Living with constraints – food quality effects on zooplankton

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

vorgelegt von

Claes Becker

Kiel, März 2004

Referent: Dr. M. Boersma ....

Korreferent: ....

Tag der mündlichen Prüfung:...........................

Zum Druck genehmigt: Kiel, den ....

....

Der Dekan

Contents

Introduction

Introduction

Resources and growth – limitations in general

All consumers need resources to fuel the metabolism, and supply the consumer with organic precursors and essential nutrients. Obviously, consumers may be restrained by food limitations at periods of lower food levels. Moreover, resources differ in nutrient composition, and an unfavourable composition for the consumer induces constraints. Naturally, there are no consumers that can allocate all energy or nutrients to simultaneously support maximal growth, reproduction and life span. All consumers have to make life-history adjustments, and trade-off investments between for example growth rate, time and size of maturity, clutch size, offspring size. Under food limitations these trade-offs become even more prominent.

Tilman (1982) defined resources as essential (non-substitutable) and substitutable. Although this theory initially was developed for plants that take up single nutrients, it is also applicable to consumers (Ciros-Peréz et al. 2001; Rothhaupt 1995). Essential nutrients cannot be substituted: single minerals cannot replace vitamins, and fatty acids cannot be used as amino acids in protein synthesis. This translates into Liebig's law of the minimum (von Liebig 1855), which states that at any one point, only one factor limits growth or reproduction. For example, in Fig. 1, nutrient α is limiting at low concentrations, which is shown, as growth increases with additions of this component. However, at a specific point another component becomes limiting (nutrient β in Fig. 1) and the growth reaches an asymptote. Liebig's law dictates that above the β limitation point, the addition of α should not influence growth levels. At this point and above, only the addition of β should positively impact growth, and additions of the β component enhance growth. The process continues until another factor becomes limiting or maximal growth is achieved. Thus, in Fig. 1 the growth supported by α is totally dependent on the concentration of β and vice versa. Substitutable resources on the other hand, imply that one resource can be replaced with another. Hence, as long as food sources contain all essential compounds the consumer can substitute one food source for another. This does not necessary mean that both resources are equally good for the consumer, but simply that it can survive on each of the resources separately. Moreover, the availability of different food packages, rich in different resources, can be beneficial for the consumers. For example, resources may act complementarily if a mixed diet supports higher growth than
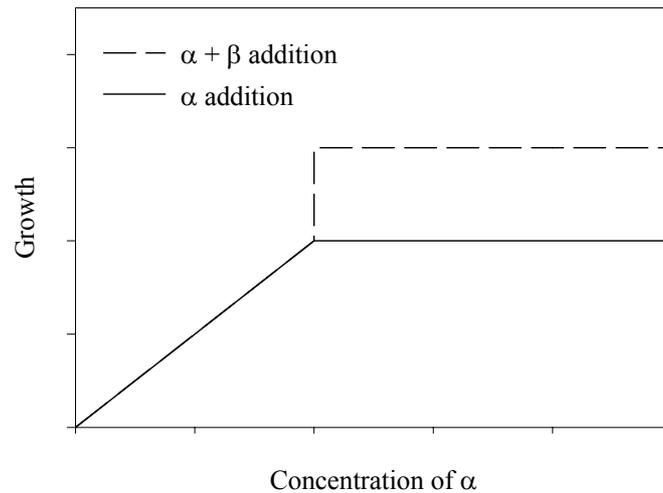
Fig. 1. Different resource limitations and the implications on growth. The nutrient α additions are provided in a concentration gradient and nutrient β is either available or not (single addition).

each of the single food items alone. The focus of this thesis will be the effects of essential resources and especially fatty acids and phosphorus.

Food quality

Most consumers take up food items as complex biochemical packages, and not as single nutrients. Because nutritional requirements differ between a consumer and its prey, the prey items are often of less than optimal quality for a consumer. This suggests that consumers may frequently encounter food sources of sub-optimal quality causing retarded development. For a food source to be considered of good quality it needs to be easily ingestible, digestible and also it has to contain all essential compounds. The food quality of animal species of commercial importance for human consumption has been studied in great detail. Only fairly recently have these investigations moved into the aquatic realm as aquaculture developed into commercial viability. Despite the enormous body of knowledge available on this small set of commercially interesting species our knowledge on fundamental aspects of effects of food quality on natural systems is still limited. Crustacean zooplankters are key organisms in aquatic ecosystems, because they are the most important link between the primary productivity of the microalgae and the production of many fish species. Although considerable progress has been made in defining the effects of food limitation of quantity and quality origins, many questions are still open, especially with respect to essential and substitutable resources. Several authors have studied food quality effects on herbivorous zooplankton (e.g. Boersma 2000; Brett et

al. 2000; DeMott 1998; Elser et al. 2000; Hessen 1992; Müller-Navarra 1995b; Sterner et al. 1993; Urabe and Sterner 2001). Numerous factors that determine food quality have been recognized and investigated, and four important ones were identified: 1) size and morphology; 2) toxicity; 3) nutrient content; 4) biochemical content.

Size and Morphology- Algal cells that are too large (Bern 1994) or too small (Brendelberger 1991) cannot be ingested by zooplankton. The dimension of the particle is also of importance, because a needle shaped algae can be ingested if oriented lengthwise. Apart from the actual cell size, prey hardness has a major impact on the ability to ingest food particles (DeMott 1995). Moreover, if the algae have defensive structures like thick cell walls (van Donk et al. 1997), or gelatinous sheaths (Porter 1976) it might not be seriously damaged by passage through the zooplankton gut, and hence, for the zooplankton the food quality decreases (DeMott and Tessier 2002). Different zooplankters have different preferences in food particle size. This is generally determined by the gape size of the mouth-parts (mandibles) or by the opening in the carapace. The lower end is set by the mesh size in the filtering apparatus.

Toxicity- Several algal species contain toxic compounds that can have detrimental impacts on zooplankton development and reproduction. For example different blue-green algae are bad food sources for zooplankton and it has been shown that different strains of *Microcystis aeruginosa* are toxic to *Daphnia* (Lampert 1982; Nizan et al. 1986; Reinikainen et al. 1994) and also to warm-blooded animals (Collins 1978). *Microcystis aeruginosa* can produce more toxins in response to both indirect and direct zooplankton grazing (Jang et al. 2003). Thus, for the algae this is an effective defence, instantly lowering the filtration rate and increasing the mortality of their predators (Lampert 1981). The interaction between copepods and diatoms has been a point of much scientific interest over the last decade especially in marine systems. Reports have shown that during diatom blooms, copepod egg production and egg viability decreases. The underlying reasons are still under debate, but effects of toxicity or nutrient deficiencies are being discussed (reviewed in Ianora et al. 2003; Paffenhöfer 2002). If this is an algal defence, the direct benefits for the algae from the interaction are less clear, since the current grazing is not seriously affected.

Mineral composition- Compounds that cannot be synthesised *de novo* are by definition essential, and the consumer depends on a satisfactory intake with the food. Essential compounds are either inorganic minerals or biochemical compounds such as amino acids, fatty acids and vitamins. The mineral composition of the food can influence

the nutritional quality and the necessity of a number of minerals for zooplankton has been investigated (DeMott and Gulati 1999; Sterner et al. 1993; Sterner and Schulz 1998). For example, if the phosphorus concentration in algae is also low, nutritional quality of the food is low (DeMott 1998; Urabe et al. 1997). Studies of phosphorus limitation often use the carbon:phosphorus molar ratio (C:P), as an indicator of food quality. An increased C:P ratio hence signifies a relative lower content of phosphorus and is usually linked to a decrease in food quality. Daphniids are assumed to be limited by phosphorus above a C:P ratio of around 80-300 (Brett et al. 2000; DeMott et al. 1998; Vrede et al. 2002). Nitrogen and the consequential C:N ratio is another predictor of food quality that affects zooplankton growth (Sterner 1993; Sterner et al. 1993). Most evidence for P limitation comes from freshwater systems where a wide range of seston C:P ratios in different lakes has been recorded (Elser and Hassett 1994). In many studies, the most abundant crustacean zooplankton genus *Daphnia*, has been used as a test organism. *Daphnia* has a higher requirement for phosphorus relative to other zooplankters (C:P = 30) (from Hessen and Lyche 1991), which makes daphniids more likely to be affected when P becomes scarce. Other zooplankters have lower P requirements (e.g. *Diaphanosoma* and *Bosmina*) and are less negatively affected by feeding on severely P limited algae (Sterner and Schulz 1998; Urabe and Watanabe 1992). Moreover, copepods in general also have a low P demand (C:P = 130) (from Hessen and Lyche 1991) but with a tendency for higher N requirements, they are more likely to be limited by this element (Hessen and Lyche 1991). Marine copepods have a lower N content relative to their freshwater counterparts (Walve and Larsson 1999) and thus, are even more likely limited by N (Checkley and Entzeroth 1985; Kiorboe 1989; Townsend and Pettigrew 1997). Although availability of essential compounds is important for consumers, a further aspect is the accessibility of those compounds. For example, phosphorus is mostly taken up through the absorption of phosphate. Several other forms (e.g. polyphosphate) cannot easily be absorbed, unless preceded by a biotransformation by bacteria (van Wazer 1973).

Phosphorus is required for skeletal growth and maintenance (Machlin 1973), and it plays a large role in energy rich compounds (ATP), as precursors for phospholipids and DNA (Mathews and van Holde 1996). Nitrogen on the other hand, is important for protein synthesis and is taken up in the form of proteins and amino acids (Alberts et al. 1994). Other elements, such as selenium (Elendt and Bias 1990) and especially calcium (Hessen et al. 2000; Hessen and Rukke 2000), have received some attention in zooplankton

research. These elements are typically used in the moulting process, and deficiencies are deleterious.

