanoid copepods are able to deplete the spring diatom bloom in Hopavågen and thus they are the equivalent to freshwater cladocerans.

Dissolved phosphorus concentrations were approximately similar in all three experiments, after around one week in copepod bags, increasing copepod density-dependently, except in Kiel Bight where only a positive trend is shown (Figure 1-10, 2-13 and 3-9). In *Daphnia* bags, however, dissolved phosphorus was lower and decreased with increasing *Daphnia* density (Figure 1-10). Accordingly, dissolved N:P ratios decreased with copepod density in Schöhsee and Hopavågen (in Kiel Bight only a decreasing trend was found at day 8), while freshwater cladocerans increased dissolved N:P ratios at day 13 and seston N:P ratios at day 7 and 13 (Table 4-2). These dissolved nutrient results confirm stoichiometric theory. In order to maintain a constant body N:P ratio, homeostasis theory predicts the ability of zooplankton to retain nutrients in shortage (Sterner, 1990). For copepods, a preferential retention of nitrogen has been shown (Hessen & Lyche, 1991), while *Daphnia* is known to retain phosphorus from nutrients taken up with their food (Andersen & Hessen, 1991). Thus, copepods are expected to excrete relatively low amounts of N and decrease dissolved N:P ratios, while *Daphnia* excrete relatively low amounts of P and increase N:P ratios. For copepods, the increase of seston N:P or N:C might be due to the decline of copepod densities in all experiments and associated small fragments of dead copepods contributing to the seston fraction. For a summary of expected and observed nutrient ratios of the dissolved and sestonic fraction, see Table 4-2.

Table 4-2: Stoichiometric increase (↑) or decrease (↓) of nutrient ratios of different compartments with zooplankton density. * denotes an impact of large copepods only. Expected (exp.) and observed (obs.) results are abbreviated.

	Freshwater *Daphnia*		**Freshwater copepods**		**Brackish water copepods**		**Marine copepods**	
	Exp.	**Obs.**	**Exp.**	**Obs.**	**Exp.**	**Obs.**	**Exp.**	**Obs.**
Dissolved N:P	↑	↑	↓	↓	↓	↓	↓	↓
Seston N:P or N:C	↑	↑	↓	—	↓	↑	↓	↑

In order to test the theory of homeostasis in the different experimental sites, C:N values of copepods, their food particles and the sediment were investigated in more detail. Unfortunately, no data of C:N ratios of brackish water copepods are available, restricting the comparison to freshwater and marine sites, lake Schöhsee and Hopavågen fjord. Copepods from both systems did not differ in their C:N ratios (t-test; t=-0.7, p>0.05) (Figure 4-2). Freshwater copepod C:N showed a mean of 6.8, similar to reported values (e.g. Urabe, 1995; Elser *et al.*, 2000). C:N ratios of marine copepods with a mean of 7.2 were slightly higher than mean values reported from copepod assemblages of the Baltic Sea of 5.8 (Pertola *et al.*, 2002), 6.6 (Walve & Larsson, 1999) and 6.8 (Gismervik, 1997). As Gismervik (1997) and Walve and Larsson (1999) stated, the high mean value may result from lipid-containing carbon rich overwintering stages of *Calanus*. Indeed, the high carbon content of *Calanus* (see Figure 2-18) elevated the marine mean copepod C:N ratio. The C:N ratios of marine copepods and seston are nearly balanced (Figure 4-2), however calculations of differences between seston and all copepod species within each bag show a rather large standard deviation (Figure 4-3). This high standard deviation is mainly caused by *Calanus*, elemental imbalance was negative for all values calculated with *Calanus*, while $C:N_{seston}$-$C:N_{copepod}$ values calculated with *Centropages*, *Temora* and *Acartoa* were positive (Figure 4-3). Thus, the similar C:N ratios from Hopavågen fjord may indicate, that copepods fulfil their stoichiometric demands from seston resources, but species not containing lipid resources face the necessity to retain nitrogen relative to carbon.

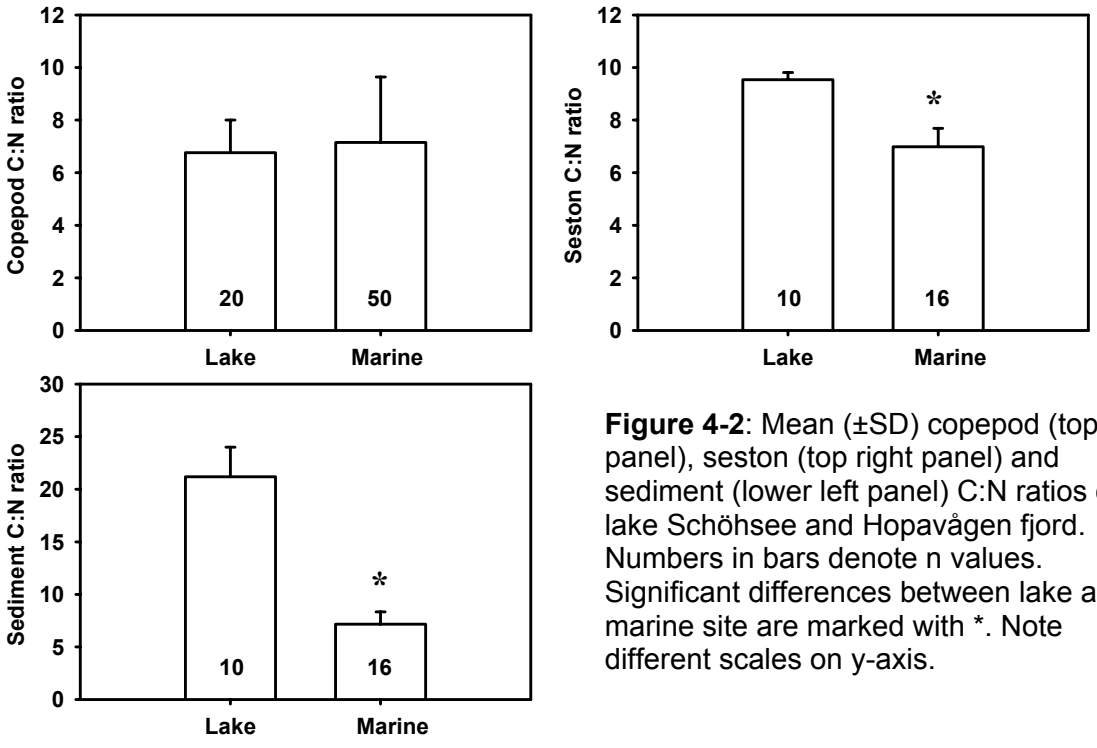


Figure 4-2: Mean (±SD) copepod (top left panel), seston (top right panel) and sediment (lower left panel) C:N ratios of lake Schöhsee and Hopavågen fjord. Numbers in bars denote n values. Significant differences between lake and marine site are marked with *. Note different scales on y-axis.

The similar C:N ratios of Hopavågen copepods and seston was not mirrored in Schöhsee, where seston values exceeded copepod C:N (Figure 4-2). The difference between freshwater seston and copepods was significantly higher than in Hopavågen (t-test; t=4.7, p<0.001, Figure 4-3). Freshwater seston is often reported to show higher C:N values of ~11 (Dobberfuhl & Elser, 2000; Pertola *et al.*, 2002), compared to marine ratios, frequently similar to the classic Redfield ratio of 6.6 (Redfield, 1958) and highlighting its marine origin (e.g. Walve & Larsson, 1999). Indeed, lake seston C:N with a mean ratio of 9.4 of the enclosure bags was significantly higher compared to the marine site (t-test; t=13.2, p<0.001) and indicates a minor shortage in nitrogen (Figure 4-2). Thus, freshwater copepods face higher seston C:N ratios compared to their body ratio, as frequently reported (e.g. Urabe & Watanabe, 1992; Elser & Foster, 1998; Dobberfuhl & Elser, 2000; Pertola *et al.*, 2002). In order to maintain their relatively low C:N ratio, homeostasis theory predicts the ability of copepods to preferentially retain nitrogen (Sterner, 1990; Hessen & Lyche, 1991). My data support these findings for freshwater and marine copepods, excluding *Calanus finmarchicus*. Copepods are able to maintain their body C:N ratio by retention of nitrogen from their ingested particles. For bulk zooplankton samples, Elser and Hassett (1994) found that freshwater zooplankton have to retain phosphorus to fulfil stoichiometric demands, while marine zooplankton are rich in nitrogen compared to their food. The main reason for this phenomenon is probably caused by the indirect comparison between cladocerans (more diverse and abundant in freshwaters) and copepods (numerically dominating in marine habitats) (Sommer, 1998; Dodson, Arnott & Cottingham, 2000). This imbalance of zooplankton guilds can affect stoichiometric ratios, since copepods and cladocerans vary considerably in their elemental stoichiometry: copepods have a high N:P ratio, while *Daphnia* contain relatively more P and thus have a lower N:P ratio than copepods, as also shown in chapter 3.1 (Andersen & Hessen, 1991; Hessen & Lyche, 1991).

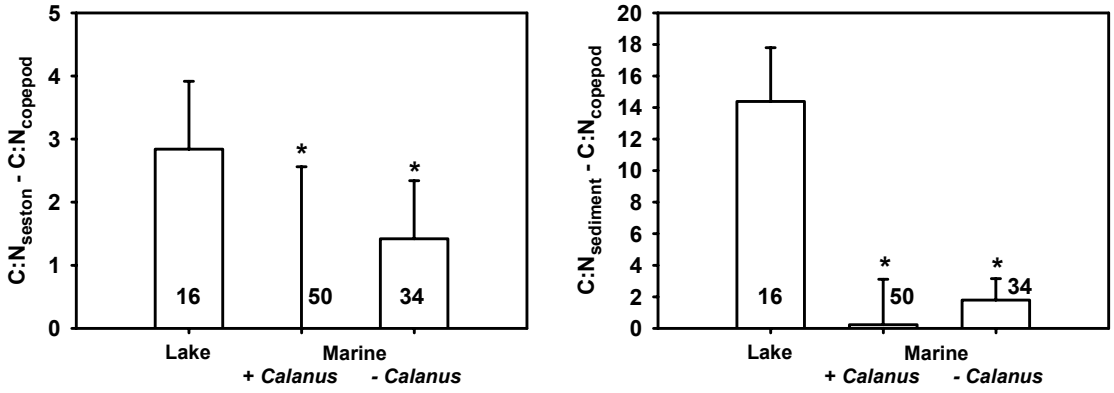


Figure 4-3: Elemental differences between seston and copepod C:N ratios (±SD, left panel) and sediment and copepod ratios (±SD, right panel). Differences were calculated with and without *Calanus* for the marine site. Numbers denote n values. Significant differences between the lake and the marine site are marked with *. Note different scales on y-axis.

By preferential retention of nitrogen, copepods are supposed to excrete material relatively depleted in N, i.e. high C:N (Sterner, 1990). Indeed, in lake Schöhsee, nutrients recycled by copepods in form of sediment particulate matter were found to be short in nitrogen, exceeding copepod C:N ratios (t-test; t=15, p<0.001; Figure 4-3). Thus, zooplankton is a key factor inducing changes in the nutrient stoichiometry of pelagic systems; copepods alter the relative availability of nitrogen in the water column for sestonic particles as also shown by Elser and Foster (1998) for N:P ratios. For Hopavågen, copepod and sediment C:N was approximately equal (see Figure 4-2 and 4-3), but calculations without *Calanus* showed higher values, i. e. sediment C:N exceeded copepod C:N (Figure 4-3). To conclude, copepods in lake Schöhsee did not recycle nitrogen efficiently and thus can influence lower trophic levels, i.e. their food sources, by a change in their nutritional elements. As predicted by theory, lake Schöhsee copepods retained relatively more nitrogen, as did non-lipid containing copepods from Hopavågen fjord. Thus, if intended to compare zooplankton stoichiometry, caution is given when lipid-storing zooplankton (here *Calanus*) is prevalent in the sample. Further, when comparing freshwater and marine systems, it can be misleading to sample bulk zooplankton for stoichiometric analyses, as this reflects the comparison of the most abundant zooplankton: mainly *Daphnia* in freshwater and copepods in the Sea.

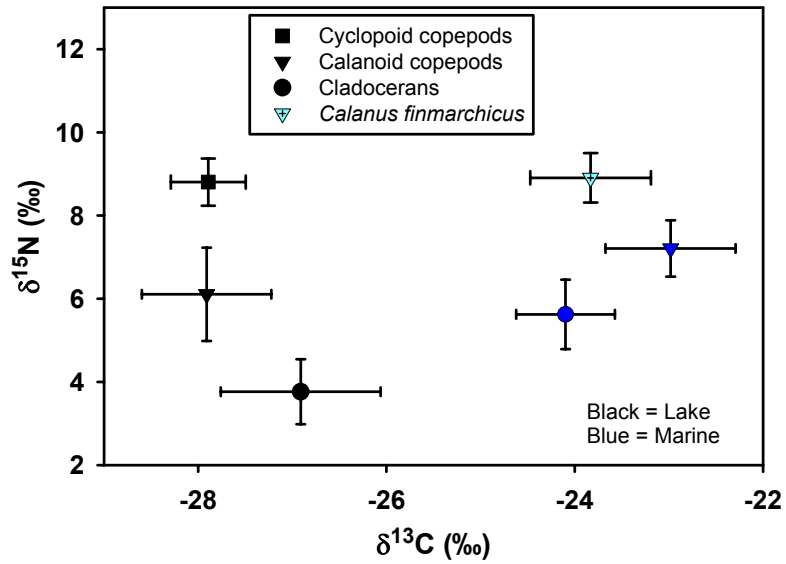


Figure 4-4: Mean (±SD) $\delta^{15}C$ and $\delta^{15}N$ values for cyclopoid and calanoid copepods and cladocerans from enclosures in fresh- and saltwater. The signature of the calanoid copepod *Calanus finmarchicus* is shown separately. Samples of the marine cladocerans *Evadne* are from Hopavågen, not from enclosure bags.

Copepods and cladocerans not only showed differences in their stoichiometry, but also in stable isotope signatures. Stable isotopes of the various mesozooplankton assemblages in Schöhsee and Hopavågen differed in their $\delta^{15}C$ values, but varied over a similar range of $\delta^{15}N$ (Figure 4-4). Highest trophic levels due to predacious feeding in freshwater were expected and measured for cyclopoid copepods. However, in the Sea, highest $\delta^{15}N$ signatures in spring enclosures were found for the calanoid copepod *Calanus finmarchicus* suggesting a more predatory feeding. The only cyclopoid copepod in Hopavågen was *Oithona*, which had a $\delta^{15}N$ of ~8‰, similar to *Centropages hamatus*, in summer enclosures

(Sommer, 2003). Freshwater calanoid copepods showed an intermediate trophic position between cyclopoids and *Daphnia* (Figure 4-4). The relatively high variability in δ^{15}N of freshwater calanoid copepods in spring enclosures, and their similar signature compared to cyclopoid copepods in summer (Sommer, 2003), suggest a more flexible and dynamic feeding behaviour. Freshwater and marine cladoceran species, *Daphnia* and *Evadne*, showed lowest δ^{15}N values of the mesozooplankton community confirming their mainly herbivorous feeding mode. While cyclopoid copepods in Schöhsee seem to be able to prey upon cladocerans (see chapter 1), calanoid copepods can do so in the sea.

Conclusions

5. Conclusions

From the 6 enclosure experiments in fresh-, brackish- and saltwater during spring and summer we gained valuable results and experience and can strongly recommend enclosure studies for investigations on food webs especially regarding lower trophic levels. Repeatability of our mesocosms was shown by the two experiments in Hopavågen, when results revealed strong similarities between experiment one and two. However, large scale enclosure experiments are also very time consuming and costly, thus, from experience, I would

- restrict experimental duration to around 2 weeks, as biofilm growth on enclosure foil may occur thereafter. The reason for the long duration of the freshwater experiment in spring was to ensure that zooplankton stable isotope analyses mirror stable isotope signatures gained during the experimental period. But, as marine copepods showed a change in signature already after 7 days, a restriction to 14 days is well founded.
- not recommend appendicularians treatments in spring. As summer enclosures successfully showed, appendicularians increased in abundance when copepods were removed from enclosure bags (Sommer, F. *et al.*, 2003a). However, appendicularians are also very sensitive to temperature, thus their growth might have been restricted in spring even when grazers were excluded.
- decide on the season for the experiment depending on the zooplankton guild of interest. If cladocerans are of main interest, the best study season with high abundances and high impacts on the lower trophic food web in freshwater is in spring. If copepods are the main focus, high impacts and most suitable food sources can be found in summer in freshwater, however for marine or brackish water sites, impacts on the spring bloom are important.
- recommend the method of bubbling wild zooplankton catches with air in order to remove cladocerans. This method can be advised if copepods are of interest, as they are rather difficult to culture in huge amounts. By the removal of cladocerans, this method approved to investigate the impact of copepods for around 2 weeks, before cladocerans became abundant. If cladocerans are of interest, however, cultures are necessary, as it is difficult to remove copepods from wild catches.
- be aware that it is difficult to stock enclosure bags with marine cladoceran species. Unfortunately, it is complicated to separate marine cladocerans from copepods, culture marine cladocerans in sufficient amounts, and transfer them into enclosure bags, due to their fragile character.
- mix enclosure bags thoroughly before sampling. Especially before sampling zooplankton, as they often accumulate near the bottom of the bags during daytime.

Further, samples should be taken immediately after mixing, due to a fast downward movement of the zooplankton.

- include different density gradients of zooplankton, allowing not only a time-dependent analyses but also a quantitative description of zooplankton impacts.

Summary & Zusammenfassung

Summary

The aim of this work was the comparison of the impact of copepods and cladocerans, the major guilds within the mesozooplankton in marine and freshwater habitats, respectively, on lower trophic food levels. Differences between copepods and cladocerans were expected because these two zooplankton guilds differ in their feeding behavior; copepods can actively select food particles, while cladocerans are unselective filter-feeders. Copepods and cladocerans are also known for their different stoichiometric N:P ratios, thus the project aimed at comparing the impacts on the nutrient ratios. Furthermore, zooplankton interactions were compared upon stable isotope analysis in order to determine differences between the zooplankton species. Mesocosm experiments were conducted at three different sites in order to compare freshwater (Lake Schöhsee), brackish (Kiel Bight) and marine (Hopavågen) systems. The mesocosm bags were stocked with natural occurring copepods or cladocerans in a logarithmically scaled density gradient in order to examine the effects qualitatively and quantitatively. Whereas copepod and cladoceran treatments were set up in the freshwater experiment, only copepods were used in the marine and in the brackish experiment due to very low abundances of cladocerans.

The first objective of this study was the change of the phytoplankton community induced by copepods and cladocerans. By conducting the experiments in spring, we aimed at investigating if the zooplankton guilds are able to reduce the phytoplankton spring bloom independent of their initial density. In freshwater, copepods were not able to reduce the phytoplankton bloom efficiently, no matter of their initially stocked abundance. This finding might be due to a strong decrease in copepod density, either caused by crowding effects, unsuitable food availability or, most likely, predation of cyclopoid copepods. In contrast, *Daphnia* were able to increase rapidly in abundance and graze intensively on the phytoplankton. The main reason for this impact was the relatively small size of the phytoplankton present, suitable for the filter-feeder *Daphnia*. After around 22 days, *Daphnia* in all different density bags managed to decrease phytoplankton abundances markedly and induce a clear water phase within the enclosure bags. The opposite was found in saltwater, where copepods of all density bags were able to considerably decrease the spring bloom, consisting mainly of long chained diatoms. This indicates that natural occurring densities of cladocerans in Lake Schöhsee and calanoid copepods in Hopavågen fjord might be able to decrease the spring phytoplankton bloom.

However, in contrast to freshwater cladocerans, marine copepods did not induce a clear water phase, due to restricted grazing on large phytoplankton, simultaneously increasing small cells. In brackish water, the phytoplankton bloom occurred previous to the experiment, but similar results as in Hopavågen were found. In confirmation, copepods in the freshwater experiment also increased phytoplankton biomass (chl *a*), mainly small species,

either be due to released grazing pressure of copepods on small species while feeding on larger cells, and/or due to an indirect effect via a trophic cascade. While copepods decreased ciliates density-dependently in all experiments (see also Zöllner, 2004) and partly fed on nauplii, ciliates and nauplii released grazing pressure on small cells. Results showed that the most important factors determining the feeding behavior of zooplankton were size and quantity of phytoplankton.

Another aim of this study was the investigation of zooplankton impacts on the nutrient stoichiometry. By different nutritional demands, copepods and cladocerans were expected to induce changes upon the dissolved, sestonic as well as sediment fraction. As copepods are relatively rich in nitrogen, I indeed found a copepod density-dependent decrease of N:P ratios in the dissolved fraction. This decrease of dissolved N:P was supposedly induced by preferential retention of nitrogen compared to phosphorus by copepods, resulting in excretion of lower N:P ratios than ingested. However, the pattern could not be confirmed within the sestonic fraction, but the decrease of copepod abundances and decomposition of dead animals probably added nitrogen to the particulate fraction. Contrastingly, *Daphnia* had a lower body N:P ratio than copepods. *Daphnia* preferentially retain phosphorus and thus increased the dissolved N:P ratio as well as the sestonic N:P ratio density-dependently. By these effects, *Daphnia* showed a feedback mechanism on their food sources, affecting phytoplankton quality, revealing intriguing seston C:P ratios above 1000. Thus, *Daphnia* affected their own growth by changing the quality of their potential food. Still, *Daphnia* increased in abundance even at these high ratios, indicating daphniids' extremely efficient capability of P retention. To my knowledge, *Daphnia* growth under these high C:P ratios was not reported before.

A comparison of the nutritional differences between freshwater and saltwater copepod C:N and the seston and sediment C:N within the different enclosure bags, revealed differences between the sites. While freshwater copepods contain relatively more nitrogen and show a lower C:N ratio than seston and sediment values, marine copepods seemed to be in balance with the particulate matter. However, this balance was caused by including a lipid rich copepod species (*Calanus finmarchicus*) in the calculations. All other species confirmed freshwater copepod results, showing a preferential retention of nitrogen. Thus, the interaction of copepods and seston did not differ qualitatively between a freshwater and a saltwater system.

The third aim, the application of stable isotope analyses to this study clearly revealed insights of the relative trophic positions of different copepod species, and differences between copepods and cladocerans. In Schöhsee, daphniids from enclosures stocked purely with differing densities of *Daphnia* showed little variability in stable isotope values, but when cladocerans developed in a copepod-mediated environment, a change in carbon food source occurred for *Daphnia*. Copepods modified the lower trophic level food web components and

abundances, and daphniids that thrived in enclosure bags together with copepods exhibited a density dependent depletion in $\delta^{13}C$ values. Increasing abundances of high nucleic acid bacteria in the copepod bags may account for the trend in *Daphnia* $\delta^{13}C$ via increased respiratory release of isotopically light CO_2 into the water column of the bags. Cyclopoid copepod stable isotope signatures from Lake Schöhsee suggest that cyclopoids preyed on the available zooplankton. In Hopavågen, stable isotope analysis revealed that calanoid copepods can feed upon other zooplankton in the sea, but show fast changes in their $\delta^{13}C$ which might result from a high flexibility to adjust to available carbon sources. In general, the study highlighted the usefulness of complementing conventional plankton study techniques with stable isotope analyses by showing complex species interactions of zooplankton, which would not be revealed by conventional techniques.

Zusammenfassung

Das Ziel dieser Arbeit war ein Vergleich zwischen den Auswirkungen von Copepoden und Cladoceren, den größten Gilden des Mesozooplanktons in Salz- bzw. Süßwasser Habitaten, auf niedrigere trophische Ebenen. Unterschiedliche Effekte zwischen Copepoden und Cladoceren wurden erwartet, da beide Zooplanktongruppen sich in ihrem Fraßverhalten unterscheiden; Copepoden können aktiv ihre Futterpartikel selektieren, während Cladoceren ihr Futter unselektiv filtrieren. Neben dieser unterschiedlichen Nahrungsaufnahme ist auch ein unterschiedliches stöchiometrisches N:P Verhältnis für Copepoden und Cladoceren charakteristisch. Deshalb sollten in diesem Projekt auch die Auswirkungen auf die Nährstoffverhältnisse vergleichend untersucht werden. Des weiteren wurden Zooplankton Interaktionen durch Stabile Isotopen Analysen untersucht, um Unterschiede zwischen den Zooplanktonarten festzustellen. Mesokosmos Experimente wurden an drei verschiedenen Standorten durchgeführt, in einem Süßwasser- (Schöhsee), Brackwasser- (Kieler Förde) und Salzwassersystem (Hopavågen fjord), und anschließend untereinander verglichen. Die Mesokosmos Säcke wurden mit den an den Standorten vorkommenden Arten von Copepoden und Cladoceren in Form eines logarithmischen Dichtegradienten bestückt, um die auftretenden Effekte sowohl qualitativ als auch quantitativ zu untersuchen . Während wir im Süßwasser Copepoden und Cladoceren auf die Säcke verteilen konnten, wurden im Salz- und Brackwasser, wegen zu geringen Dichten der marinen Cladoceren, nur Copepoden verwendet.

Das erste Ziel dieser Arbeit war es, die von Copepoden und Cladoceren verursachte Veränderung der Phytoplanktonzusammensetzung zu erfassen. Die Durchführung der Experimente im Frühjahr ermöglichte es mir, die Auswirkungen unterschiedlicher Zooplanktongruppen und –dichten auf die Frühjahres Phytoplanktonblüte genauer zu untersuchen. Süßwasser-Copepoden waren, unabhängig von ihrer anfänglichen Dichte, nicht in der Lage das Phytoplankton effektiv zu dezimieren. Dieses Ergebnis könnte auf die starke Abnahme der Copepodendichten zurückzuführen sein. Die Dichteabnahme kann entweder durch einen „crowding“ Effekt, ungeeignete Futterverfügbarkeit oder, am wahrscheinlichsten, durch Prädation von cyclopoiden Copepoden verursacht worden sein. Im Gegensatz dazu waren Daphnien in der Lage, sehr schnell zu wachsen und das Phytoplankton stark zu reduzieren. Dieser starke Einfluss der Daphnien wurde noch durch die relativ kleine Größe des Phytoplankton begünstigt, das sich gut für *Daphnia* eignet. Nach 22 Tagen hatten die Daphnien in allen Säcken die Abundanzen des Phytoplanktons deutlich verringert und induzierten ein Klarwasserstadium. Im Gegensatz zu den Süßwasser-Ergebnissen, waren im Salzwasser alle verschiedenen Copepodendichten in der Lage, das Phytoplankton der Frühjahresblüte zu reduzieren, dass hauptsächlich aus langkettigen Kieselalgen bestand. Dies läßt darauf schließen, dass natürlich vorkommende Dichten von

Cladoceren im Schöhsee und calanoiden Copepoden im Hopavågen wahrscheinlich in der Lage sind, die Phytoplanktonblüte im Frühjahr zu reduzieren.