Biochemical composition- Biochemical composition of the food is an important determinant of food quality and different components appear to have differing roles. Guisande and co-workers (1999; 2000) found that egg numbers mainly were determined by food abundance, yet the availability of essential amino acids had a strong effect on egg hatching. Thus, the reproductive success was determined by the interaction of food quantity and quality, in terms of amino acids. Amino acids are rich in nitrogen and therefore amino acid limitations might be closely linked to N limitations (Anderson et al. 2004). Others have investigated the importance of sterols in the food. Cyanobacteria normally provide a poor food source for zooplankton, and recently that poor food quality was linked to the low levels of sterols in these algae (von Elert et al. 2003). Over all, however, the biochemical compounds receiving the largest proportion of scientific interest have been the polyunsaturated fatty acids (PUFA). Among these, the importance of the fatty acids from the ω3 family (eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) has been highlighted (Brett and Müller-Navarra 1997; Müller-Navarra 1995a; Müller-Navarra 1995b; Sundbom and Vrede 1997; Wacker and von Elert 2001; Weers and Gulati 1997). One aspect that makes certain fatty acids essential is their role as precursors for prostaglandins, leukotrienes and tromboxanes, which are involved in inflammatory and immune responses. Especially, arachidonic acid (ARA), EPA and DHA are important in this process. Furthermore, PUFAs are essential in cell membrane fluidity, where less fluid membranes are dysfunctional (Rock and Cronan 1985). Deficiencies of essential fatty acids during periods when neural tissue is developed (e.g. eyes and brain) are detrimental (Olsen 1999). To avoid these negative effects, reproducing females usually allocate high contents of these fatty acids into the eggs. It has been shown for example that malpigmentation in fish, which can be used as an indicator of environmental stress, was very closely linked to DHA:EPA ratios in the fish larvae, especially in the eyes of developing fish fry, higher DHA:EPA ratios have been found (McEvoy et al. 1998). Another, yet non-essential, trait of fatty acids is that they prove effective as energy stores. Such storage could be used to compensate for periods of low food, and is also important for *Daphnia* egg production, in which each egg production cycle is preceded by a visible accumulation of fat droplets (Tessier and Goulden 1982).

The essentiality of a fatty acid is dependent upon the location of the double bonds on a fatty acid molecule. Most animals lack specific desaturases to insert double bonds

between the methyl end and the 9th carbon atom of a fatty acid molecule (Cook 1985). Hence, there are different families of fatty acids that a consumer needs in a pre-fabricated form, such as ω3 and ω6 fatty acids, which have the first double bond on the 3rd and the 6th carbon atom respectively (Cook 1985). However, when these essential bonds are available, most consumers have the capability to elongate and (beyond the 9th carbon) desaturate these compounds into desired PUFAs.

The freshwater food quality controversy- Of all these potential food quality determinants, the importance of phosphorus and fatty acids has attracted most attention in freshwater systems. Phosphorus has been suggested to be the main limiting nutrient in most lakes and rivers (Elser and Hassett 1994). Moreover, negative effects of phosphorus limitation on daphniids have been described (DeMott 1998; Urabe et al. 1997). On the other hand, different studies found *Daphnia* growth to be closely linked to the PUFA concentrations of the food (Müller-Navarra 1995b; Wacker and von Elert 2001). In these studies positive effects between *Daphnia* growth and the content of two different ω3 fatty acids were found. The most likely explanation for these different patterns is that different food quality aspects play variable roles in different ecosystems and at different times. However, it is also possible that the focus of the different studies was such that they inevitably led to different conclusions. As a result, different schools exist promoting different "most-important" factors. Interestingly, the various studies have used different techniques to prove their points. For phosphorus, studies were mainly performed experimentally in the laboratory with algae grown under different phosphorus conditions (DeMott 1998; Sterner et al. 1993). However, such algae not only differ in P-content, but also undergo morphological and biochemical changes. For instance, P-limited algal cells form thicker cell walls (Tillberg et al. 1984a), which makes them less digestible for *Daphnia* (van Donk et al. 1997). Moreover, the fatty acid profile of P-limited algae changes, with a higher total concentration of fatty acids, yet the proportion of EPA decreases (Boersma 2000; Müller-Navarra 1995a). Therefore, the negative effects on zooplankton growth due to feeding on P-limited algae might be explained by separate co-varying constraints and hence only reflect the indirect effect of P-limitation. On the other hand, the studies propagating the importance of polyunsaturated fatty acids mainly used correlative evidence from field studies (Müller-Navarra 1995b; Wacker and von Elert 2001). Hence, also in these studies unknown factors could co-vary with the appointed limiting fatty acid. In recent years, these problems have been acknowledged, and new techniques to experimentally manipulate the fatty acids and phosphorus concentrations in

the food developed. For phosphorus, researchers have used variable mixtures of different P-rich algae in order to assess effects of direct P-limitation (DeMott 1998). Urabe et al. (1997) used the fact that daphniids can take up dissolved phosphorus from the water. Others addressed the problem from the algal perspective, and used the fact that P-limited algae rapidly take up dissolved P from the surrounding water, resulting in a direct change in the C:P ratio (Boersma 2000; DeMott 1998; Elser et al. 2001; Plath and Boersma 2001). These authors argue, that when production is low (e.g. no photosynthesis) the biochemical and morphological features are responding slowly. To manipulate fatty acids, several techniques exist, such as fatty acid emulsions, microencapsulated lipids or PUFA-rich algae (Plath and Boersma 2001; Sundbom and Vrede 1997; Weers and Gulati 1997). However, in these techniques, mixtures of fatty acids were used, and it was not possible to attribute the effects of single fatty acids. Further new methods have been developed, which allow specific additions of single fatty acids to algal cells (von Elert 2002; von Elert and Stampfl 2000). Combining the recently developed techniques, allows for the manipulation of both single fatty acids and the P content of the algae, as well as using the algal cells as a vehicle to study the effects on the zooplankton grazers.

The study organisms

Aquatic zooplankters have a great impact on the transfer of energy and nutrients throughout the food web, as they link primary production with higher trophic levels. However, different zooplankton taxa play roles of differing importance.

Cladocerans- Cladoceran zooplankton occurs both in freshwaters and in marine systems. Only eight species of cladocerans have been found in marine systems compared to the more than 600 species recorded in freshwaters. Marine cladocerans are typically difficult to culture in laboratory. Therefore, information of the biology of these zooplankters is still meagre. Their feeding behaviour is still not completely understood, but a raptorial feeding pattern has been suggested for most (Kim et al. 1989). At certain times in the year marine cladocerans increase in numbers and should make an important link between primary and secondary production during these periods. It is generally accepted that cladocerans play a more dominant role in freshwater compared to marine ecosystems. In the majority limnic ecosystems several cladoceran genera exist, and one of the most common is the genus *Daphnia*, which often dominates zooplankton biomass. Daphniids are general filter feeders, with limited possibilities to select their food. In fact, selection is restricted to rejection of already captured food particles. This is an ineffective

procedure, firstly because the feeding is interrupted during the removal of the clogging particle (Lampert 1987), and secondly in the removal process the animals are likely to remove edible captured particles. Even though daphniids lack the possibility to actively select single food particles, they are able to localise patches with high food abundance (e.g. Cuddington and McCauley 1994; Jensen et al. 2001). Throughout the summer *Daphnia* normally reproduce parthenogenetically, and females produce identical genetic copies (clones). This is rapid a process that only takes 2-4 days under favourable conditions. Hence, daphniids can quickly respond to increased food abundance. The effect of this is normally visible in spring, when the phytoplankton biomass peak is followed by an increase of rapidly reproducing *Daphnia*. The daphniids actually increase to such a high total biomass, that the phytoplankton biomass is suppressed resulting in a "clear water phase". On the other hand, daphniids can also switch to reproduce sexually, which is preceded by the production of males and eggs that need to be fertilised. The onset of this process is often related to environmental factors such as shorter day length, lower temperatures, high predation and less food, all indicating periods of lower survival probabilities. Hence, the sexual eggs represent a diapausing stage that enables survival of *Daphnia* populations during unsuitable conditions. A shell of chitin, called the ephippium, protects the resting eggs. These can survive long periods of diapause and harsh conditions, before the eggs are "activated" and develop into new daphniids.

Copepods- They occur in freshwater as well as in marine ecosystems. In contrast to cladocerans, copepods seem to play a more prominent role in marine systems. Their feeding process cannot easily be generalised, as copepods exhibit herbivorous, omnivorous, carnivorous and detrivorous. However, adult cyclopoid copepods are commonly accepted to be predaceous (e.g. Gliwicz 1994; Gliwicz and Umana 1994; Kerfoot 1977). Nevertheless, many copepods feed on protists and algae (Jürgens et al. 1996; Santer and van den Bosch 1994), but use a different feeding behaviour compared to the cladocerans. Herbivorous and omnivorous copepods are very selective in their feeding process. The selectivity is sensitive and copepods can distinguish between food particles of different nutritional status (Cowles et al. 1988), taste (DeMott 1986) and toxicity (DeMott and Watson 1991). Copepods reproduce sexually, however, there is some evidence that some copepod species can undergo parthenogenesis (reviewed in Dussart and Defaye 2001). After fertilization the eggs are either dropped, or carried in characteristic egg sacs attached to the female abdomen. The eggs hatch and the neonates develop through several naupliar and copepodite stages before maturation into adults. As a

consequence, copepods have longer generation times compared to the parthenogenetic cladocerans. This, in combination with their lower feeding efficiency (Santer and van den Bosch 1994) suggests that copepods are less prone to deplete their food resources in a similar manner to daphniids. Some copepod species also undergo diapause as a process for an individual or its offspring to overcome poor conditions, specific resting eggs as well as resting stages have been described (Dussart and Defaye 2001).

Zooplankton interaction- As a result of the differences in feeding preferences and feeding modes and life cycles, copepods and cladocerans affect the plankton community to a different extent (Sommer et al. 2001). Interestingly, the potential interactions between copepods and cladocerans have received little attention, even though these zooplankton taxa co-occur in many freshwater bodies. A few studies have shown that in ecosystems where herbivorous copepods are dominant, smaller sized phytoplankton species increase in abundance; this is linked to the larger copepodites and adults preferring larger particles. Conversely, in a system dominated by the cladoceran *Daphnia*, the larger phytoplankton species are most abundant, since these species are outside the range filtered by *Daphnia* (Rothhaupt 1997; Sommer et al. 2001). Moreover, Rothhaupt (1997) observed not only differences in the phytoplankton species composition, but also that the nutrient dynamics changed with differing herbivore dominance. This could be explained by the dissimilar nutrient requirements of the grazers. Daphniids have a higher requirement of phosphorus (C:P = 30) compared to copepods (C:P = 135) (from Hessen and Lyche 1991). Thus, in order to fulfil this higher requirement, daphniids should more efficiently retain phosphorus from the phytoplankton. Indeed Rothhaupt (1997) found daphniids drove the algae to P-limitation. On the other hand, both N and P remained limiting under copepod grazing (*Eudiaptomus* sp.). The changes induced by the herbivore on the phytoplankton composition and nutrient availability should most likely also affect other aspects of the plankton, such as the biochemical composition (fatty acids, amino acids). However, so little is known about the requirements of copepods and cladocerans for such substances, it is not possible to predict the direction of these changes, nor the consequences.

In this thesis I studied the effects of different environmental factors on zooplankton. The most direct approach to detect changes should be to provide a measure of the relative fitness directly with an ecosystem. Fitness is defined as the relative contribution of one genotype to the next generation relative to other genotypes. In other words, the investment in reproduction one individual makes in relation to investments of other individuals. However, not only the input in reproduction is of importance but also

the offspring survival (Lampert and Sommer 1997). Including all these parameters makes fitness determination directly in the field impossible; hence, proxies for fitness have to be used. The most direct estimate of fitness is the measurement of instantaneous rate of increase (r) in laboratory. It accounts for the maximal fitness excluding non-intrinsic mortality in a population of stable age distribution. Establishing r can be time consuming, especially in copepods with their long generation times, since developmental times are included in the calculations (Stearns 1992). Several methods have been developed to link r with short-term measures. More traditional proxies for fitness include egg production (S.E.P. Hebert 1977), lipid index (Tessier and Goulden 1982) and standard carbon content (Boersma and Vijverberg 1994). More sophisticated ones include the juvenile growth rate, and the ratio of RNA to DNA (Lampert and Trubetskova 1996; Vrede et al. 2002).

Hypotheses

The co-occurrence of various zooplankton guilds, exhibiting different feeding patterns and nutritional requirements, suggests several possible interactions. One of the most exciting interactions is the possibility that different guilds could show mutualistic interactions. This follows the ideas of previous reports (Rothhaupt 1997; Sommer et al. 2001) that showed large differences induced by diverse food preferences between calanoid copepods and daphniids. Such a pattern suggests that these two guilds could represent separate niches, or at least niches of minor overlap. Hence, the first hypothesis I wanted to test was that plankton communities manipulated by one guild should provide a better food for the second guild. Further, I expected the guild interaction to improve the food quality compared to when offered a food source not manipulated by meso-zooplankton. Moreover, I propose that seston previously manipulated by one guild should result in a food of lower quality for members of the same guild. I expect these patterns to act beneficial on both guilds and study these interactions both in a limnic and a marine ecosystem.

Recent reports have suggested daphniids to be co-limited by fatty acids and phosphorus (Plath and Boersma 2001). This pattern suggests that zooplankton can substitute these resources, and hence not essential. However, in the above study, it was not possible to separate the energy effect from the effect of the essential fatty acid (EPA). In the last years, new techniques were developed (von Elert 2002; von Elert and Stampfl 2000), thus, allowing a detailed study of the P to EPA interaction. I hypothesise that essential nutrients, in accordance with von Liebig (1855), cannot be substituted and that

daphniids are only limited by essential resource sequentially. Moreover, I suppose that different resources play variably important roles throughout the ontogenesis. I expect that EPA should be more important for reproduction, as each egg production cycle is preceded by an accumulation of lipids that decrease when the eggs are released (Tessier and Goulden 1982). Phosphorus on the other hand, should play a larger role during earlier life stages.

Life history experiments can demonstrate when and how limitation pressures shift during *Daphnia* life history. However, to understand how various essential compounds are allocated within daphniids, *Daphnia* elemental stoichiometry and biochemical composition has to be investigated. Daphniids are generally believed to be homeostatic in terms of P (Sterner 1990), which suggests that their internal C:P ratio should be stabile independent of the food C:P ratio. Lately however, evidence against this theory has been put forward (e.g. DeMott et al. 1998). Nevertheless, the fat storage varies much more and can make up as much as 70% of the dry weight. Hence, in comparison with the fat storage the P content is stable. I investigate how phosphorus and different fatty acids are allocated by the daphniids during various life stages. I suppose that P, for which daphniids only have a limited storage capacity, should be relatively stable compared to fatty acids over the *Daphnia* life cycle. I expect essential fatty acids on the other hand, to a greater extent to be stored for later use, e.g. during period of low food abundance, or to enhance the reproductional outcome.

Thesis outline

In the present thesis, five chapters concerning the interaction between phytoplankton and zooplankton are presented. The chapters are:

    I   Differential impacts of copepods and cladocerans on lake seston, and resulting effects on zooplankton growth

    II   Impacts of copepods on marine seston and resulting effects on *Calanus finmarchicus* RNA:DNA ratios

    III Resource quality effects on life histories of *Daphnia*

    IV Differential impacts of phosphorus and fatty acids on *Daphnia* growth and reproduction

    V   Discussion

The first two chapters deal with the interactions between zooplankton and seston from one freshwater and one marine mesocosm experiment. I used different techniques to determine the growth potentials of the marine zooplankton and the freshwater daphniids. An identical mesocosm set-up was used in both field studies. Chapter three and four describe the outcome from two sets of experiments conducted in laboratory.

(I) In the first chapter, I discuss a parallel laboratory-mesocosm experiment. In a large-scale mesocosm experiment, the impacts by the different feeding behaviour of both copepods and daphniids on the seston community were investigated. The mesocosm consisted of 24 enclosure bags that were enriched with two separate density gradients of copepods and daphniids. I investigated how the expected changes affected the zooplankton growth. Observed differences were linked to food quantity/quality changes induced by the manipulating of the various zooplankton and densities.

(II) In chapter two, I describe how various densities of marine copepods affect the seston community and how these differences affect the *Calanus finmarchicus* nutritional status. In this particular study, nutritional status was approximated with RNA:DNA measurements, where a higher ratio indicates a higher nutritional status(Wagner et al. 1998). The RNA:DNA technique, is a useful tool that allows measurements on organisms directly from the field. The RNA:DNA changes were over time in the various mesocosm treatments continuously monitored. Observed differences in growth potential were linked to changes in food quantity and quality. Interestingly, the niche that is filled by *Daphnia* in the freshwater seemed to be unoccupied in these experiments.

(III) Chapter three deals with the interaction between nutrient and biochemical limitations on *Daphnia* life histories. I investigated how *Daphnia magna* responded to a concentration gradient of phosphorus (P) and the availability of eicosapentaenoic acid (EPA), a fatty acid of the ω3 family. These resources (P and EPA) are essential to daphniids and should not be substitutable. The life history study enabled me to assess if the limitation pressure of each resource is the same throughout the *Daphnia* ontogeny and thus, has the same impact on development and reproduction.

In the fourth chapter (IV), I describe the continued work with biochemical and fatty acid limitations. I set out to investigate the level of fatty acid demand *Daphnia magna* requires for growth by using concentration gradients of fatty acids. Moreover, I determined how daphniids use fatty acids in ample supply and investigated if excess fatty

acids were stored for future use (reproduction, starvation buffer) or were they merely used up (e.g. for energy purposes or in biosynthesis).

The last chapter (V) the discussion, takes all of the results obtained in this thesis, summarises these and gives suggestions for further research directions.

Chapter I

Differential impacts of copepods and cladocerans on lake seston, and resulting effects on zooplankton growth

Abstract

In an enclosure study in Schöhsee, a small mesotrophic lake in Northern Germany, the impact of copepods and daphniids on the seston community was studied. In general, these two guilds differ in their feeding behaviour. Copepods actively select their food, with a preference for larger particles, whereas most cladocerans are unselective filter feeders. In this study we investigate how the impact of the two different grazers affects zooplankton growth. We combine results obtained in the laboratory with results measured *in situ* in the enclosures. Copepods and cladocerans were cultured on seston from enclosures that were inhabited by density gradients of copepods or daphniids. We observed that *Daphnia* grew faster on seston that was manipulated by copepods than on seston that was manipulated by daphniids, and that somatic growth decreased with increasing densities of daphniids in the enclosures. In contrast, we observed no differences in development rates for copepods grown on the different media. The population growth rates of *Daphnia* in the *Daphnia* treatments were determined in the enclosures. Growth differences in both somatic- and population growth of *Daphnia* were correlated to food quality aspects of the seston. In the laboratory we found that *Daphnia* growth was correlated with several fatty acids. The strongest regression was with the concentration of 20:4ω3 ($r^2 = 0.37$). This particular fatty acid also showed the highest correlation with growth after normalisation of the fatty of the fatty acids to the carbon content of the enclosures ($r^2 = 0.33$). On the other hand, in the enclosure the population growth correlated most to the particulate nitrogen content ($r^2 = 0.78$) and only to the N:C ratio, when normalised to carbon ($r^2 = 0.51$).