Marine Copepoden waren, im Gegensatz zu Cladoceren im See, jedoch nicht in der Lage, ein Klarwasserstadium zu induzieren. Die Copepoden fraßen hauptsächlich große Zellen, wobei sich gleichzeitig kleine Zellen durch verminderten Fraßdruck vermehren konnten. Im Brackwasser fand ich ähnliche Ergebnisse, allerdings war die Frühjahresblüte in der Kieler Förde vor dem eigentlichen Beginn des Experimentes. Die Ergebnisse wurden auch durch die Süßwasser-Copepoden bestätigt. Es konnte eine Zunahme der Phytoplanktonbiomasse (Chlorophyll *a*) und kleiner Phytoplanktonarten beobachtet werden. Dies könnte entweder durch die direkte Entlastung des Fraßdruckes der Copepoden auf kleine Arten, oder durch einen indirekten Effekt einer trophische Kaskade verursacht worden sein. Während Copepoden dichteabhängig in allen Experimenten Ciliaten verringerten (siehe auch Zöllner, 2004) und auch teilweise Nauplien fraßen, wurde der Fraßdruck auf kleine Zellen verringert. Die Ergebnisse zeigen, dass Größe und Quantität des Phytoplanktons die wichtigsten Faktoren sind, die das Fraßverhalten von Zooplankton bestimmen.

Das zweite Ziel dieser Arbeit war die Untersuchung des Einflusses von Zooplankton auf die Nährstoffstoichiometrie. Aufgrund der verschiedenen Anforderungen von Copepoden und Cladoceren an die Ernährung,wurde erwartet, dass sie Veränderungen in der gelösten, partikulären und sedimentären Fraktion hervorrufen. Copepoden besitzen einen relativ hohen Stickstoffgehalt, weshalb ich eine Copepoden-dichteabhängige Abnahme des gelösten N:P Verhältnisses in den Säcken fand. Diese Abnahme wurde wahrscheinlich von Copepoden durch eine bevorzugte Zurückhaltung von Stickstoff, verglichen zu Phosphor, verursacht, weshalb Copepoden ein geringeres N:P Verhältnis ausschieden als mit der Nahrung aufgenommen wurde. Allerdings konnte dieses Muster nicht in der partikulären Fraktion nachgewiesen werden. Durch die abnehmenden Copepodenabundanzen über die Zeit sowie dem damit verbundenen Abbau von toten Tieren könnte aber Stickstoff der partikuläre Fraktion zugeführt worden sein. Daphnien hingegen haben ein geringeres N:P Verhältnis als Copepoden. Daphnien hielten vorzugsweise mehr Phosphor in ihrem Körper zurück und erhöhten dadurch das gelöste N:P Verhältnis sowie das partikuläre N:P Verhältnis dichteabhängig in den Mesokosmen. So beeinflußten Daphnien indirekt die Qualität des Phytoplanktons und damit ihres eigenen Futters, und erhöhten sogar das C:P Verhältnis des Sestons auf über 1000. Es wurde erwartet, dass durch diese Veränderung der Futterqualität das Wachstum der Daphnien limitiert ist. Trotz dieser extrem hohen C:P Werte nahmen die Daphnien aber in ihrer Abundanz zu und zeigten dadurch ihre extrem gute Fähigkeit zur Phosphor-Rückhaltung. Meines Wissens nach wurde Wachstum von Daphnien unter diesen extrem hohen C:P Verhältnissen noch nicht beschrieben.

Bei einem Vergleich der C:N Verhältnisse von Süß- und Salzwasser Copepoden mit dem Seston und dem Sediment in den Enclosure Säcken, wurden Unterschiede zwischen

den Standorten festgestellt. Während Copepoden aus dem Süßwasser relativ mehr N beinhalten und ein geringeres C:N Verhältnis als das Seston und das Sediment hatten, schienen marine Copepoden im Gleichgewicht mit der partikulären Fraktion zu sein. Allerdings war dieses Gleichgewicht durch eine Copepodenart (*Calanus*) verursacht, die einen hohen Fettgehalt besaß. Alle anderen Copepodenarten bestätigten die Ergebnisse aus dem Süßwasser und zeigten ebenfalls eine bevorzugte Rückhaltung von Stickstoff. Dies zeigt, dass die Interaktion zwischen Copepoden und Seston im Süß- und Salzwasser sich qualitativ nicht unterschied.

Der dritte Aspekt dieser Arbeit war die Analyse von Stabilen Isotopen, die Einblicke auf die relativen trophischen Ebenen der verschiedenen Copepodenarten, und die Unterschiede zwischen Copepoden und Cladoceren geben sollten. Im Schöhsee zeigten Daphnien eine geringe Variabilität ihrer stabilen isotopen Werte zwischen den verschiedenen Daphniendichten. Wenn Daphnien jedoch in Säcken wuchsen, in denen das Seston von Copepoden beeinflusst wurde, änderten die Daphnien ihre Kohlenstoff-Futterquelle. Copepoden veränderten die Komponenten und Abundanzen der niedrigeren trophischen Ebenen des Futternetzes, wodurch Daphnien, die in Enclosure Säcken zusammen mit Copepoden auftraten, eine Copepoden-dichteabhängige Erniedrigung ihres $\delta^{13}C$ zeigten. In den Copepodensäcken könnten zunehmende Dichten von Bakterien, die einen hohen Anteil an Nukleinsäure besaßen, für den Trend von Daphnien $\delta^{13}C$, durch zunehmende Freisetzung von isotopisch leichtem CO_2 in den Enclosuresäcken durch Atmung, verantwortlich sein. Stabile Isotopen Signaturen von cyclopoiden Copepoden im Schöhsee deuten darauf hin, dass Cyclopoide fähig sein können, anderes Zooplankton zu fressen. Im Hopavågen machte die Analyse von stabilen Isotopen deutlich, dass calanoide Copepoden im Meer anderes Zooplankton fressen können. Marines Zooplankton zeigte eine hohe Flexibilität, und damit die Fähigkeit, sich an schnell verändernde Kohlenstoffquellen anzupassen. Im allgemeinen verdeutlichte diese Studie die Nützlichkeit, anhand komplexer Interaktionen zwischen Zooplanktonarten, die mit herkömmlichen Methoden nicht entdeckt worden wären, Techniken von konventionellen Planktonstudien mit Stabilen Isotopen Analysen zu ergänzen.

Appendix

Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton

This chapter is published in Rapid Communications in Mass Spectrometry (2003), 17, pp.2605-2610.

Abstract

A literature survey of zooplankton stable isotope studies revealed inconsistencies between authors concerning a) fixation and b) allowance for gut clearance of zooplankton prior to $\delta^{13}C$ and $\delta^{15}N$ determinations. As stable isotope analyses was applied for enclosure zooplankton, it was crucial to address whether commonly used preservation techniques induce changes in stable isotope values. Additionally, zooplankton gut clearance is rarely performed: the gut content assumed to be negligible relative to organism mass. Although larger organisms are routinely eviscerated, or specific tissues are dissected, and analysed for stable isotopes to reduce errors introduced via the gut contents.

Introduction

Recent advances in understanding of aquatic ecosystem structure and functioning have benefited greatly from the application of stable isotope techniques. Stable isotope analyses are particularly useful in determining trophic relationships of small bodied aquatic organisms (e.g. crustacean zooplankton) when other techniques such as observation or gut content analysis are impractical. Understanding zooplankton trophic relationships is fundamental since zooplankton plays a pivotal role linking basal resources to organisms at higher trophic levels. Many studies have analysed composite zooplankton samples to examine large-scale ecosystem processes (e.g. del Giorgio & France, 1996). However, it has been demonstrated that zooplankton interspecific stable isotopic variability even within just one lake can span 16‰ for carbon and 10‰ for nitrogen.(Zohary *et al.*, 1994; Grey *et al.*, 2001) Thus it is often necessary to perform stable isotope analyses on separate species (Meili *et al.*, 1996; Grey & Jones, 1999).

Separation of zooplankton from seston samples to provide material of sufficient quantity and purity for stable isotope analyses is time consuming. This is a function of the community composition, the morphological similarity of closely related species, relative abundance and/or the organism size. In addition, the quantity of samples required in zooplankton studies (e.g. depth profiles or multiple mesocosm experiments) is generally high. Immediate separation of bulk samples and further preparation is often not feasible in remote fieldwork

locations or on cruises. Therefore, samples are generally preserved with the addition of a fixative and stored indefinitely for subsequent determination of parameters such as community composition. Yet studies involving other organisms have revealed that fixatives generally affect stable isotope integrity (Junger & Planas, 1993; Hobson, Gibbs & Gloutney, 1997; Gloutney & Hobson, 1998; Bosley & Wainright, 1999; Ponsard & Amlou, 1999; Kaehler & Pakhomov, 2001; Arrington & Winemiller, 2002; Edwards, Turner & Sharp, 2002; Sarakinos, Johnson & Vander Zanden, 2002). An alternative is to store samples frozen without a chemical fixative. Freezing is suitable for larger organisms such as fish, but may again be impractical in some field situations or its physical action may destroy smaller, more delicate animals and preclude its use. We undertook a survey of published zooplankton stable isotope studies (50) to determine how fresh- and saltwater zooplankton samples have been treated prior to stable isotope analyses: 46% preserved the samples, with freezing being the most widespread technique (32% of total). The complete literature source list is available from the authors upon request.

Studies involving preservatives (mainly formalin and/or ethanol) and their effects on isotopes in animal tissues as diverse as invertebrates (Junger & Planas, 1993), fish muscle tissue (Bosley & Wainright, 1999; Arrington & Winemiller, 2002; Edwards *et al.*, 2002), *Drosophila* (Ponsard & Amlou, 1999), quail muscle, quail and sheep blood (Hobson *et al.*, 1997) have often used freezing as a control treatment. To date, the effects of commonly used preservatives such as formalin, ethanol, methanol or gluteraldehyde on zooplankton stable isotope composition have not been clearly defined. The notable exception was a report by Mullin et al. (Mullin, Rau & Eppley, 1984) of a long-term (two years) storage effect of formalin on zooplankton δ^{13}C and δ^{15}N.

Unlike more conventional methods such as gut content analysis which provide a dietary snap-shot, the isotopic composition of a consumer tissue represents an integrated measure of contributions from, and assimilation of, different food sources over time. Since differing tissues may reflect dietary assimilation or isotopic routing at differing rates, particular tissues are often chosen to standardise between studies; for example, white muscle has been adopted as the tissue of choice to represent isotopic dietary relationships in fish (Pinnegar & Polunin, 1999). Tissue separation cannot be applied to small bodied organisms. Invertebrates and young of year fish are generally analysed whole, but gut clearance or visceral removal is commonly practiced in order to remove any bias induced by the gut contents themselves. Crustacean zooplankton individuals are typically too small to perform gut removal upon. Thus Grey et al. (Grey & Jones, 1999; Grey *et al.*, 2001) have previously advocated gut clearance in filtered water prior to further preparation for stable isotope analyses. When we compiled the zooplankton stable isotopes studies by other authors, 7 (Toetz, 1992; Kaehler, Pakhomov & McQuaid, 2000; Hobson *et al.*, 2002; O'Reilly *et al.*, 2002; Jones & Waldron, 2003; Karlsson *et al.*, 2003; Schmidt *et al.*, 2003) from the 50

published (14 %) allowed for gut clearance. Most of the remainder (64%) did not give specific information regarding sample treatment between collection and fixation or live preparation. We assume that the authors considered the effect of gut content to be negligible.

The aims of this study were then twofold. Firstly to determine whether commonly used fixatives or preservation techniques induced changes in the $\delta^{13}C$ and $\delta^{15}N$ values of zooplankton relative to unpreserved (fresh) material that was immediately prepared for isotopic analyses. Secondly, to address whether the gut content does influence the bulk zooplankton isotopic signature.

Methods

Fixation

6 different types of preservation were applied to bulk zooplankton samples, analysed for carbon and nitrogen stable isotopes and compared to unpreserved control values. Zooplankton was collected from mesotrophic lake Schöhsee by repeated tows of a 250 µm mesh net through the epilimnion. Species composition comprised *Daphnia hyalina x galeata*, *Mesocyclops leuckarti, Cyclops* spp., *Thermocyclops oithonoides*, *Diacyclops bicuspidatus* and *Eudiaptomus* spp. The bulk zooplankton sample was maintained alive at ambient temperature until return to the laboratory when it was well mixed and divided into equal sub-samples for treatment. One was immediately filtered onto a pre-combusted (550°C, 24 h) Whatman GF/F filter, rinsed with distilled water, oven dried overnight at 60°C and stored in a desiccator (C - control). Sub-samples for freezing were concentrated on 50µm gauze to remove excess water, placed in Cryovials and frozen at –20°C (F). Sub-samples for shock-freezing were similarly concentrated but the sealed Cryovials were immersed in liquid nitrogen for ~10 seconds prior to storage at –20°C (SF). For chemical fixation, 96% ethanol (E), methanol (M), 37% formaldehyde (FO) or gluteraldehyde (G), were added to further sub-samples to make final concentrations of 30%, 30%, 10%, and 4% respectively. The six treatments and the control were replicated five times. After four days, preserved zooplankton was concentrated, washed and dried in an identical manner to that described for the control treatment.