Introduction

Ever since Hutchinson formulated his paradox of the plankton (1961), and Tilman (1982) investigated the conditions necessary for the coexistence of species in a certain habitat, many researchers have investigated the interactions between planktonic organisms. This was often done with phytoplankton (Flöder and Sommer 1999; Huisman and Weissing 1999; Interlandi and Kilham 2001), most likely since resources are easily defined for phytoplankton. The interactions between freshwater zooplankters have also been investigated in some detail, especially the (competitive) interactions between members of one of the most obvious taxa in freshwater environments, the Cladocera (Bengtsson 1986; Boersma 1995; DeMott and Kerfoot 1982; Hessen 1990; Matveev 1983; Vanni 1986). Interestingly, the potential interactions between copepods and cladocerans have received much less attention, even though members of these guilds co-occur in many freshwater bodies. Whereas feeding of cladocerans, especially daphniids is well studied and these animals are normally classified as herbivorous, the nutrition of copepods is less clear. Adult cyclopoid copepods are commonly accepted to be predaceous, and as a result the predator-prey interaction between cyclopoid copepods and cladocerans has been studied in some detail (e.g. Gliwicz 1994; Gliwicz and Umana 1994; Kerfoot 1977). Nevertheless, not all of the copepod species and stages are predaceous, and many feed on protists and algae (Jürgens et al. 1996; Santer and van den Bosch 1994), but with a different feeding behaviour compared to herbivorous cladocerans. Copepods actively select their food, while most cladocerans are filter feeders and do not discriminate between food particles (Butler et al. 1989; DeMott 1986). Food selection by copepods is very sensitive and copepods can even distinguish (probably via chemoreception) between food particles of different nutritional status (Cowles et al. 1988) or toxicity (DeMott and Moxter 1991). Members of the genus *Daphnia,* the most important cladoceran genus in many temperate lakes, on the other hand, can only reject already captured food particles. This procedure is ineffective since feeding is interrupted until the clogging particle is removed (Lampert 1987). Moreover, edible particles may be removed together with the rejected particles. Even though daphniids lack the possibility to actively select single food particles, they are able to localise patches with high food abundance (e.g. Cuddington and McCauley 1994; Jensen et al. 2001). As a result of their high feeding efficiency and their parthenogenetic mode of reproduction, with the resulting rapid potential of population growth they can rapidly exploit such food sources. In contrast, copepods go through several naupliar and copepodite stages, show low feeding efficiency (Santer and van den Bosch 1994), and rely

on sexual reproduction. This implies that copepods are probably much less effective in depleting their resources. In short, as a result of the differences in feeding preferences and feeding modes and life cycles, copepods and cladocerans affect the seston community to a different extent (Sommer et al. 2001).

A few studies have shown that in ecosystems where herbivorous copepods are dominant, smaller sized phytoplankton species prevail, since the larger copepodite stages and the adults prefer larger particles. On the other hand, in a *Daphnia* dominated system the larger phytoplankton species are most abundant, since these species are outside the range filtered by *Daphnia* (Rothhaupt 1997; Sommer et al. 2001). Moreover, Rothhaupt (1997) also showed that not only differences in the phytoplankton species composition could be observed, but also that the main herbivores caused differences in the nutrient dynamics. Whereas daphniids, with their high requirement for phosphorus, drove the algae to P-limitation, in systems with copepods both N and P remained limiting. This effect is explained by the fact that the different grazers differ in their nutrient content and requirements. Daphniids generally have a higher requirement for phosphorus than copepods, with molar C:P ratios of around 30 and 135 respectively (Hessen and Lyche 1991). Therefore, daphniids will retain much of the phosphorus and thus cause P-limitation in the phytoplankton. Most likely, as a result of shifts in phytoplankton composition and nutrient availability when exposed to different herbivores also other aspects of the seston will change, such as the biochemical composition (fatty acids, amino acids). However, as still very little is known about the requirements of copepods and cladocerans for such substances it is not possible to predict the direction of these changes, nor the consequences.

In short, there are many differences between the cladocerans and copepods, which can cause differential effects on the food web. As in many lakes members of these taxa co-exist, it is very interesting to study how they affect each other. Possibly, given their different mode of feeding, the competition between the taxa will not be large, and they fill different niches. If this is the case, it could well be that by removing a food source, which is not readily available to the other crustaceans in the system, but competes with these food sources for nutrients, the presence of one taxon could actually be of benefit to the other one. This we aim to investigate in this paper. Moreover, by correlating several seston characteristics with the growth and reproduction of the organisms we aim to identify the parameters of the food that play a role in this interaction.

Materials and Methods

This work was done as a part of a larger project, in which we aim to determine the impact of different herbivorous zooplankton on the food web. These effects are studied in detail on various levels ranging from bacteria to zooplankton (Sommer et al. 2001). In this paper we focus on the interaction between the seston community and the zooplankton growth.

A mesocosm experiment was conducted in mesotrophic Schöhsee in May 2001. 24 mesocosms (2.2 m long; 1.6 m^3) were installed and filled with lake water, which was sieved through 55 μm plankton gauze in order to remove mesozooplankton, and enriched with a logarithmic density gradient of different zooplankton species (for more information about the set-up see (Sommer et al. 2001). The *Daphnia* density gradient was obtained by adding a clone of *Daphnia galeata x hyalina* originally isolated from lake Schöhsee. For the copepod density gradient we used wild-caught zooplankton samples (<250 μm) from Schöhsee, which were dominated by the calanoid copepods *Eudiaptomus gracilis* and *Eudiaptomus graciloides* and the cyclopoid copepods *Mesocyclops leuckarti*, *Diacyclops bicuspidatus* and *Thermocyclops oithonoides*. To remove the cladocerans from the samples, the water was vigorously bubbled with air for several hours, during which the floating cladocerans were repeatedly removed from the water surface. The inoculated copepod samples contained calanoid and cyclopoid copepods in a ratio of about 1:1. The copepod enclosures were enriched with copepods to obtain densities of 5, 10, 20, 40 and 80 individuals per litre. The *Daphnia* density gradient was inoculated to achieve the enclosure densities of 1.25, 2.5, 5, 10 and 20 individuals per litre. We had a mixed treatment, which was inoculated with 20 copepods and 5 daphniids per litre. There was also a control treatment that did not receive any zooplankton. All together we had 12 different treatments that all were replicated yielding 24 enclosures. Different numbers were used for copepods and cladocerans to obtain similar biomass rather than similar numbers in the enclosures (see Sommer et al. 2001). Water and zooplankton samples were taken every three to four days. In order to avoid sampling errors due to aggregation or sedimentation, the enclosures were thoroughly mixed with a Secchi disk before sampling. Filtrates of the water samples were analysed for nutrient (C:N:P) and fatty acid composition. For seston C:N:P analyses, pre-filtered (100 μm) water was collected on pre-combusted and, for phosphorus, acid washed, GF/F glass fibre micro filters and dried overnight at 60°C. Total nitrogen and carbon was measured using a FISONS® NA2000 elemental analyser, total phosphorus was determined by an alkaline persulphate oxidation (Grasshoff et al. 1983). Fatty acid spectra were also established for the seston in the

enclosures, and at the end of the experiment also for the zooplankton. For the seston analyses we filtered 2-5L on a GF/C filter that was subsequently stored under N_2 gas at – 70°C until further processing. For the analysis of the zooplankton, we collected animals (1-5mg dry weight) from each enclosure at the end of the experiment, sorted them, and stored them under nitrogen. Fatty acids were extracted, esterified and analysed on a gas chromatograph according to (Wiltshire et al. 2000). To quantify the fatty acid content we used an internal standard of odd-chained fatty acid methyl esters (13:0-21:0; Restek).

Zooplankton samples were taken with two vertical tows through the entire water column of the enclosure with a 55μm mesh size plankton net. The samples were fixed with ethanol. The zooplankton was identified and counted under a dissecting stereomicroscope. From those counts, we calculated the population growth rates of the zooplankters in the enclosures.

During this mesocosm study we conducted two laboratory experiments, in order to determine how the differences between copepod and *Daphnia* treatments affect growth and development of different zooplankton taxa. The two laboratory experiments were conducted during the periods 9-12 and 13-18 days after the start of the enclosures. In both experiments, 2.5-litre water was taken daily from both replicate bags from the enclosure treatments. In the first experiment we used water from the following treatments: control, mixed, copepod 10 and 80, *Daphnia* 10 and 20 (number corresponds to individuals per litre). The second experiment was conducted with water from control, mixed, copepod 5 and 20, *Daphnia* 1.25 and 5. The water from each treatment was mixed and sieved over a 100 μm gauze to remove most of the mesozooplankton. The water was kept dark and gently stirred to prevent sedimentation. The laboratory temperature during both experiments was 16°C, which was the ambient temperature of the enclosures. For the laboratory experiment we used *Daphnia magna*, our standard test clone to test for food quality effects (Boersma 2000). This clone was originally collected from a pond in Frankfurt and has been kept at the Max Planck Institute for Limnology for many years. The experimental mothers were fed *Scenedesmus obliquus* (1mgC L^{-1}) every day and the medium was changed twice weekly. Third brood offspring were collected from these mothers within 24 hours after birth and randomly divided in 120ml flow-through vessels, filled with water from the separate enclosure treatments, with a flow rate of 1L d^{-1}. Each vessel contained five animals and we had five replicates per treatment. Six juveniles were randomly selected and placed on an aluminium weighing boat to determine initial dry mass. After three or five days for the first and the second experiment respectively, the

experiment was terminated. All animals from each experimental vessel were harvested, and put together on an aluminium boat. These were then dried at 60°C overnight and weighed to the nearest 0.1μg. The increase in *Daphnia* body biomass per day (somatic growth rate) was computed for each treatment.

For the copepods, we determined the development of calanoid juveniles that were caught from Lake Schöhsee on the start day of the experiment. The copepodites were anaesthetised with carbonated water and 20 copepodites were randomly pooled together and placed in 120ml flow-through vessels in a quadruplicate set-up. Four vessels were additionally filled and harvested immediately to establish initial stage distributions, the other vessels were emptied after five days. All animals were fixed with 4% formaldehyde solution and the copepodite stages were determined under a stereomicroscope. The average copepodite stage was calculated with a start and an end value.

Growth of *Daphnia* was tested in both experiments, whereas the development of the copepods was tested in the second experiment only. Since copepods have a longer generation time compared to daphniids they should be given more time to show a noticeable response, therefore the second experiment lasted five days instead of three days, as was the case with the first experiment.

The results from all growth studies (somatic and population) were correlated with the seston parameters in the enclosures in order to determine potential limitations. For these computations we used the average values of the seston parameters over the duration of the laboratory experiments.

Results

In the first growth experiment, conducted 9 days after the start of the enclosure experiment, there were only small differences in *Daphnia* growth when fed seston from different enclosures. However, there was a tendency that the daphniids grew faster on seston manipulated by copepods compared to *Daphnia* manipulated seston (ANOVA; $P = 0.06$; Fig. I, 1, top panel). In the second experiment, which started 13 days after the zooplankton had been added to the enclosures, the results became clearer. Interestingly, overall growth of the daphniids was higher in the second experiment. The daphniids continued to grow faster on seston manipulated by copepod grazers (ANOVA; $P < 0.001$; Fig. I, 1, bottom panel). We also observed that growth of *D. magna* declined with increasing densities of daphniids in the enclosures (ANOVA; Duncan's multiple range test; $P = 0.0116$), which was not the case for the growth of the daphniids in the copepod
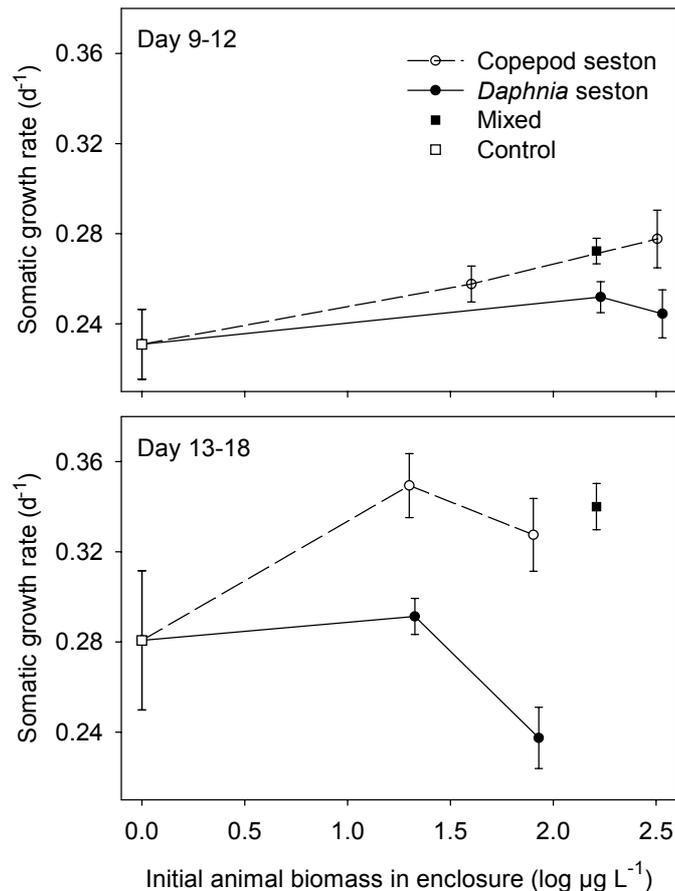
Fig. I, 1. Somatic growth rate over three days for *Daphnia magna* fed seston from enclosures that were inoculated with different densities of zooplankton and which had been pre-handled by the zooplankton community. Top panel: first period (day 9-12), bottom panel: second period (day 13-18). The enclosure seston used as experimental food, was at day zero enriched with copepods, daphniids, mixture of both copepods and daphniids or without zooplankton addition (control). Error bars denote ±SE for five replicates.

gradient (ANOVA; Duncan's multiple range test; $P = 0.2631$; Fig. I, 1). Post-hoc comparisons showed that in the second experiment, the copepod treatments were significantly different from both the control and the *Daphnia* treatments. In both experiments seston pre-treated by both grazers (mixed treatment) supported high *Daphnia* growth rates comparable to the growth on copepod water.

In contrast to what we found for *Daphnia*, we did not find significant differences in the developmental rate of the *Eudiaptomus*. The copepodites developed into higher stages but the development seemed to be independent of any pre-handling.

In the enclosures that were inoculated with *Daphnia* we established population growth rates between the different sampling dates. This was conducted over the last part of the experiment. The copepod densities did not change very much over this period. We found that *Daphnia* population growth rates were high with relatively large variations
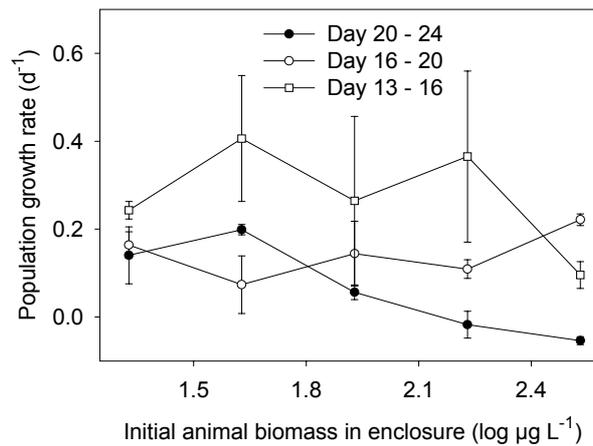
Fig. I, 2. The impact of initial *Daphnia* densities on the population growth rates in the *Daphnia* enclosures, over three consecutive periods. Error bars denote ±SE from 2 replicate enclosures.

between the two replicate treatments in the period between day 13-16 (Fig. I, 2). In the following period (day 16-20) the growth rates declined somewhat but seemed to be independent of the initial densities. Only in the last period (day 20-24) we observed a significant decrease in growth rates relative to the period before (day 16-20 *versus* day 20-24; repeated measures analysis, $F_{1,5} = 8.27$; $P = 0.0348$). Moreover, in this period we also observed a stronger decrease in growth rates in those enclosures inoculated with the higher *Daphnia* densities.

When *Daphnia* somatic growth rates from the laboratory were compared with the population growth rates within the enclosures, we observed no significant correlation when the overlapping time intervals were used. However, since population growth is the numerical response of food quality changes, we expected the visible response to be delayed about one egg production time. This was indeed the case, and, the somatic growth was positively correlated with the population growth from the consecutive period ($y = 0.65x-0.058$; $r^2 = 0.15$; $P = 0.020$).

During the two laboratory experiments the animals faced different amounts of food in terms of carbon (Fig. I, 3), with generally higher values in the first experiment. However, only in the first experiment there was significantly lower carbon in the *Daphnia* treatment (ANOVA; $P = 0.0098$) compared to the copepod treatment. The content of particulate nitrogen and phosphorus followed a similar pattern to carbon.
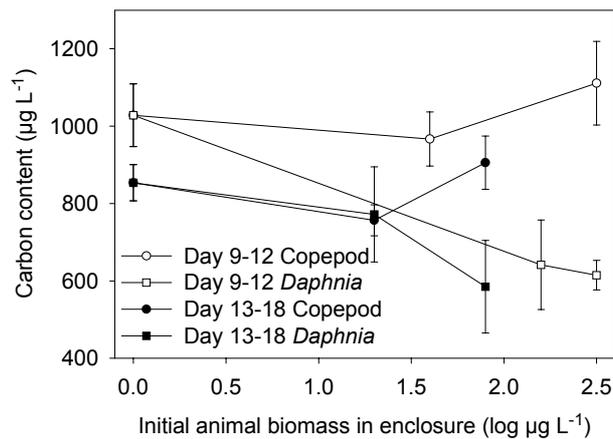
Fig. I, 3. The average particulate carbon content (<100μm) during two laboratory experiments, conducted between day 9-12 and 13-18. Error bars are ±SE from two respectively three measurements.

At the end of the enclosure experiment the total fatty acid content per litre of the seston in the *Daphnia* treatments was considerably lower than in the copepod treatments, and also the content of the essential ω3 fatty acids at day 20 and 24 were significantly affected by the type of grazer (*Daphnia versus* copepod; repeated measurements analysis, $F_{1,9} = 31.8$; $P < 0.001$). When the content of the ω3 fatty acids of the animals was studied we found the reverse pattern. Copepods (mixtures of both calanoids and cyclopoids) had a significantly lower content of these fatty acids compared to daphniids (ANOVA; $P = 0.039$), but a higher content of ω6 fatty acids. Moreover, generally the copepods had lower concentrations of the longer fatty acids (Table I, 1). We computed the ω3:ω6 ratio of both
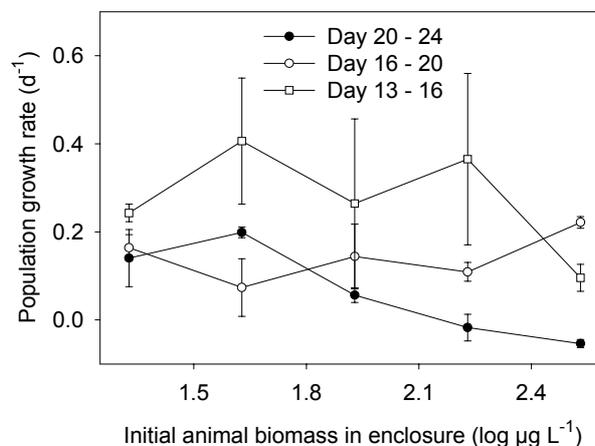

Fig. I, 4. The ω3:ω6 ratio of enclosure seston and zooplankton at the end of the enclosure experiment (day 24). The seston values are the averages from the two last samplings (day 20 and 24). Error bars are ±SE from two replicate enclosures.

animals and seston, a measure often used to define food quality of zooplankton, with higher ratios normally considered to represent higher quality. As expected, there were differences between zooplankton taxa, with daphniids having higher ω3:ω6 ratios than copepods (Fig. I, 4; ANOVA; $P < 0.001$), but no significant differences between animals taken from different densities. The ω3:ω6 ratios in the seston did not seem to vary much between the enclosures, only in the highest copepod density the ω3:ω6 ratios were elevated. This was due to the small amounts of both fatty acid families in this treatment. Thus, we found that seston manipulated by copepods had significantly higher ω3:ω6 ratios than seston manipulated by daphniids (*Daphnia* versus copepod; repeated measures analysis over the last two sampling dates, $P = 0.006$; Fig. I, 4).