Gut evacuation

Further zooplankton was similarly collected from Schöhsee at a later date to test for gut content effects. Animals were maintained in 0.45µm filtered lake water for three hours to allow gut clearance. Microscopic examination of gut colouration was used as a surrogate measure of evacuation. Sub-samples of zooplankton were taken before and after gut clearance, screened on gauze to remove excess water and immediately frozen (control). The remaining animals were then introduced into 2 L beakers (~ 700 individuals L^{-1}) containing a

^{15}N-enriched culture of *Cryptomonas* sp. (~10 mg carbon L^{-1}) and allowed to graze undisturbed. The algae had been cultured for three days in WC-medium(Guillard & Lorenzen, 1972) containing 1% labelled ammonium nitrate ($NH^{15}NO_3$). Gut filling was monitored (by microscopy) in small subsamples every 10 minutes, since the gut filling time of copepods is known to be extremely variable (30 to 130 min,(Arashkevich, 1977) depending on species, time of the day, temperature, pH, food availability and quality). After 40 minutes of grazing, microscopic re-examination revealed the guts of *Eudiaptomus* spp. and *Cyclops kolensis* to appear full. *Cyclops abyssorum* and *Daphnia hyalina x galeata* were allowed to feed for 65 minutes. Zooplankton was then removed, repeatedly screened through filtered water to remove excess algae and further prepared for storage in a similar manner to controls. After three days, samples were thawed and zooplankton species separated before rinsing with distilled water and drying on GF/F filters for 16 h. Sufficient material was collected during the experiment to provide three or four replicates from each species.
All dried samples were pulverised with mortar and pestle and weighed into tin cups for subsequent analyses of ^{15}N and ^{13}C. Tin cups were oxidised in a Carlo Erba NA1500 elemental analyser coupled to a Micromass IsoPrime continuous flow isotope ratio mass spectrometer. Isotope ratios are expressed conventionally using the δ notation in per mil (‰) relative to secondary standards of known relation to the international standard of Vienna Pee Dee Belemnite and atmospheric nitrogen for carbon and nitrogen respectively. Repeated measurement of an internal standard exhibited a precision of <0.2‰ for both carbon and nitrogen.

ANOVA was used to test for differences between treatment means. Dunnett's post-hoc tests were applied to determine if mean δ^{15}N and mean δ^{13}C from each of the six treatments differed significantly from the control values. Independent samples t-tests were used to analyse replicates of zooplankton isotopic signals between natural lake, gut evacuated and post ^{15}N-addition. Gut content (mass) relative to body mass, was calculated from a single isotope, two source model according to Phillips & Gregg (2001): gut evacuated zooplankton δ^{13}C or δ^{15}N (for each species) and labelled algal δ^{13}C or δ^{15}N were substituted into the model as the two sources, post-grazing zooplankton with refilled guts as the mixture. Our model assumes that any shift of isotopic signatures is exclusively due to gut content and neglects zooplankton assimilation of the diet, validated by the high prey densities used and continuous monitoring of zooplanktons' gut fullness to ensure shortest grazing times.

Results

Fixation

The mean (±1 SD) value of control zooplankton $\delta^{13}C$ was –29.6 ± 0.2‰ (see Figure 1) and $\delta^{15}N$, 6.7 ± 0.2‰, typical for Schöhsee (Grey, unpublished data). Treatments resulted in zooplankton $\delta^{13}C$ differing from the control values by 0.1 to 1.1‰, and $\delta^{15}N$ by 0.6 to 1.5‰ (Figure 1). Standard deviation from the mean of five replicates of each treatment was <0.4‰ for both carbon and nitrogen. Ethanol and shock-freezing treatments did not significantly alter zooplankton $\delta^{13}C$ ($\alpha>0.05$). Formalin (+1.1‰) and freezing (–0.9‰) treatments resulted in the greatest deviation from control carbon values. All treatments resulted in ^{15}N-enrichment relative to control values ($\alpha<0.05$).

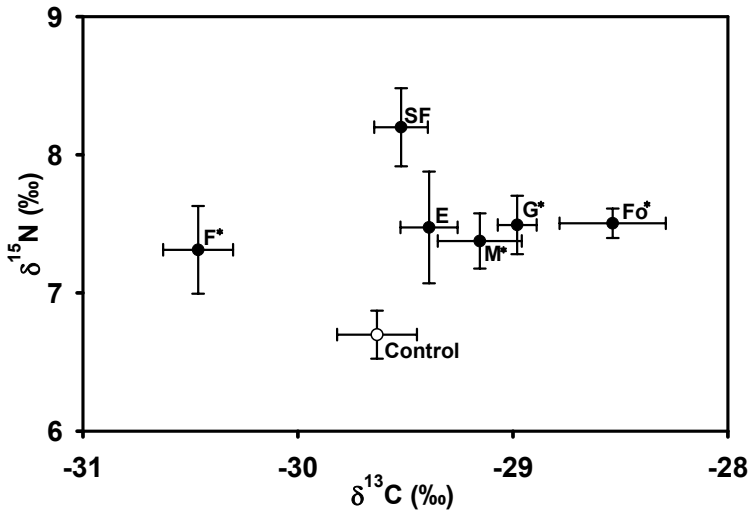


Figure 1: Mean zooplankton $\delta^{13}C$ and $\delta^{15}N$ values (‰ ± 1SD; n = 5) of control samples compared to six treatments: F – frozen, SF – shock-frozen, E – ethanol, M – methanol, G – gluteraldehyde and Fo – Formalin. Asterisks denote significant deviation from control $\delta^{13}C$ (Dunnett's test; α = 0,05). All treatments resulted in significant ^{15}N-enrichment.

Gut evacuation

There was no significant difference in either $\delta^{13}C$ or $\delta^{15}N$, between zooplankton collected and prepared directly from the lake and those allowed to gut evacuate in particle free water (t-tests; $p>0.05$; Table 1). After grazing on ^{15}N-labelled algae ($\delta^{13}C$, –26.6 ± 0.1‰; $\delta^{15}N$, 6035 ± 170.6‰), all four zooplankton species were significantly ^{15}N-enriched (t-tests; $p<0.05$), but there was considerable interspecific variability. *Cyclops kolensis* exhibited the greatest ^{15}N-enrichment (509.0 ± 16.5‰). The $\delta^{13}C$ values of *Daphnia* grazing on the *Cryptomonas* were significantly ^{13}C-depleted relative to the gut evacuated samples ($p = 0.03$), but the copepod species were statistically unaltered ($p>0.05$). The model of Phillips and Gregg (2001) predicted gut content ranging from 0.1 to 8.8% relative to body mass for the copepods using either $\delta^{13}C$ or $\delta^{15}N$ (Table 1). There was a marked discrepancy between the gut mass of *Daphnia* calculated using $\delta^{13}C$ (7.6%) compared to using $\delta^{15}N$ (1.0%).

Table 1. Mean $\delta^{13}C$ and $\delta^{15}N$ (‰ ± 1SD) of Schöhsee zooplankton prepared fresh from the lake, after gut evacuation in particle free water and after grazing on ^{15}N-labelled *Cryptomonas*. Gut content mass (% relative to body mass) is calculated from a mixing model according to Phillips & Gregg (2001). See text for details.

	Lake		Gut evacuated		Post grazing		Gut content mass	
Species	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**From $\delta^{13}C$**	**From $\delta^{15}N$**
Daphnia hyaline x galeata	-32.5 ± 0.1	12.6 ± 0.3	-32.8 ± 0.2	12.3 ± 0.4	-32.3 ± 0.2	71.5 ± 6.4	7.6 ± 2.6	1.0 ± 0.1
Eudiaptomus spp.	-30.9 ± 0.1	11.3 ± 0.1	-31.1 ± 0.2	11.2 ± 0.1	-30.9 ± 0.0	268.6 ± 15.8	4.6 ± 0.8	4.3 ± 0.3
Cyclops kolensis	-30.3 ± 0.2	11.7 ± 0.1	-30.8 ± 0.0	11.4 ± 0.3	-30.5 ± 0.2	509.0 ± 16.5	8.8 ± 5.7	8.3 ± 0.3
Cyclops abyssorum	-32.0	11.6	-31.8 ± 0.3	11.3 ± 0.2	-32.0 ± 0.2	18.9 ± 1.3	-	0.1 ± 0.0

Table 2. Estimates of maximum induced shift in stable isotope values caused by inclusion of gut contents from two dietary sources – see test for details of modeling.

Species	**Gut content (%)**	**100% at -35‰ $\delta^{13}C$ (‰)**	**100% at -20‰ $\delta^{13}C$ (‰)**	**100% at 2.3‰ $\delta^{15}N$ (‰)**	**100% at 18.2‰ $\delta^{15}N$ (‰)**	**Max. induced shift for carbon (‰)**	**Max. induced shift for nitrogen (‰)**
Daphnia hyaline x galeata	1.0	-34.01	-33.86	5.67	5.83	0.14	0.13
Daphnids [a]	5.0	-34.05	-33.30	5.53	6.33	0.70	0.63
Eudiaptomus spp.	4.3	-34.04	-33.40	5.55	6.24	0.60	0.54
Cyclops kolensis	8.3	-34.08	-32.84	5.42	6.74	1.16	1.04
Copepods [b]	20.0	-34.20	-31.20	5.02	8.20	2.80	2.50
Calanus finmarchicus [c]	26.0	-34.26	-30.36	4.82	8.95	3.64	3.25

Gut content data sources:

[a] calculated from gut passage time and feeding rate (Lampert, 1987).
[b] V. Alekseev, personal communication.
[c] T. Jarvis, unpublished data .

Discussion

Fixation

The zooplankton samples used in our experimental test for treatment effect on stable isotope integrity were derived from a natural lake, mixed species assemblage. However, the standard deviation between five replicates of each treatment was remarkably small (generally <0.2‰) despite each replicate comprising many individual animals. Thus we are confident that any significant deviation from the control stable isotope values was a function of treatment and not of differing species composition.

Zooplankton carbon stable isotope values were significantly affected by four out of six treatments. Formalin addition resulted in a ^{13}C-enrichment of 1.1‰. This contrasts with previous studies where formalin has resulted in ^{13}C-depletion in tissues as diverse as quail and sheep (Hobson *et al.*, 1997), *Drosophila melanogaster* (Ponsard & Amlou, 1999), quail egg albumen (Gloutney & Hobson, 1998), flounder and shrimp (Bosley & Wainright, 1999), minnow (Edwards *et al.*, 2002), cob, octopus and kelp (Kaehler & Pakhomov, 2001) and most interestingly, zooplankton after 2 years preservation (Mullin *et al.*, 1984). Sarakinos et al.(2002) proposed this was due to the binding of formalin (with an isotopic signature of –32 ‰) to animal tissue. Significant losses of carbon after formaldehyde fixation were reported for *Daphnia magna* (17%) (Boersma & Vijverberg, 1994) and *Megacyclops gigas* (35%) after 6 d (Salonen & Sarvala, 1980), sufficient to cause changes in δ^{13}C values. Gluteraldehyde and formalin are both toxic and alkylating agents so affect tissues in a similar manner. They also both contain carbon atoms and thus introduce a potential contaminatory carbon source. Alcohols are lipid solvents so preservation with either ethanol or methanol involves not only the addition of a carbon source but also loss of lipids, likely to result in heavier δ^{13}C (DeNiro & Epstein, 1977). However, ethanol was actually the only chemical preservative to retain zooplankton stable isotope integrity in our study.

Zooplankton was ^{13}C-depleted by 0.9‰ after the freezing treatment. This again contrasts with some previous studies. Bosley and Wainright (1999) showed that freezing did not influence δ^{13}C or δ^{15}N stable isotope values of winter flounder and mud shrimps. Unlike the chemical preservatives, freezing does not introduce a contaminatory source of carbon, but significant losses of carbon after freezing were described by Salonen and Sarvala (1980). The effect is likely derived from mechanical breakdown of the cells and loss of carbon components via leaching when thawed or filtering during our preparation procedure. The immediacy of shock-freezing under liquid nitrogen reduces the time ice crystals form within the tissues and may explain why shock-freezing resulted in minimal change in zooplankton δ^{13}C. Our data certainly question the use of frozen samples as a control against which other different preservation methods can be tested, the procedure used in the following studies of invertebrates (Junger & Planas, 1993), fish muscle tissue (Arrington & Winemiller, 2002;

Edwards *et al.*, 2002), *Drosophila melanogaster* (Ponsard & Amlou, 1999), and zooplankton (Mullin *et al.*, 1984). Since the trophic transfer of carbon is relatively conservative, the introduction of a 1‰ shift from formalin or freezing, two commonly used preservation techniques, is highly undesirable.

All preservation treatments resulted in ^{15}N-enrichment relative to control values, more consistent with other studies of fixation (Bosley & Wainright, 1999; Ponsard & Amlou, 1999; Arrington & Winemiller, 2002; Edwards *et al.*, 2002). None of the experimental treatments involved the addition of nitrogen, so δ^{15}N alteration must be related to the protein denaturant properties of the alcohols, or protein and nucleic acid binding in the alkylating agents. It is not clear how either freezing treatment alters δ^{15}N unless it is via leaching in the preparation process and/or thawing, causing loss of the lighter isotope.