Neither carbon, nitrogen nor phosphorus showed a strong correlation with *Daphnia* growth in our laboratory study. The strongest positive correlation with *Daphnia* somatic growth and food quantity aspects was with the concentration of 20:4ω3. At higher concentrations the animals showed significantly higher growth rates (Fig. I, 5; y = 0.171x+0.21, $r^2 = 0.37$, $P < 0.001$). In a backward stepwise multiple regression with *D.*

Table I, 1. The average fatty acid content (µg mg dryweight^{-1}) and SE (two measures, one for each replicate enclosure) for copepods and *Daphnia* at the end of the enclosure experiment (day 24).

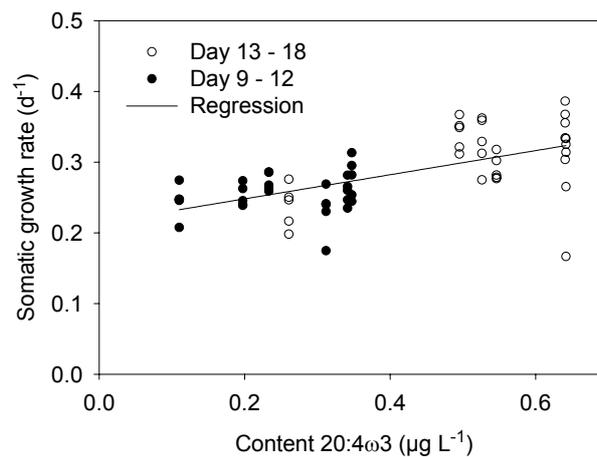| Parameters	*Daphnia*		Copepods	
	µg mgDW^{-1}	SE	µg mgDW^{-1}	SE
14:0	10.09	2.81	8.90	1.38
16:0	18.25	7.33	20.04	2.99
16:1ω7	2.30	0.19	0.30	0.16
18:0	15.28	8.63	5.41	1.63
18:1ω12/ω9	5.77	1.88	4.49	3.67
18:1ω7	2.01	0.48	1.35	0.88
18:2ω6	7.83	0.24	1.67	1.12
18:3ω6	0.55	0.01	0.30	0.15
18:3ω3	5.09	0.72	0.71	0.36
18:4ω3	4.08	0.35	0.90	0.64
20:00	0.64	0.17		
20:1ω7	0.11	0.03	0.39	0.10
20:2ω6	0.85	0.03		
20:3ω6	0.30	0.03		
20:4ω6	8.87	0.00	2.50	1.81
20:3ω3	0.14	0.03		
20:4ω3	1.01	0.03		
20:5ω3	9.09	0.17	2.31	1.75
22:0	0.37	0.14		
22:2ω6	0.09	0.09		
22:4ω6	0.09	0.09		
22:5ω3	1.18	0.22		
22:6ω3	9.90	0.03		
24:0	0.63	0.34		
24:1ω9	0.73	0.12		

Fig. I, 5. The correlation between somatic growth rates for *Daphnia magna* and the average content of eicosatetraenoic acid (20:4ω3) in the food. The daphniids were fed seston from enclosures that were inoculated with different densities of zooplankton. The enclosure seston was pre-handled by the zooplankton community over either 9 (●) or 13 (○) day period. The regression equation is y = 0.171x+0.21, $r^2 = 0.37$, $P<0.001$.

magna somatic growth as the dependent variable, 54% of the variance was explained by the content of 20:4ω6, 20:4ω3 and 20:5ω3. On the other hand, in the enclosures the population growth rates during the last growth period (day 20-24) correlated most strongly with the particulate nitrogen content per litre (Fig. I, 6; y = 0.0040x-0.184; $r^2 = 0.78$; $P <0.001$).

We calculated the ratios of the different fatty acids to carbon as a measure of food quality rather than quantity, and computed the correlations of the different parameters with both somatic and population *Daphnia* growth. In the laboratory experiment several of the factors had a positive correlation with somatic growth. Neither P:C nor N:C had a
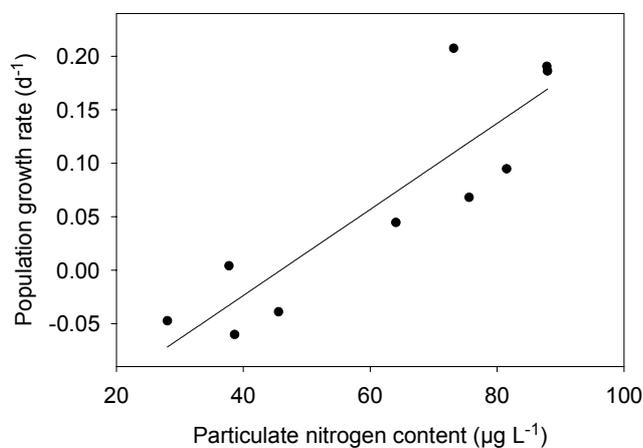

Fig. I, 6. The linear regression between population growth rate and particulate nitrogen content. The population growth between day 20-24 in correlation with the particulate nitrogen content from day 22. The regression equation is y = 0.0040x-0.184, $r^2 = 0.78$, $P = 0.001$.

Table I, 2. Correlation between seston quality parameters and *Daphnia* growth in laboratory (Somatic) and in enclosures (Population). In all correlations the ratio of the specific compound to carbon is used (e.g. fatty acid:C or N:C).

Parameters	Somatic growth, g		Population growth, r	
	r^2	P	r^2	P
P:C	0.17	<0.001	0.30	0.10
N:C	0.19	<0.001	0.51	0.021
16:0	0.16	0.0014	0.10	0.38
16:1ω7	0.19	<0.001	0.38	0.054
18:0	0.0004	0.87	0.23	0.15
18:1ω12/ω9	0.16	0.002	0.026	0.65
18:1ω7	0.17	0.001	0.21	0.17
18:2ω6	0.01	0.36	0.14	0.28
18:3ω6	0.06	0.05	0.0022	0.90
18:3ω3	0.004	0.63	0.0095	0.79
18:4ω3	0.06	0.05	0.14	0.27
20:0	0.13	0.01	0.12	0.57
20:1ω7	0.01	0.64	0.07	0.72
20:2ω6	0.04	0.15	0.05	0.77
20:3ω6	0.03	0.21	nd	
20:4ω6	0.29	<0.001	0.14	0.28
20:3ω3	0.03	0.17	0.096	0.50
20:4ω3	0.33	<0.001	0.008	0.81
20:5ω3	0.22	<0.001	0.28	0.11
22:6ω3	0.10	0.014	0.22	0.17
24:0	0.20	0.001	0.006	0.92

significant impact on growth, but several of the fatty acids seemed to be important. Again, 20:4ω3 showed the strongest correlation with somatic growth (Table I, 2). In a backward stepwise multiple regression with *Daphnia* somatic growth as the dependent variable 52% of the variance was explained by the ratio of 18:3ω3 and 20:4ω6 to the total carbon. The population growth was only positively related to the N:C ratio (Table I, 2).

Discussion

A few studies have shown that herbivorous copepods and daphniids have different impacts on phytoplankton communities. In a laboratory experiment, (Rothhaupt 1997) showed that different algae became dominant when different grazers were present. Whereas small sized algae became dominating in a system with *Eudiaptomus,* larger algae prevailed under a regime of *Daphnia* grazing. Sommer et al. (2001) reported a similar result from an enclosure study comparing grazing effects of daphniids and copepods on the phytoplankton community. They found a strong impact on the size structure of the phytoplankton community dependent on the different zooplankton taxa. These differences

can be linked to the different feeding behaviour of copepods and cladocerans. Many studies have described that copepods can detect and select favourable particles (e.g. Butler et al. 1989; DeMott 1986). Daphniids on the other hand feed unselectively, but are able to reject unsuitable particles (DeMott 1982; Kerfoot and Kirk 1991). There are several explanations for the increase in smaller particles under copepod dominance: the copepods primarily feed on the larger algae, thus leaving more nutrients available for the smaller-sized algae to grow. Alternatively, it could be the case that with copepod grazing, nutrients are released in the water column, which can rapidly be taken up by small-size phytoplankton biomass with their shorter generation times and larger surface to volume ratios. A third possible explanation is that the effect is indirect via the micro-zooplankton. Copepods also feed on ciliates, and the densities of these potential algal grazers on the smaller phytoplankton species decreases as a result of the presence of copepods (Zöllner et al. 2003).

We found *Daphnia* growth to be strongly influenced by the pre-treatment of the seston community by different guilds (Fig. I, 1), and observed a positive effect of copepod pre-handling on growth. Our experiments were conducted during a spring bloom, in which the seston community already largely consists of smaller sized algae (Sommer et al. 1986). However, the copepod grazing still had a positive impact on *Daphnia* growth. In phytoplankton counts (from day 11) of this enclosure experiment, we indeed observed that the smaller algae (<100 μm) decreased with *Daphnia* density, while no effect was visible for the copepods (Feuchtmayr, unpublished results). In contrast to the effects observed for *Daphnia* growth, we observed no differences in the development of the copepodites grown under different conditions. This could be interpreted as no impact of *Daphnia* grazing on the food for copepods, but it could well be the case that we did not detect any differences since we used too blunt a technique, determining development in a mixture of copepodite stages over 5 days. Preferably in such a short interval, we should have used more sensitive methods such as egg production experiments or RNA/DNA measurements (Saiz et al. 1998; Vrede et al. 2002). Therefore we cannot draw any real conclusions from this part of the experiment.

Previous studies have tried to link somatic growth rates of *Daphnia* with seston characteristics in a range of different lakes (Elser et al. 2001; Müller-Navarra 1995b; Wacker and von Elert 2001). In Schöhsee, *Daphnia* growth over an entire season was closely linked to the content of the polyunsaturated fatty acid EPA (20:5ω3) (Müller-Navarra 1995b). In a similar study in Lake Constance the content of α-linolenic acid

(18:3ω3) was the strongest factor correlating with *Daphnia* growth (Wacker and von Elert 2001). These different fatty acids are members of the ω3 fatty acid family, which are essential for most consumers. However, since many consumers have the possibility to desaturate and elongate the fatty acids on other positions (Olsen 1999), they are not totally dependent on the content of a single ω3 fatty acid, although the conversion of the fatty acid may b slow (von Elert 2002). In this study we also found that somatic growth of *Daphnia* was positively related to the content of a few polyunsaturated fatty acids. The strongest single regression was with the absolute concentration of 20:4ω3. This fatty acid is similar to EPA, differing only in one (non-essential) double bond. It is often found in low concentrations and therefore seldom mentioned in other studies. Both EPA and 20:4ω6 also correlated positively to our measures of growth, not only in absolute concentrations, but also their ratio to carbon (Table I, 2). Nevertheless, this is correlative evidence for the importance of certain fatty acids, and it cannot be ruled out that a different factor correlating with the fatty acids is in fact the quality-determining factor (Becker and Boersma 2003; Boersma and Kreutzer 2002).

In the *Daphnia* enclosures, the population growth showed considerable fluctuations, with a general decrease over the last sampling intervals (Fig. I, 2). Surprisingly, the growth of the populations in the enclosures seemed to be independent of the initial animal biomass between day 13-16 and 16-20, which, if indeed the daphniids exerted the predation pressure on the phytoplankton as suggested, is difficult to explain. Only in the interval between day 20-24, we observed a decline of population growth rate with density. The patterns found for the somatic growth rates in the laboratory and the population growth rates in the enclosures were similar, although not significantly correlated when the same time period was chosen for both measures. The correlation of the somatic growth rates in the laboratory with the population growth rate in the period immediately afterwards was, however, significant. This is understandable since the population growth in our study was a numerical response. Therefore we would expect that the response due to food changes could be seen not earlier as one egg production cycle later than the food effect.

Surprisingly, we did observe different correlations of food quality parameters between laboratory growth and population growth in the enclosures, even though the two growth measurements are correlated. From the laboratory microcosms we would infer that several fatty acids are limiting, whereas in the enclosures we would conclude that the N:C ratio was the most important limiting factor. From these results an interesting question

arises: are results linking growth differences to food quality in laboratory comparable to what happens in the field? Microcosm experiments have been used in several studies to investigate food quality limitations (Boersma et al. 2001; Elser et al. 2001; Müller-Navarra 1995b; Wacker and von Elert 2001). The method is sensitive, easy to perform and needs only a few days. Moreover, it is closely linked to population growth in the laboratory (Lampert and Trubetskova 1996). Our results here, however, indicate that we might be measuring two different things. Of course, under normal field conditions potential population growth rates as measured in the laboratory will never be attained because predation is present in the field, a factor largely excluded in our enclosures. The most straightforward explanation of the differences in results is that in a correlation analysis one factor has to show the highest correlation, the exact nature of which might be coincidental. This might be true, but does not explain our observation that only N:C ratio correlates significantly with population growth, and many other factors correlate with somatic growth. Where establishing population growth rates under (semi-) natural conditions has the advantage that one does not have to take the water into the laboratory with possible changes the nature of the seston, it has the disadvantage that variability in estimates of population growth rates is usually high. In contrast to somatic growth estimates showing direct responses (weight increase), numerical responses involve several more factors. Where somatic growth rates only include somatic growth of juvenile animals the population growth rates also include egg production, moulting success etc, and it could well be the case that different factors are of important in different life-stages (see also Becker and Boersma 2003). Interestingly enough, in a previous enclosure study on the effect of fatty acid additions on population growth of *Daphnia* also no effect was found of the addition of emulsions of highly unsaturated fatty acids (Boersma and Stelzer 2000). These differences in results between laboratory studies and field observations certainly warrant future investigations.

At the end of the enclosure experiments (day 20 and 24) we observed distinct differences in the fatty acid concentration per litre of the different treatments, with lower fatty acid content in the *Daphnia* seston. In the animals we found the opposite, with higher concentrations of both ω3 and ω6 fatty acids in the daphniids than in the copepods. There were also differences in the fatty acid composition between the two taxa, and especially the long chained fatty acids were found in much lower concentrations in the copepods (Table I, 1). Interestingly, even though the ω3:ω6 ratios of the seston were similar in the enclosures (only the highest copepod density differed), the ratio between ω3:ω6 fatty acids

was significantly higher in the daphniids than in the copepods (Fig. I, 4). It appears as if these ratios were stable as it did not decrease with increasing animal densities, and also not during less optimal conditions when growth was retarded (Fig. I, 2; last period), which suggests some kind of homeostasis for fatty acids (Anderson and Pond 2000). These differences have also been found in laboratory experiments. In two separate studies copepods and daphniids were cultured with *Cryptomonas* sp., and the fatty acid pattern of each zooplankton was determined. Daphniids showed a high ω3:ω6 ratio of 12.8 (from Weers et al. 1997) and *Eudiaptomus* a lower ω3:ω6 ratio 5.8 (von Elert and Stampfl 2000). These patterns suggest different requirements for different fatty acids between copepods and cladocerans (Boersma and Stelzer 2000), which could have as a consequence that different fatty acids are left in the seston in a similar way that macronutrients are recycled differently be cladocerans and copepods (Rothhaupt 1997), although it seems to be the case that the daphniids with their non-selective feeding mode reflect the seston much more than the copepods.

Daphniids in high densities had a negative impact on *Daphnia* growth. However, the question is whether the presence of copepods is really beneficial to the daphniids, or whether the absence of *Daphnia* is the more important factor. In that case the control treatments (without copepods and daphniids) should yield the same growth as the ones with copepods. This was clearly not the case. In the first experiment the control supported low growth rates and the effect of copepods on the growth rate was marginally significant (ANOVA; $P = 0.06$). In the second experiment the growth of the control was on an intermediate level between the copepod and Daphnia treatments, and significantly different from the copepod treatments. Interestingly, growth of Daphnia on the mixed-treatments was relatively high even though we had an initial *Daphnia* density of 5 ind. L^{-1}. The probable reason for this was that the *Daphnia* development in this treatment was much slower compared to only-*Daphnia* treatment. The effective *Daphnia* density development in the mixed treatment was more similar to the lowest *Daphnia* treatment. Most likely, the higher predation pressure of the cyclopoid copepods caused the slower development in this treatment.

We conclude that the presence of copepods indeed increased the feeding conditions for *Daphnia*. Even though our copepod growth data were too weak to state in which direction the interaction would have affected the copepods, there is a good chance that the interaction is beneficial for both organisms, caused by the different feeding behaviours with preferences for differently sized particles. Our experiment was conducted during

spring conditions where the seston community consists largely of small particles. It is interesting that already under these conditions we can detect differences between which zooplankton taxa that have manipulated the seston. This effect should probably even stronger still in late summer when larger particles and colonies are more common in the seston.

Chapter II

Impacts of copepods on marine seston, and resulting effects on *Calanus finmarchicus* RNA:DNA ratios

Abstract

We investigated the impact of copepods on the seston community in a mesocosm set-up, and assessed how the changes in food distribution affect the condition of the grazers, by measuring the RNA:DNA ratios in different developmental stages of *Calanus finmarchicus*. Increasing copepod densities did not affect the particulate carbon content. On the other hand, chlorophyll *a* content increased with higher copepod densities. Moreover, higher copepod densities increased the seston food quality in the mesocosms (measured as C:N and $\omega3{:}\omega6$ ratios). These food quality indicators were significantly correlated to the nutritional status of *C. finmarchicus*. Interestingly, the relationship was in contrast to our expectations, suggesting a lower growth potential on higher quality food. Moreover, in concordance with earlier studies, we found that when copepods were in high densities the large particles ($>1000\mu m^3$) decreased and that the smaller particles ($<1000\mu m^3$) increased in number. These patterns were closely linked to the condition of *C. finmarchicus* that had a higher growth potential with increasing biovolumes of large particles. The opposite was found with increasing biovolumes of smaller particles. Moreover, we found that the increase of smaller plankton was responsible for the food quality increase. Thus, we conclude that the decreasing conditions of *C. finmarchicus* were a result of a decrease of favourable sized food particles, induced by the copepod grazing.

Introduction

Zooplankton production can be constrained by many factors of different origin. Obviously, food quantity has a major impact. However, many studies have found retarded developmental rates even on high food concentrations, suggesting limitation by food quality rather than limitation by food quantity (e.g. Kleppel and Burkart 1995; Villar-Argaiz and Sterner 2002). Several characteristics of the food can affect quality and thus induce changes in zooplankton development. Toxicity of various species of phytoplankton can have a great impact on the copepod community (Frangoulos et al. 2000; Schmidt et al. 2002; Turner et al. 1998). Several diatoms have proven to have negative impacts on copepod fecundity and egg viability (reviewed in Ianora et al. 2003; Paffenhöfer 2002). The exact causes of these negative impacts are still under discussion, both toxicity as well as nutritional inadequacy have been invoked. Other constraints on copepod development can be caused by the size and morphology, as well as by the mineral and biochemical composition of the food (Kleppel and Burkart 1995; Villar-Argaiz and Sterner 2002). Especially the content of various fatty acids, and more specifically the ratio between two classes (ω3 and ω6 fatty acids) have been shown to correlate closely with egg production in a number of copepod species (Jónasdóttir 1994; Jónasdóttir et al. 1995), whereas especially low-nitrogen foods have a negative impact on copepod growth and reproduction (Kiorboe 1989).