Our experiment was not designed to determine the causative mechanisms altering the stable isotope ratios. Our data clearly demonstrate that no single preservation treatment of zooplankton was ideal for the integrity of both carbon and nitrogen stable isotope ratios. It would appear prudent to avoid the use of a preservation method and prepare dried material immediately from fresh. If preservation were a necessary requirement of the study, then we would advise complementary testing of preservatives on the organisms in question. Our study was only of short duration and further changes in isotope values may occur with longer storage, although Salonen and Sarvala (1980) noted highest carbon losses within 6 days of preservation. Additionally, zooplankton composition, life stage and amount of lipid stores vary temporally and may be differentially affected by preservation.

Gut evacuation

The effect of gut content on the bulk organism stable isotope signature was effectively investigated twice during our experimental ^{15}N-tracer experiment; once when comparing gut evacuated zooplankton to the same species collected and prepared immediately from the lake, and a second time when comparing the gut evacuated zooplankton to those allowed to graze the ^{15}N-labelled algae (Table 1). Since there was no significant difference between natural lake and gut evacuated samples, the obvious conclusion to draw is that gut evacuation is an unnecessary procedure. However, we advise caution before accepting this conclusion. The zooplankton was collected from Schöhsee in late October and the productivity in the water column during this period is rather low, so zooplankton guts may have been partly empty already before gut evacuation. Also, zooplankton species may have been in isotopic equilibrium with the putative diet at this time. If either, or indeed both of these suppositions were true, then gut evacuation would have had minimal impact on zooplankton isotope values. Moreover, our judgement of whether the gut was truly empty after gut evacuation, based upon microscopy observations, was rather subjective. Indeed it was noted that there was little visual difference in gut content of *Cyclops abyssorum* before

and after gut clearance. Porter (1975) observed retention of gut material in *Daphnia galeata* for up to 5 days when placed in filtered water, so visual interpretation of gut evacuation may have been inaccurate, especially for daphnids.

The second part of the experiment (after allowing zooplankton to graze on ^{15}N-labelled algae) resulted in significant ^{15}N-enrichment of the zooplankton (Table 1). Our zooplankton gut content mass data calculated using both δ^{13}C and δ^{15}N values are comparatively low at 1 % for daphnids and <1 to 8.3 % for copepods. These values likely underestimate naturally occurring gut content masses for the following reasons. 1) Zooplankton was only allowed to prey on ^{15}N -labelled Cryptomonas as a single algal food source. 2) We had initially selected *Cryptomonas* as a suitable prey, which should be ably ingested by the zooplankton assemblage (Santer, 1994). However, the low uptake of ^{15}N exhibited by *Cyclops abyssorum* probably reflects a more predatory mode of feeding and suggests *Cryptomonas* was unsuitable prey. 3) Again, our gut-fullness estimations based upon microscopy may have been inaccurate and the time allowed for feeding on the ^{15}N-labelled algae therefore too short. Conversely, the discrepancy in *Daphnia* gut content mass calculated from carbon (7.6%) and nitrogen (1%), may actually arise from the *Daphnia* grazing for too long and some differential excretion of carbon and nitrogen products occurring (Andersen & Hessen, 1991; Hessen & Lyche, 1991). We chose the conservative measure for our model. As far as we are aware, there are no published values of the relative gut mass with which to compare our experimental data, partly due to the high variability of zooplankton ingestion rates. The gut mass of daphnids can be estimated from knowledge of the gut passage time (dependent upon food concentrations, environmental conditions) and the feeding rate being approximately 5% of body weight ind^{-1} h^{-1} (Lampert, 1987). A conservative estimate of gut passage time (GPT) of 30 to 60 minutes (Geller, 1975) results in a gut content of no more than 5% of body weight. Estimates for various freshwater copepods are higher (20%; V.R. Alekseev, personal communication) and calculated as 26% for the marine copepod *Calanus finmarchicus* (T.Jarvis, unpublished data). Fryer (1957) reported visual observations of *Acanthocyclops viridis* ingesting complete chironomid larvae trunks, and the gut content of an individual *Macrocyclops albidus* (< 2.5 mm in length) comprising the remains of copepodid stages of two cyclopoids copepods, engulfed whole and remaining entire within the gut; the remains of two further cyclopoid copepods; the remains of a chydorid cladoceran; nine oligochaete chaetae; and the skeleton of a single diatom. From these observations, it would appear that the gut content of certain copepod species can contribute a substantial proportion to total body mass.

To determine whether the gut content may influence bulk zooplankton stable isotope ratios, we again used a single isotope, two source mixing model (Phillips & Gregg, 2001), but for this calculation substituted a range of relative gut mass values. Particulate organic matter from freshwaters typically exhibits δ^{13}C between –35 and –20‰ (del Giorgio & France, 1996),

and patchy, estuarine POM δ^{15}N has been recorded varying from 2.3 to 18.2‰ (Bax *et al.*, 2001). We used these extreme values as theoretical dietary end-members for the model. Although our experiment was conducted using freshwater zooplankton, there is little reason to limit the implications of gut contents affecting bulk isotopic signature to freshwater species. Hence we have used estuarine POM data as an end member and included data on gut mass from a marine copepod, *Calanus finmarchicus*, within our model. Rather, our experimental results likely represent the lower end of a spectrum of zooplankton gut content masses, since saltwater zooplankton tends to be larger-bodied than its freshwater counterparts.

The zooplankton consumer δ^{13}C and δ^{15}N was fixed at –34‰ and 5.7‰, respectively, representing isotopic equilibrium with the –35‰ and 2.3‰ POM diet end-member, including a 1‰ for carbon and 3.4‰ for nitrogen trophic fractionation (Peterson & Fry, 1987). The maximal isotopic zooplankton shift induced by switching the gut content from 100% at –35‰ (or 2.3‰), to 100% at –20‰ (or 18.2‰) was then calculated from the model (Table 2). Our theoretical modelling suggests that inclusion of the gut content in both carbon and nitrogen stable isotope analysis of *Daphnia* is likely unimportant (maximum induced shift: 0.7‰), but for copepods can introduce an error of up to 3.6‰ for carbon and 3.25‰ for nitrogen. Obviously the model makes many assumptions and the extreme values used will generate maximum potential shifts. We believe this approach is justified when the putative basal resource of zooplankton comprises multiple components and has highly variable stable isotope signatures, and when zooplankton are known to feed selectively and/or switch diets (Grey *et al.*, 2001). Basal resource isotope signatures can fluctuate over a reduced temporal scale and may be very different from a consumer signature (O'Reilly *et al.*, 2002). Our data suggest that even the gut content of small organisms like copepods can influence the bulk organism stable isotope ratios and thus the simple step of gut clearance should be practiced routinely to reduce this potential source of variation.

References

Acuna J. L., Bedo A. W., Harris R. P. & Anadon R. (1995) The seasonal succession of appendicularians (Tunicata, Appendicularia) off Plymouth. *Journal of the Marine Biological Association, U.K.*, **75**, 755-758

Adams T. S. & Sterner R. W. (2000) The effect of dietary nitrogen content on trophic level N-15 enrichment. *Limnology and Oceanography*, **45**, 601-607

Adrian R. & Schneider-Olt B. (1999) Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. *Journal of Plankton Research*, **21**, 2175-2190

Ærtebjerg G. (2003) Monitoring Cruise Report with r/v Gunnar Thorson in the Sound, Kattegat, Belt Sea and Arkona Sea. *National Environmental Research Institute, Denmark.*, http://www.bsh.de/en/Marine%20data/Observations/MURSYS%20reporting%20system/mursys203.jsp

Aksnes D. L. & Magnesen T. (1983) Distribution, development, and production of *Calanus finmarchicus* (Gunnerus) in Lindaspollene, western Norway, 1979. *Sarsia*, **68**, 195-207

Albjerg J., Behrends G., Giesenhagen H., Hansen H.-P. & Wikner J. (1996) 3rd Periodic Assessment of the state of the marine environment of the Baltic Sea Regions. *The Baltic Marine Environment Protection Commission (Helsinki Commission - HELCOM)*, http://www.baltic.vtt.fi/balticinfo/text_files/chapter4.5.4.html

Albright L. J., Sherr E. B., Sherr B. F. & Fallon R. D. (1987) Grazing of ciliated protozoa on free and particle-attached bacteria. *Marine Ecology Progress Series*, **38**, 125-129

Andersen T. & Hessen D. O. (1991) Carbon, nitrogen, and phosphorus-content of freshwater zooplankton. *Limnology and Oceanography*, **36**, 807-814

Arashkevich E. G. (1977) Duration of food digestion in marine copepods. *Polskie Archiwum Hydrobiologii*, **24**, 431-438

Arrington D. A. & Winemiller K. O. (2002) Preservation effects on stable isotope analysis of fish muscle. *Transaction of the American Fisheries Society*, **131**, 337-342

Atkinson A. (1995) Omnivory and feeding selectivity in 5 copepod species during spring in the Bellingshausen-Sea, Antarctica. *Ices Journal of Marine Science*, **52**, 385-396

Båmstedt U. (1986) Chemical composition and energy content. *In: Corner, E.D.S. and O'Hara, S.C.M. (editors): The Biological Chemistry of Marine Copepods. Oxford University Press*, 1-58

Bautista B., Harris R. P., Tranter P. R. G. & Harbour D. (1992) *In situ* copepod feeding and grazing rates during a spring bloom dominated by *Phaeocystis* sp. in the English-Channel. *Journal of Plankton Research*, **14**, 691-703

Bax N. J., Burford M., Clementson L. & Davenport S. (2001) Phytoplankton blooms and production sources on the south-east Australian continental shelf. *Marine and Freshwater Research*, **52**, 451-462

Becker C. & Boersma M. (2003) Resource quality effects on life histories of *Daphnia*. *Limnology and Oceanography*, **48**, 700-706

Becker C. (2004) Living with constraints - food quality effect on zooplankton. PhD Thesis, University of Kiel

Behrends G. & Schneider G. (1995) Impact of *Aurelia aurita* Medusae (Cnidaria, Scyphozoa) on the standing stock and community composition of mesozooplankton in the Kiel-Bight (Western Baltic-Sea). *Marine Ecology Progress Series*, **127**, 39-45

Bern L. (1987) Zooplankton grazing on [methyl-^{3}H] thymidine-labelled natural particle assemblages: determination of filtering rates and food selectivity. *Freshwater Biology*, **17**, 151-159

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

Beutler M. (2003) Spectral fluorescence of chlorophyll and phycobilins as an *in situ* tool of phytoplankton analysis - models, algortihms and instruments. PhD Thesis, University of Kiel

Boersma M. & Vijverberg J. (1994) The effect of preservation methods on the carbon content of *Daphnia*. *Archiv für Hydrobiologie*, **130**, 241-247

Bollens G. C. R. & Penry D. L. (2003) Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). *Marine Ecology Progress Series*, **257**, 139-158

Bollens S. M. & Frost B. W. (1989) Zooplanktivorous fish and variable diel vertical migration in the marine planktonic copepod *Calanus pacificus*. *Limnology and Oceanography*, **34**, 1072-1082

Bosch F. v. d. & Santer B. (1993) Cannibalism in *Cyclops abyssorum*. *Oikos*, **67**, 19-28

Bosley K. L. & Wainright S. C. (1999) Effects of preservatives and acidification on the stable isotope ratios (N-15 : N-14, C-13 : C-12) of two species of marine animals. *Canadian Journal of Fisheries and Aquatic Sciences*, **56**, 2181-2185

Bottrell H. H., Duncan A., Gliwicz Z. M., Grygierek E., Herzig A., Hillbrichtilkowska A., Kurasawa H., Larsson P. & Weglenska T. (1976) Review of some problems in zooplankton production studies. **24**, 419-456

Brendelberger H. (1985) Filter mesh-size and retention efficiency for small particles: comparative studies with Cladocera. *Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie*, **21**, 135-146

Brendelberger H. & Geller W. (1985) Variability of filter structures in 8 *Daphnia* species - mesh sizes and filtering areas. *Journal of Plankton Research*, **7**, 473-486

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

Brett M. T. & Müller-Navarra D. C. (1997) The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biology*, **38**, 483-499

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

Burns C. W. (2000) Crowding-induced changes in growth, reproduction and morphology of *Daphnia*. *Freshwater Biology*, **43**, 19-29

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

Carrillo P., Reche I. & CruzPizarro L. (1996) Intraspecific stoichiometric variability and the ratio of nitrogen to phosphorus resupplied by zooplankton. *Freshwater Biology*, **36**, 363-374

Chaudron Y., Poulet S. A., Laabir M., Ianora A. & Miralto A. (1996) Is hatching success of copepod eggs diatom density-dependent? *Marine Ecology Progress Series*, **144**, 185-193

Confer J. L. (1971) Intrazooplankton predation by *Mesocyclops edax* at natural prey densities. *Limnology and Oceanography*, **16**, 663-&

Conley W. J. & Turner J. T. (1985) Omnivory by the coastal marine copepods *Centropages hamatus* and *Labidocera aestiva*. *Marine Ecology Progress Series*, **21**, 113-120

Cowles T. J. & Strickler J. R. (1983) Characterization of feeding-activity patterns in the planktonic copepod *Centropages typicus* Kroyer under various food conditions. *Limnology and Oceanography*, **28**, 106-115

Cowles T. J., Olson R. J. & Chisholm S. W. (1988) Food selection by copepods - Discrimination on the basis of food quality. *Marine Biology*, **100**, 41-49

Cuddington K. M. & McCauley E. (1994) Food-dependent aggregation and mobility of the water fleas *Ceriodaphnia dubia* and *Daphnia pulex*. *Canadian Journal of Zoology - Revue Canadienne de Zoologie*, **72**, 1217-1226

Daan R., Gonzalez S. R. & Breteler W. (1988) Cannibalism in omnivorous calanoid copepods. *Marine Ecology Progress Series*, **47**, 45-54

Dagg M. (1977) Some effects of patchy food environments on copepods. *Limnology and Oceanography*, **22**, 99-107

Davis C. S. & Alatalo P. (1992) Effects of constant and intermittent food-supply on life-history parameters in a marine copepod. *Limnology and Oceanography*, **37**, 1618-1639

Dawidowicz P. (1990) The effect of *Daphnia* on filament length of blue-green-algae. *Hydrobiologia*, **191**, 265-268

del Giorgio P. A. & France R. L. (1996) Ecosystem-specific patterns in the relationship between zooplankton and POM or microplankton $\delta^{13}C$. *Limnology and Oceanography*, **41**, 359-365

DeMott W. R. (1986) The role of taste in food selection by freshwater zooplankton. *Oecologia*, **69**, 334-340

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

DeMott W. R. (1988b) Discrimination between algae and detritus by freshwater and marine zooplankton. *Bulletin of Marine Science*, **43**, 486-499

DeMott W. R. & Watson M. D. (1991) Remote detection of algae by copepods - Responses to algal size, odors and motility. *Journal of Plankton Research*, **13**, 1203-1222

DeMott W. R. (1995) Optimal foraging by a suspension-feeding copepod: responses to short-term and seasonal variation in food resources. *Oecologia*, **103**, 230-240

DeMott W. R., Gulati R. D. & Van Donk E. (2001) Effects of dietary phosphorus deficiency on the abundance, phosphorus balance, and growth of *Daphnia cucullata* in three hypereutrophic Dutch lakes. *Limnology and Oceanography*, **46**, 1871-1880

DeNiro M. J. & Epstein S. (1977) Mechanism of carbon isotope fractionation associated with lipid-synthesis. *Science*, **197**, 261-263

DeNiro M. J. & Epstein S. (1978) Influence of diet on distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, **42**, 495-506

Dobberfuhl D. R. & Elser J. J. (2000) Elemental stoichiometry of lower food web components in arctic and temperate lakes. *Journal of Plankton Research*, **22**, 1341-1354

Dodson S. I., Arnott S. E. & Cottingham K. L. (2000) The relationship in lake communities between primary productivity and species richness. *Ecology*, **81**, 2662-2679

Donaghay P. L. & Small L. F. (1979) Food selection capabilities of the estuarine copepod *Acartia clausi*. *Marine Biology*, **52**, 137-146

Durbin E. G. & Durbin A. G. (1992) Effects of temperature and food abundance on grazing and short-term weight change in the marine copepod *Acartia hudsonica*. *Limnology and Oceanography*, **37**, 361-378

Dussart B. H. & Defaye D. (2001) Introduction to Copepoda. Backhuys Publishers, Leiden

Edwards M. S., Turner T. F. & Sharp Z. D. (2002) Short- and long-term effects of fixation and preservation on stable isotope values (delta C-13, delta N-15, delta S-34) of fluid-preserved museum specimens. *Copeia*, 1106-1112

Ehret H. (2000) Experimentelle Untersuchungen zum Nahrungsspektrum von *Eudiaptomus gracilis*. MSc Thesis, University of Konstanz

Einsle U. (1993) Crustacea, Copepoda, Calanoida und Cyclopoida. Gustav Fischer Verlag, Stuttgart

Elser J. J. & Hassett R. P. (1994) A stoichiometric analysis of the zooplankton-phytoplankton interaction in marine- and freshwater ecosystems. *Nature*, **370**, 211-213

Elser J. J. & Foster D. K. (1998) N : P stoichiometry of sedimentation in lakes of the Canadian shield: Relationships with seston and zooplankton elemental composition. *Ecoscience*, **5**, 56-63

Elser J. J. (2000) Ecological stoichiometry: from sea lake to land. *Trends in Ecology and Evolution*, **15**, 393-394

Elser J. J., Fagan W. F., Denno R. F., Dobberfuhl D. R., Folarin A., Huberty A., Interlandi S., Kilham S. S., McCauley E., Schulz K. L., Siemann E. H. & Sterner R. W. (2000) Nutritional constraints in terrestrial and freshwater food webs. *Nature*, **408**, 578-580

Fernandez F. (1979) Nutrition studies in the nauplius larva of *Calanus pacificus* (Copepoda, Calanoida). *Marine Biology*, **53**, 131-147

Feuchtmayr H. & Grey J. (2003) Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. *Rapid Communications in Mass Spectrometry*, **17**, 2605-2610

Feuchtmayr H., Zöllner E., Santer B., Sommer U. & Grey J. (2004) Zooplankton interactions in an enclosure experiment: Insights from stable isotope analyses. *Freshwater Biology*, submitted

Friedman M. M. & Strickler J. R. (1975) Chemoreceptors and feeding in calanoid copepods (Arthropoda-Crustacea). *Proceedings of the National Academy of Sciences of the United States of America*, **72**, 4185-4188

Frost B. (1972) Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. *Limnology and Oceanography*, **17**, 805-814

Frost B. W. (1977) Feeding behavior of *Calanus pacificus* in mixtures of food particles. *Limnology and Oceanography*, **22**, 472-491

Fry B. & Sherr E. B. (1984) $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. *Contributions in Marine Science*, **27**, 13-47

Fry B. (1988) Food web structure on Georges Bank from stable C, N, and S isotopic compositions. *Limnology and Oceanography*, **33**, 1182-1190

Fryer G. (1957) The food of some freshwater cyclopoid copepods and its ecological significance. *Journal of Animal Ecology*, **26**, 263-286

Fußmann G. (1996) Die Kontrolle der Rotatorien im Pelagial eines mesotrophen Sees durch Bottom-up und Top-down-Prozesse: Freilandbeobachtungen und Enclosure-Experimente. PhD Thesis, University of Kiel

Geller W. (1975) Die Nahrungsaufnahme von *Daphnia pulex* in Abhängigkeit von der Futterkonzentration, der Temperatur, der Körpergrösse und dem Hungerzustand der Tiere. *Archiv für Hydrobiologie - Supplement*, **48**, 47-107

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

Ghadouani A., Pinel-Alloul B. & Prepas E. E. (2003) Effects of experimentally induced cyanobacterial blooms on crustacean zooplankton communities. *Freshwater Biology*, **48**, 363-381

Gismervik I. (1997) Stoichiometry of some marine planktonic crustaceans. *Journal of Plankton Research*, **19**, 279-285

Gismervik I., Olsen Y. & Vadstein O. (2002) Micro- and mesozooplankton response to enhanced nutrient input - a mesocosm study. *Hydrobiologia*, **484**, 75-87

Gliwicz Z. M. (1977) Food size selection and seasonal succession of filter feeding zooplankton in an eutrophic lake. *Ekologia Polska-Polish Journal of Ecology*, **25**, 179-225

Gliwicz Z. M. & Siedlar E. (1980) Food size limitation and algae interfering with food collection in *Daphnia*. *Archiv für Hydrobiologie*, **88**, 155-177

Gloutney M. L. & Hobson K. A. (1998) Field preservation techniques for the analysis of stable-carbon and nitrogen isotope ratios in eggs. *Journal of Field Ornithology*, **69**, 223-227

Gophen M. & Geller W. (1984) Filter mesh size and food particle uptake by *Daphnia*. *Oecologia*, **64**, 408-412

Grasshoff K., Kremling K. & Erhardt M. (1999) Methods of seawater analysis. 3rd edition, Wiley-VCH, Weinheim

Grey J. & Jones R. I. (1999) Carbon stable isotopes reveal complex trophic interactions in lake plankton. *Rapid Communications in Mass Spectrometry*, **13**, 1311-1314

Grey J., Jones R. I. & Sleep D. (2000) Stable isotope analysis of the origins of zooplankton carbon in lakes of differing trophic state. *Oecologia*, **123**, 232-240

Grey J., Jones R. I. & Sleep D. (2001) Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnology and Oceanography*, **46**, 505-513

Guillard R. & Lorenzen C. (1972) Yellow-green algae with chlorophyllide c. *Journal of Phycology*, **8**, 10-14

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

Hansen F. C., Reckermann M., Breteler W. & Riegman R. (1993) *Phaeocystis* blooming enhanced by copepod predation on protozoa - evidence from incubation experiments. *Marine Ecology Progress Series*, **102**, 51-57

Hart R. C. (1990) Copepod postembryonic durations - pattern, conformity, and predictability - the realities of isochronal and equiproportional development, and trends in the copepodid-naupliar duration ratio. *Hydrobiologia*, **206**, 175-206

Heath M. R. (1999) The ascent migration of *Calanus finmarchicus* from overwintering depths in the Faroe-Shetland Channel. *Fisheries Oceanography (Suppl. 1)*, **8**, 84-99

Hessen D. O. & Lyche A. (1991) Inter- and intraspecific variations in zooplankton element composition. *Archiv für Hydrobiologie*, **121**, 343-353

Hessen D. O. & Rukke N. A. (2000) The costs of moulting in *Daphnia*; mineral regulation of carbon budgets. *Freshwater Biology*, **45**, 169-178

Hillebrand H., Durselen C. D., Kirschtel D., Pollingher U. & Zohary T. (1999) Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, **35**, 403-424

Hirche H. J. (1996) Diapause in the marine copepod, *Calanus finmarchicus* - A review. *Ophelia*, **44**, 129-143

Hobson K. A., Alisauskas R. T. & Clark R. G. (1993) Stable nitrogen isotope enrichment in avian-tissues due to fasting and nutritional stress - implications for isotopic analyses of diet. *Condor*, **95**, 388-394

Hobson K. A., Gibbs H. L. & Gloutney M. L. (1997) Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. *Canadian Journal of Zoology - Revue Canadienne de Zoologie*, **75**, 1720-1723

Hobson K. A., Fisk A., Karnovsky N., Holst M., Gagnon J. M. & Fortier M. (2002) A stable isotope (delta C-13, delta N-15) model for the North Water food web: implications for evaluating trophodynamics and the flow of energy and contaminants. *Deep Sea Research II*, **49**, 5131-5150

Huntley M. E., Barthel K. G. & Star J. L. (1983) Particle rejection by *Calanus pacificus* - discrimination between similarly sized particles. *Marine Biology*, **74**, 151-160

Hydrographie B. f. S. u. (2000) North Sea and Baltic Sea Report. http://www.bsh.de/en/Marine%20data/Observations/MURSYS%20reporting%20system/mursys2002.jsp

Hygum B. H., Rey C. & Hansen B. W. (2000) Growth and development rates of *Calanus finmarchicus* nauplii during a diatom spring bloom. *Marine Biology*, **136**, 1075-1085

Ianora A., Poulet S. A. & Miralto A. (2003) The effects of diatoms on copepod reproduction: a review. *Phycologia*, **42**, 351-363

Jensen K. H., Larsson P. & Hogstedt G. (2001) Detecting food search in *Daphnia* in the field. *Limnology and Oceanography*, **46**, 1013-1020

Jones J. I. & Waldron S. (2003) Combined stable isotope and gut contents analysis of food webs in plant-dominated, shallow lakes. *Freshwater Biology*, **48**, 1396-1407

Jonsson P. R. & Tiselius P. (1990) Feeding-behavior, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. *Marine Ecology Progress Series*, **60**, 35-44

Junger M. & Planas D. (1993) Alteration of trophic interactions between periphyton and invertebrates in an acidified stream - a stable carbon isotope study. *Hydrobiologia*, **262**, 97-107

Kaehler S., Pakhomov E. A. & McQuaid C. D. (2000) Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by delta C-13 and delta N-15 analysis. *Marine Ecology Progress Series*, **208**, 13-20

Kaehler S. & Pakhomov E. A. (2001) Effects of storage and preservation on the delta C-13 and delta N-15 signatures of selected marine organisms. *Marine Ecology Progress Series*, **219**, 299-304

Karlsson J., Jonsson A., Meili M. & Jansson M. (2003) Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. *Limnology and Oceanography*, **48**, 269-276

Kattner G. & Krause M. (1987) Changes in lipids during the development of *Calanus finmarchicus* s.l. from copepodid I to adult. *Marine Biology*, **96**, 511-518

Kerfoot W. C. (1977) Implications of copepod predation. *Limnology and Oceanography*, **22**, 316-325
Kiefer F. (1978) Freilebende Copepoda. Die Binnengewässer. Schweizerbarth, Stuttgart
Kim S. W., Onbe T. & Yoon Y. H. (1989) Feeding habits of marine cladocerans in the inland Sea of Japan. *Marine Biology*, **100**, 313-318
Kirchner M. (1995) Microbial colonization of copepod body surfaces and chitin degradation in the sea. *Helgoländer Meeresuntersuchungen*, **49**, 201-212
Kleppel G. S. (1993) On the diets of calanoid copepods. *Marine Ecology Progress Series*, **99**, 183-195
Koehl M. A. R. (1984) Mechanisms of particle capture by copepods at low Reynolds numbers: Possible modes of selective feeding. *In: Meyer, D.G. and Strickler, J.R. (editors): Trophic interactions within aquatic ecosystems. AAAS Selected Symposium. Series 85. Westview, Boulder*, 135-166
Lair N. & Hilal M. (1992) *Acanthodiaptomus denticornis* another omnivorous calanoid copepod - description of its mouth appendages and feeding experiments on animal prey. *Hydrobiologia*, **248**, 137-142
Lampert W. (1978) Climatic conditions and planktonic interactions as factors controlling the regular succession of spring algal bloom and extremely clear water in Lake Constance. *Verhandlungen der internationalen Vereinigung für theoretische und angewandte Limnologie*, **20**, 969-974
Lampert W. (1978) Release of dissolved organic carbon by grazing zooplankton. *Limnology and Oceanography*, **23**, 831-834
Lampert W. (1981) Inhibitory and toxic effects of blue-green algae on *Daphnia*. *Internationale Revue der Gesamten Hydrobiologie*, **66**, 285-298
Lampert W. (1987) Feeding and nutrition in *Daphnia*. *In: Peters RH, De Bernardi R: Daphnia*, 143-192
Lampert W. (1988) The relationship between zooplankton biomass and grazing - a review. *Limnologica*, **19**, 11-20
Lampert W. & Loose C. J. (1992) Plankton Towers - Bridging the Gap between Laboratory and Field Experiments. *Archiv für Hydrobiologie*, **126**, 53-66
Lampert W. & Sommer U. (1997) Limnoecology. Oxford University Press
Landry M. R. (1981) Switching between herbivory and carnivory by the planktonic marine copepod *Calanus pacificus*. *Marine Biology*, **65**, 77-82
Lee B. G. & Fisher N. S. (1992) Decomposition and release of elements from zooplankton debris. *Marine Ecology Progress Series*, **88**, 117-128
Lonsdale D. J., Heinle D. R. & Siegfried C. (1979) Carnivorous feeding-behavior of the adult calanoid copepod *Acartia tonsa* Dana. *Journal of Experimental Marine Biology and Ecology*, **36**, 235-248
Lürling M., Roozen F., Van Donk E. & Goser B. (2003) Response of Daphnia to substances released from crowded congeners and conspecifics. *Journal of Plankton Research*, **25**, 967-978
Lynch M. (1980) *Aphanizomenon* blooms: Alternate control and cultivation by *Daphnia pulex*. In: W. C. Kerfoot (ed.), Evolution and ecology of zooplankton communities. University Press of New England, Hanover (N. H.); London, 299-304
Maier G. (1993) An example of niche partitioning in three coexisting freshwater cyclopoid copepods. *Journal of Plankton Research*, **15**, 1097-1102
Marshall S. M. & Orr A. P. (1955) On the biology of *Calanus finmarchicus* .8. Food uptake, assimilation and excretion in adult and stage-V *Calanus*. *Journal of the Marine Biological Association, U.K.*, **34**, 495-529
Matsakis S. & Conover R. J. (1991) Abundance and feeding of medusae and their potential impact as predators on other zooplankton in Bedford Basin (Nova-Scotia, Canada) during spring. *Canadian Journal of Fisheries & Aquatic Sciences*, **48**, 1419-1430
Mauchline J. (1998) The biology of calanoid copepods. Academic Press, London
Mayzaud P., Tirelli V., Bernard J. M. & Roche-Mayzaud O. (1998) The influence of food quality on the nutritional acclimation of the copepod *Acartia clausi*. *Journal of Marine Systems*, **15**, 483-493

McKee D., Atkinson D., Collings S. E., Eaton J. W., Gill A. B., Harvey I., Hatton K., Heyes T., Wilson D. & Moss B. (2003) Response of freshwater microcosm communities to nutrients, fish, and elevated temperature during winter and summer. *Limnology and Oceanography*, **48**, 707-722

Meili M., Kling G. W., Fry B., Bell R. T. & Ahlgren I. (1996) Sources and partitioning of organic matter in a pelagic microbial food web inferred from the isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of zooplankton species. *Archiv für Hydrobiologie - Special Issues in Advanced Limnology*, **48**, 53-61

Meyer-Harms B., Irigoien X., Head R. & Harris R. (1999) Selective feeding on natural phytoplankton by *Calanus finmarchicus* before, during, and after the 1997 spring bloom in the Norwegian Sea. *Limnology and Oceanography*, **44**, 154-165

Miller C. B., Morgan C. A., Prahl F. G. & Sparrow M. A. (1998) Storage lipids of the copepod *Calanus jinmarchicus* from Georges Bank and the Gulf of Maine. *Limnology and Oceanography*, **43**, 488-497

Minagawa M. & Wada E. (1984) Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, **48**, 1135-1140

Mullin M. M., Rau G. H. & Eppley R. W. (1984) Stable nitrogen isotopes in zooplankton - some geographic and temporal variations in the North Pacific. *Limnology and Oceanography*, **29**, 1267-1273

Nejstgaard J. C., Witte H. J., Vanderwal P. & Jacobsen A. (1994) Copepod grazing during a mesocosm study of an *Emiliania huxleyi* (Prymnesiophyceae) bloom. *Sarsia*, **79**, 369-377

Niehoff B., Klenke U., Hirche H. J., Irigoien X., Head R. & Harris R. (1999) A high frequency time series at Weathership M, Norwegian Sea, during the 1997 spring bloom: the reproductive biology of *Calanus finmarchicus*. *Marine Ecology Progress Series*, **176**, 81-92

Nielsen T. G. (1991) Contribution of zooplankton grazing to the decline of a *Ceratium* bloom. *Limnology and Oceanography*, **36**, 1091-1106

Norrman B., Zweifel U. L., Hopkinson C. S. & Fry B. (1995) Production and utilization of dissolved organic carbon during an experimental diatom bloom. *Limnology and Oceanography*, **40**, 898-907

Ojala A., Kankaala P., Kairesalo T. & Salonen K. (1995) Growth of *Daphnia longispina* L. in a polyhumic lake under various availabilities of algal, bacterial and detrital food. *Hydrobiologia*, **315**, 119-134

Olsen Y. (1998) Lipids and essential fatty acids in aquatic food webs: What can freshwater ecologists learn from mariculture? In: Arts, M.T. and Wainman, B.C. (editors): Lipids in Freshwater Ecosystems

Ong J. E. (1969) Fine structure of mandibular sensory receptors in brackish water calanoid copepod *Gladioferens pectinatus* (Brady). **97**, 178-&

O'Reilly C. M., Hecky R. E., Cohen A. S. & Plisnier P.-D. (2002) Interpreting stable isotopes in food webs: Recognizing the role of time averaging at different trophic levels. *Limnology and Oceanography*, **47**, 306-309

Paffenhöfer G. A. (1982) Grazing by copepods in the Peru upwelling. *Deep Sea Research I*, **29**, 145-146

Paterson M. J., Findlay D. L., Salki A. G., Hendzel L. L. & Hesslein R. H. (2002) The effects of *Daphnia* on nutrient stoichiometry and filamentous cyanobacteria: a mesocosm experiment in a eutrophic lake. *Freshwater Biology*, **47**, 1217-1233

Perez-Martinez C. & Cruz-Pizarro L. (1995) Species-specific phytoplankton responses to nutrients and zooplankton manipulations in enclosure experiments. *Freshwater Biology*, **33**, 193-203

Pertola S., Koski M. & Viitasalo M. (2002) Stoichiometry of mesozooplankton in N- and P-limited areas of the Baltic Sea. *Marine Biology*, **140**, 425-434

Peterson B. J. & Fry B. (1987) Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, **18**, 293-320

Peterson W. T. (2001) Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints, and evolutionary significance. *Hydrobiologia*, **453**, 91-105

Phillips D. L. & Gregg J. W. (2001) Uncertainty in source partitioning using stable isotopes. *Oecologia*, **127**, 171-179

Pinnegar J. K. & Polunin N. V. C. (1999) Differential fractionation of delta C-13 and delta N-15 among fish tissues: implications for the study of trophic interactions. *Functional Ecology*, **13**, 225-231

Poggensee E. & Lenz J. (1981) On the population dynamics of two brackish-water Cladocera *Podon leuckarti* and *Evadne nordmanni* in Kiel Fjord. *Meeresforschung Sonderheft*, **5**, 268-273

Ponsard S. & Amlou M. (1999) Effects of several preservation methods on the isotopic content of *Drosophila* samples. *Comptes Rendus de l'Académie des Sciences - Série III - Sciences de la vie*, **322**, 35-41

Porter K. G. (1975) Viable gut passage of gelatinous green algae ingested by *Daphnia*. *Verhandlungen der internationalen Vereinigung für theoretische und angewandte Limnologie*, **19**, 2840-2850

Porter K. G., Pace M. L. & Battey J. F. (1979) Ciliate protozoans as links in freshwater planktonic food-chains. *Nature*, **277**, 563-565

Poulet S. A. & Marsot P. (1978) Chemosensory grazing by marine calanoid copepods (Arthropoda Crustacea). *Science*, **200**, 1403-1405

Rai H. (1982) Primary production of various size fractions of natural phytoplankton communities in a North German lake. *Archiv für Hydrobiologie*, **95**, 395-412

Redfield A. C. (1958) The biological control of chemical factors in the environment. *American Scientist*, **46**, 205-221

Richardson K., Jonasdottir S. H., Hay S. J. & Christoffersen A. (1999) *Calanus finmarchicus* egg production and food availability in the Faroe-Shetland channel and northern North Sea: October-March. *Fisheries Oceanography (Suppl. 1)*, **8**, 153-162

Ringelberg J., Flik B. J. G., Lindenaar D. & Royackers K. (1991) Diel vertical migration of *Daphnia hyalina* (sensu latiori) in Lake Maarsseveen: Part 1. Aspects of seasonal and daily timing. *Archiv für Hydrobiologie*, **121**, 129-145

Roff J. C., Turner J. T., Webber M. K. & Hopcroft R. R. (1995) Bacterivory by tropical copepod nauplii - extent and possible significance. *Aquatic Microbial Ecology*, **9**, 165-175

Roman M. R. (1977) Feeding of copepod *Acartia tonsa* on diatom *Nitzschia closterium* and brown-algae (*Fucus vesiculosus*) detritus. *Marine Biology*, **42**, 149-155

Rothhaupt K. O. (1997) Grazing and nutrient influences of *Daphnia* and *Eudiaptomus* on phytoplankton in laboratory microcosms. *Journal of Plankton Research*, **19**, 125-139

Saage A. (2003) Populationsentwicklungen des Zooplanktons in Mesokosmosexperimenten. MSc Thesis, University of Kiel

Salonen K. & Sarvala J. (1980) The effect of different preservation methods on the carbon content of *Megacyclops gigas*. *Hydrobiologia*, **72**, 281-285

Santer B. (1990) Lebenszyklusstrategien cyclopoider Copepoden. PhD Thesis, University of Kiel

Santer B. (1994) Influences of food type and concentration on the development of *Eudiaptomus gracilis* and implications for interactions between calanoid and cyclopoid copepods. *Archiv für Hydrobiologie*, **131**, 141-159

Santer B. & Lampert W. (1995) Summer diapause in cyclopoid copepods - adaptive response to a food bottleneck. *Journal of Animal Ecology*, **64**, 600-613

Santer B. (1996) Nutritional suitability of the dinoflagellate *Ceratium furcoides* for four copepod species. *Journal of Plankton Research*, **18**, 323-333

Sarakinos H. C., Johnson M. L. & Vander Zanden M. J. (2002) A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. *Canadian Journal of Zoology - Revue Canadienne de Zoologie*, **80**, 381-387

Schmidt K., Atkinson A., Stubing D., McClelland J. W., Montoya J. P. & Voss M. (2003) Trophic relationships among Southern Ocean copepods and krill: Some uses and limitations of a stable isotope approach. *Limnology and Oceanography*, **48**, 277-289

Schneider G. (1989) Estimation of food demands of *Aurelia aurita* Medusae populations in the Kiel Bight Western Baltic. *Ophelia*, **31**, 17-27

Sherr E. B., Sherr B. F. & McDaniel J. (1991) Clearance rates of less-than-6 µm fluorescently labeled algae (Fla) by estuarine protozoa - potential grazing impact of flagellates and ciliates. *Marine Ecology Progress Series*, **69**, 81-92

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

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

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

Sommer U., Gliwicz Z. M., Lampert W. & Duncan A. (1986) The PEG-model of seasonal succession of planktonic events in freshwaters. *Archiv für Hydrobiologie*, **106**, 433-471

Sommer U. (1998) Biologische Meereskunde. Springer Verlag, Berlin Heidelberg

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

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

Spencer C. N. & Ellis B. K. (1998) Role of nutrients and zooplankton in regulation of phytoplankton in Flathead Lake (Montana, USA), a large oligotrophic lake. *Freshwater Biology*, **39**, 755-763

Sterner R. W. (1990) The ratio of nitrogen to phosphorus resupplied by herbivores - zooplankton and the algal competitive arena. *The American Naturalist*, **136**, 209-229

Sterner R. W. & Hessen D. O. (1994) Algal nutrient limitation and the nutrition of aquatic herbivores. *Annual Review of Ecology and Systematics*, **25**, 1-29

Sterner R. W. & Elser J. J. (2002) Ecological stoichiometry - the biology of elements from molecules to the biosphere. Princeton University Press

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

Strømgren I. (1973) Zooplankton investigations in Trondheimsfjorden, 1963-1966. *Det. Kongelige Norske Videnskabers Selskab*, **13**, 1-149

Sullivan B. K., Suchman C. L. & Costello J. H. (1997) Mechanics of prey selection by ephyrae of the scyphomedusa *Aurelia aurita*. *Marine Biology*, **130**, 213-222

Tande K. S. (1982) Ecological investigations on the zooplankton community of Balsfjorden, Northern Norway - generation cycles, and variations in body-weight and body content of carbon and nitrogen related to overwintering and reproduction in the copepod *Calanus finmarchicus* (Gunnerus). *Journal of Experimental Marine Biology and Ecology*, **62**, 129-142

Titelman J. (2001) Swimming and escape behavior of copepod nauplii: implications for predator-prey interactions among copepods. *Marine Ecology Progress Series*, **213**, 203-213

Todd C. D., Laverack M. S. & Boxshall G. A. (1996) Coastal marine zooplankton - a practical manual for students. Cambridge University Press, Cambridge

Toetz D. (1992) Use of mass-spectroscopy to investigate the diet of brook trout (*Salvelinus fontinalis*) in an alpine lake. *Journal of Freshwater Ecology*, **7**, 251-256

Touratier F., Field J. G. & Moloney C. L. (2001) A stoichiometric model relating growth substrate quality (C : N : P ratios) to N : P ratios in the products of heterotrophic release and excretion. *Ecological Modelling*, **139**, 265-291

Turner J. T. (1984a) Zooplankton feeding ecology - contents of fecal pellets of the copepods *Temora turbinata* and *Temora stylifera* from continental shelf and slope waters near the mouth of the Mississippi River. *Marine Biology*, **82**, 73-83

Turner J. T. (1984b) The feeding ecology of some zooplankters that are important prey items of larval fish. *NOAA Technical Reports National Marine Fisheries Service*, **7**, 1-28

Turner J. T., Ianora A., Esposito F., Carotenuto Y. & Miralto A. (2002) Zooplankton feeding ecology: does a diet of *Phaeocystis* support good copepod grazing, survival, egg production and egg hatching success? *Journal of Plankton Research*, **24**, 1185-1195

Turriff N., Runge J. A. & Cembella A. D. (1995) Toxin accumulation and feeding behavior of the planktonic copepod *Calanus finmarchicus* exposed to the red-tide dinoflagellate *Alexandrium excavatum*. *Marine Biology*, **123**, 55-64

Urabe J. & Watanabe Y. (1992) Possibility of N-limitation or P-limitation for planktonic cladocerans - an experimental test. *Limnology and Oceanography*, **37**, 244-251

Urabe J. (1995) Direct and indirect effects of zooplankton on seston stoichiometry. *Ecoscience*, **2**, 286-296

Utermöhl H. v. (1958) Zur Vervollkommnung der quantitativen Phytoplankton Methodik. *Mitteilungen Internationaler Theoretischer und Angewandter Limnologie*, **9**, 1-38

Vanderploeg H. A. (1981) Seasonal particle-size selection by *Diaptomus sicilis* in offshore Lake Michigan. *Canadian Journal of Fisheries & Aquatic Sciences*, **38**, 504-517

Vidal J. (1980) Physio-ecology of zooplankton .1. Effects of phytoplankton concentration, temperature, and body size on the growth-rate of *Calanus pacificus* and *Pseudocalanus* sp. *Marine Biology*, **56**, 111-134

Vitousek P. M. & Howarth R. W. (1991) Nitrogen limitation on land and in the sea - how can it occur. *Biogeochemistry*, **13**, 87-115

Vrede T., Andersen T. & Hessen D. O. (1999) Phosphorus distribution in three crustacean zooplankton species. *Limnology and Oceanography*, **44**, 225-229

Wagner M. M., Campbell R. G., Boudreau C. A. & Durbin E. G. (2001) Nucleic acids and growth of *Calanus finmarchicus* in the laboratory under different food and temperature conditions. *Marine Ecology Progress Series*, **221**, 185-197

Walve J. & Larsson U. (1999) Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. *Journal of Plankton Research*, **21**, 2309-2321

Wiadnyana N. N. & Rassoulzadegan F. (1989) Selective feeding of *Acartia clausi* and *Centropages typicus* on microzooplankton. *Marine Ecology Progress Series*, **53**, 37-45

Wickham S. A. (1995) *Cyclops* predation on ciliates: species-specific differences and functional responses. *Journal of Plankton Research*, **17**, 1633-1646

Williamson C. E. (1995) What role does UV-B radiation play in freshwater ecosystems. *Limnology and Oceanography*, **40**, 386-392

Wiltshire K. H., Harsdorf S., Smidt B., Blocker G., Reuter R. & Schroeder F. (1998) The determination of algal biomass (as chlorophyll) in suspended matter from the Elbe estuary and the German Bight: A comparison of high-performance liquid chromatography, delayed fluorescence and prompt fluorescence methods. *Journal of Experimental Marine Biology and Ecology*, **222**, 113-131

Xu Y. & Wang W. X. (2003) Fates of diatom carbon and trace elements by the grazing of a marine copepod. *Marine Ecology Progress Series*, **254**, 225-238

Zankai N. P. (1991) Feeding of nauplius stages of *Eudiaptomus gracilis* on mixed plastic beads. *Journal of Plankton Research*, **13**, 437-453

Zohary T., Erez J., Gophen M., Bermanfrank I. & Stiller M. (1994) Seasonality of stable carbon isotopes within the pelagic food- web of Lake Kinneret. *Limnology and Oceanography*, **39**, 1030-1043

Zöllner E., Santer B., Boersma M., Hoppe H.-G. & Jürgens K. (2003) Cascading predation effects of *Daphnia* and copepods on microbial food web components. *Freshwater Biology*, **48**, 2174-2193

Zöllner E. (2004) Structuring effects of mesozooplankton on freshwater and marine microbial food webs. PhD Thesis, University of Kiel

Danksagung

Mein besonderer Dank gilt meiner Betreuerin Barbara Santer für ihre Unterstützung und Hilfe in jeglicher Hinsicht, die Vermittlung ihres Wissens und ihrer langjährigen Erfahrungen über Copepoden, die nette Arbeitsatmosphäre und natürlich für ihre Entscheidung, mich als Doktorandin zu betreuen. Vielen Dank auch an Winfried Lampert für die Möglichkeit, am MPI zu arbeiten und für seinen wissenschaftlichen Rat. Für die finanzielle Unterstützung gilt mein Dank der MPG und der Deutschen Forschungsgemeinschaft (Projekt SA 638/1). Der Aufenthalt in Norwegen wurde zusätzlich unterstützt durch das Programm „Improving Human Potential - Transnational Access to Research Infrastructures“ der Europäischen Kommission.

Ohne die Hilfe des MPI Plön - IFM Kiel food web teams wären diese Experimente nicht möglich gewesen. Mein Dank gilt besonders unserem Projektleiter Ulrich Sommer u.a. für die Hilfe bei der Phytoplanktonbestimmung, aber auch Barbara Santer, Maarten Boersma, Claes Becker, Eckart Zöllner, Frank Sommer, Daniela Brepohl, Thomas Hansen, Klaus Jürgens, Hans-Georg Hoppe, Klaus Gocke und Regine Koppe. Ohne tatkräftige Unterstützung von Karin Wiedenhöft, Yvonne Funck, Julia Wohlers, Kerstin Maczassek, Andrea Saage, Sonja Dembeck, Sabine Flöder, Nadine Ryk, Uta Zschiesche, Raquel Melero, Rene Gergs, Markus Tank und den Studenten des Plankton-Praktikumskurses aus Kiel hätten die Experimente nicht in diesem Ausmaß stattfinden können. Heinke Buhtz für Hilfe bei den stabilen Isotopen Messungen, Dieter Albrecht für die Stöchiometriemessungen, Connie Reineke für Hilfe mit der HPLC, Ineke van der Veen für Statistik-Tipps, Martin Beutler und BBE Moldaenke für schnelle Hilfe bei Sondenproblemen und Herrn Ballaschek für die Zugangsgenehmigung zum Tonnenhof - Vielen Dank! Auch an die Computerfachleute am MPI, die Wege fanden, meinen Computer auszutricksen, und mir 2 Wochen vor Abgabe noch einen neuen PC zur Verfügung stellten. Die Zeit in Norwegen wäre nicht so abwechslungsreich gewesen ohne Nils Tokle, Øystein Leiknes und Jon’s Kochkünsten und der guten Organisation vor Ort von Alexandra Neyts.

Danke auch an Jon Grey „for polishing my english“, aber vor allem für seine Expertise und der Fähigkeit, mich mit seiner Begeisterung für Stabile Isotope anzustecken; und Roger Jones für hilfreiche Vorschläge zu Manuskripten und dafür, dass er mein Interesse für die Limnologie geweckt und damit meinen Weg entscheidend beeinflusst hat.

Mein Dank gilt auch der Abteilung Evolutionsökologie für die Genehmigung zur Benutzung des Umkehrmikroskopes. Danke an Eckart für die Bereitstellung von HNA Bakteriendaten und Barbara für die Genehmigung, die Daphnien-Eier Zählung mit in diese Arbeit zu integrieren. Auch bei ASLO möchte ich mich für das Student Travel Grant bedanken, das es mir ermöglichte, nach Salt Lake City zu fahren.

Vielen lieben Dank an alle meine Freunde aus Plön, es war eine geniale Zeit mit schwedischen, spanischen, holländischen und norddeutschen Traditionen. Fiskebölla, Kabelbinder, Helangor, das Preetzer Volleyball Team, Wokrodeln, (an)beachen, Whiskyessenz, Murata, Molteberries, BBQ im März, das Salt Lake City Hospital, segeln und Kajaktouren haben dazu beigetragen, das PhD Leben zu verbessern und von Zooplankton und Enclosures abzulenken ☺. Mycket tack an meinen officemate Claes, es hat riesigen Spaß gemacht mit dir das Büro zu teilen, und auch an Ineke, die immer ein offenes Ohr für mich hatte.

Mein besonderer Dank gilt meinen Eltern und Großeltern, die mir jederzeit zur Seite standen und mir alle Möglichkeiten boten, mich meinen Weg gehen zu lassen. Vielen Dank auch an Florian, der über viele Jahre immer für mich da war, und an Peter, der mich vor allem in letzter Zeit unterstützt hat.

Curriculum vitae

Name	Heidrun Feuchtmayr
Geburtstag und -ort	24.02.1977 in Burgau
Nationalität	Deutsch

Ausbildung

11/2000 – 07/2004	Promotion am Max Planck Institut für Limnologie in Plön, Abteilung Ökophysiologie
10/1999 - 09/2000	“Master of Science in Biological and Environmental Sciences” an der Lancaster University, U.K; Titel der Masterarbeit: “Bacterial bioassay of nutrient limitation in some upland lakes”
09/1996 - 09/1999	Studium der Geoökologie an der Technische Universität Bergakademie Freiberg, Vertiefungsrichtungen: Hydrologie, Pedologie, Umweltmikrobiologie/Biotechnologie

Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation selbstständig angefertigt habe und keine anderen als die angegebenen Quellen und Hilfsmittel von mir eingesetzt worden sind. Des weiteren versichere ich, dass die vorliegende Dissertation weder ganz noch zum Teil bei einer anderen Stelle im Rahmen eines Prüfungsverfahren vorgelegen hat.

Plön, 25.05.2004

Teile dieser Arbeit oder einzelne Datensätze sind bereits veröffentlicht oder bei wissenschaftlichen Zeitschriften eingereicht:

Feuchtmayr H, Zöllner, E., Santer, B., Sommer, U., Grey J (2004), Zooplankton interactions in an enclosure experiment: Insights from stable isotope analyses, *Freshwater Biology*, submitted

Feuchtmayr H, Grey J (2003), Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton, *Rapid Communications in Mass Spectrometry*, 17 (23): 2605-2610

Becker C, Brepohl D, Feuchtmayr H, Zöllner E, Sommer E, Clemmesen C, Sommer U, Boersma, M. (2004) Impacts of copepods on marine seston, and resulting effects on *Calanus finmarchicus* RNA:DNA ratios, *Marine Biology*, in press

Becker C, Feuchtmayr, H, Brepohl, D, Santer, B, Boersma, M (2004), Differential impacts of copepods and cladocerans on lake seston, and resulting effects on zooplankton growth, *Hydrobiologia*, in press

List of abbreviations and symbols

^{13}C, ^{12}C, ^{15}N, ^{14}N	isotopes of carbon and nitrogen
chl *a*	chlorophyll a
$\delta^{13}C$	carbon isotopic ratio ($^{13}C/^{12}C$)
$\delta^{15}N$	nitrogen isotopic ratio ($^{15}N/^{14}N$)
DIN	dissolved inorganic nitrogen
DIP	dissolved inorganic phosphorus
FCM	Flow cytometer
HNA	high nucleic acid
N	nitrogen
no.	number
NO_2^-	nitrite
NO_3^-	nitrate
P	phosphorus
POM	particulate organic matter
PO_4^{3-}	phosphate
PSU	practical salinity unit
‰	unit for notation of stable isotopes
SD	standard deviation
SI	stable isotope
SIA	stable isotope analyses
TDN	total dissolved nitrogen
TDP	total dissolved phosphorus
TDSi	total dissolved silicate
TN	total nitrogen
TP	total phosphorus