Calanoid copepods generally have a preference for larger particles, and do not feed on protists <5μm in the laboratory and exert little grazing on food particles <10μm, when natural plankton contains larger food. This behaviour has been identified for freshwater copepods (Rothhaupt 1997; Sommer et al. 2001) as well as for marine copepods (Frost 1972; Frost 1977). As a result, in copepod dominated ecosystems larger particles are grazed, while mostly small particles prevail (Rothhaupt 1997; Sommer et al. 2001; Sommer et al. 2003b). This pattern is most likely a combination of grazing pressure and the longer generation times for larger phytoplankton cells. Alternatively, the increase of smaller particles could be a consequence of copepod grazing fractionating larger particles into smaller ones (O'Connors et al. 1976). Copepods do not only select their food according to size, but can also distinguish between similar sized particles of different nutritional quality and mobility (Cowles et al. 1988; Paffenhöfer and Van Sant 1985). Moreover, zooplankton grazing can impact the plankton nutrient stoichiometry, resulting in increased C:N or C:P ratios (Rothhaupt 1997; Sterner and Hessen 1994), which is generally accepted as a decrease in food quality. Hence, copepods can impose large

impacts on the composition of the plankton community. Active food selection induces a higher grazing pressure on the "better" food particles and the proportion of these decreases. Hence, increasing copepod densities should imply that food competition increases and should be reflected in slower copepod development.

Copepod growth, or nutritional status can be determined by several methods. Egg production is a common technique in both laboratory and field studies (e.g. Jónasdóttir et al. 1995; Kleppel and Burkart 1995). This trait can serve as a good indicator of current nutritional status of wild caught copepods by studying the egg production over a short period of time (~24h). However, this is not an effective method when the copepod reproduction is low. Another method is to study the effects of various treatments on life-history traits (e.g. Twombly et al. 1998; Villar-Argaiz et al. 2002). This procedure is time consuming and can last for weeks, thus making it unsuitable to test for food quality effects fluctuating over short time periods. A third possibility is through measurements of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The ratio of the two (RNA:DNA) and the RNA content alone have proven to be a useful tool in determination of nutritional status of various zooplankton organisms (e.g. Båmstedt 1983; Båmstedt and Skjoldal 1980; Saiz et al. 1998; Vrede et al. 2002; Wagner et al. 1998) and fish larvae and juveniles (Clemmesen 1993; Clemmesen 1994; Clemmesen 1996; Clemmesen et al. 2003). Moreover, food quality changes can induce a RNA:DNA response in freshwater daphniids within 5 hours (Vrede et al. 2002). For copepods, this response is probably slower, due to lower growth rates and longer generation times of copepods. In marine field studies, significant correlations between chlorophyll *a* concentrations (indicator of food), copepod egg production and the copepod RNA:DNA ratios have been described (Laabir et al. 1998; Nakata 1990; Nakata et al. 1994; Saiz et al. 1998). In laboratory studies *Calanus finmarchicus* RNA:DNA ratios are good indicators of food availability (Wagner et al. 1998; Wagner et al. 2001).

When investigating the impacts of different feeding conditions on the plankton community in a close-to-natural environment, mesocosms are the method of choice. Mesocosm studies combine the benefits of field and laboratory studies. Thus, allowing alterations of specific environmental conditions (e.g. nutrient additions) on a large-scale community, under otherwise "natural" conditions. The large-scale is advantageous when running repeated sampling over long time or when large water quantities are being sampled by minimising the sampling effects on the plankton community.

In a mesocosm study we investigated the impact of various densities of copepods on the phytoplankton community. Moreover, we determined how these changes affect the copepod nutritional status. For this, we selected the dominating copepod, *Calanus finmarchicus*, a key species in northern ecosystems, being an important part of the diet for several fish species of commercial interest around the northern Atlantic seaboard. Hence, the biology of *C. finmarchicus* has been of considerable scientific interest that resulted in a better understanding of *C. finmarchicus*: physiology, population dynamics, distribution, reproduction and growth-rates (e.g. Hirche 1996; Kaartvedt 2000; Richardson et al. 1999).

We studied the effects of copepod grazing on the plankton food quality and how different feeding conditions affect the *C. finmarchicus* nutritional status (RNA:DNA).

Material and methods

This study was performed as a part of a larger project where we aimed at determining the impact of different zooplankton organism on freshwater, brackish and marine food webs. The effects are studied on various levels from bacteria to zooplankton (see Becker et al. in press; Sommer et al. 2003a; Sommer et al. 2001; Sommer et al. 2003b; Zöllner et al. 2003). In this paper we focus on the interaction between the plankton community and the nutritional status of the zooplankton.

Mesocosms – Two mesocosm studies were conducted at Trondheim Marine Systems Research Infrastructure in Hopavågen, Sletvik, Norway, during April and May 2002. The first experiment was performed between the 20th and the 26th of April and the second between 2nd and the 8th of May. In both experiments 10 transparent polyethylene mesocosms were installed (2.0 m long; 1.7m^3) and filled with the natural plankton community by lowering the bags down to about 3 metres depth and pulling them to the surface, for more information on the general set-up see Sommer et al. (2001). We reduced the meso-zooplankton in the mesocosms with repeated hauls with 250μm and 150μm plankton nets. Afterwards the mesocosms where enriched with a logarithmic density gradient of wild caught zooplankton. These were captured with a relatively large meshed plankton-net (500μm), which in both experiments enabled us to create a grazing community of "larger" zooplankton, consisting of *Calanus finmarchicus* (70-80%) (copepodite stage 3-5), *Centropages hamatus and C. typicus* (together 3-11%) and *Oithona* sp. (6-10%) and a few percent *Acartia* sp., *Temora longicornis* and *Pseudocalanus elongatus* as described by Saage (2003). From this mixture, inoculates were taken in order to establish a density gradient for Experiment 1 with: 0 (control), 0.3,

0.9, 2.7, and 8.1 individual copepods L^{-1} and for Experiment 2 with: 0 (control), 1.3, 2.5, 5 and 10 individual copepods L^{-1}. The mean copepod density directly outside the mesocosms in the fjord was 2.25 (SD±2.6) copepods per litre during both experiments. On the other hand, in a depth profile down to 15 metres the densities increased with depth and over the whole water body averaged around 12 copepods per litre (from Saage 2003). The mesocosms were monitored for plankton and organism density and composition at day 0 (start), day 3 and day 6. After sampling on day 6 the experiments were terminated. Before sampling, all mesocosms were mixed with a Secchi-disk, in order to avoid sampling errors due to aggregations or sedimentation. The mean water temperature in both experiments was 8.3°C, and the salinity differed slightly between experiment one (31.7 psu) and two (33.1 psu). At each sampling, 10 litre of water was collected, from which several plankton parameters were monitored: 1) composition of phytoplankton, ciliates and heterotrophic nano-flagellate 2) mineral composition of particulate matter (C:N:P) 3) fatty acid (FA) composition. Parallel to the sampling the chlorophyll *a* content was measured at one metre depth with a "bbe FluoroProbe" (Beutler et al. 2002). Zooplankton samples were taken with a vertical tow through the entire water column of the mesocosm with a 55μm plankton-net. The samples were fixed in formaldehyde, and identified and counted under a dissecting stereomicroscope. In order to investigate the nutritional status of the copepods we focused on the dominating copepod, *C. finmarchicus* and used RNA:DNA ratios as a proxy of nutritional condition. These animals were sampled at the same time and with the same technique as the zooplankton described above. The captured zooplankton from each mesocosm was concentrated in a 200ml bottle and kept dark and cooled on ice before sorting into *C. finmarchicus* copepodite stages. The sampling process and the succeeding identification took about 4 hours. All individuals were stored solitary in Eppendorf tubes at −18°C, transported on dry ice to the home institutes and then stored at −74°C until further analysis.

Laboratory study – Parallel to the first mesocosm field experiment, we performed a laboratory experiment to investigate how differences in food quantity and quality affect the nutritional condition of *C. finmarchicus*. This laboratory study was conducted in order to experimentally produce controlled environments: one treatment with filtered water (starved) and one treatment with a mixture of good food algae (*Rhodomonas* sp./*Tetraselmis* sp. (Rho/Tet)) in unlimited amounts. These two treatments should serve as the two extremes, in comparison with the samples from the mesocosms. Thus, indicate if the nutritional condition of *C. finmarchicus* followed the expected pattern by being higher

at good feeding conditions and lower under starving situations. The other important goal of the laboratory experiment was to establish which copepodite stages to focus on in the mesocosm studies, as RNA:DNA ratios in copepods are strongly stage dependent (Wagner et al. 1998; Wagner et al. 2001), and we wanted to establish which of the stages present in the water column was most responsive. We started the laboratory experiment on the 3rd day of the first mesocosm experiment and continued for 5 days. Since the first mesocosm experiment was terminated after 6 days and replaced by new bags after 7 days we stored the last food suspension at 8°C in darkness for 24 hours before replacing the treatment suspension. We had 5 different treatments in triplicates, achieving totally 15 experimental vessels. The various food conditions were established by using water from 3 different mesocosm treatments namely; Low-cop, Medium-cop and High-cop (these treatments corresponds to: 0; 0.3 and 8.1 copepods L^{-1} respectively). Two additional treatments were created artificially (starved and *Rhodomonas* sp./*Tetraselmis* sp. (Rho/Tet)), by sterile filtering (0.2μm) water from Hopavågen by adding a mixture of Rho/Tet to establish a concentration of about 1mgC L^{-1} for the enriched treatment. The algae were obtained from stock cultures of Trondheim Marine Systems Research Infrastructure, Norway. At the start of the laboratory experiment we captured copepods from the natural environment as above, which were concentrated in a beaker. From this we added aliquots corresponding to 15 individuals of mixed stages of *C. finmarchicus* per replicate. The animals were cultivated in 1.5L PET-bottles, which were stored in a tank with continuous flow of fjord water, with the same temperature as in the fjord (8.3°C). The different food suspensions were changed daily by gently siphoning the water through a small tube covered with 100μm gauze to avoid loss of animals. Around 200ml of the "old" suspension remained, before the bottles were gently refilled with fresh food suspensions. After 5 days the experiment was terminated and the animals were sampled for RNA:DNA analysis as above.

Analyses- The phytoplankton species composition in the mesocosms was monitored using an inverted microscope with identification of most organisms to genus level. In order to investigate the effects of the total plankton size distribution on the nutritional status of the copepods (RNA:DNA), we combined the phytoplankton data with the measurements of ciliates and heterotrophic nano-flagellates. The total biovolumes were calculated as sum parameters for all particles with a mean cell volume smaller and larger than 1000μm^3.

The particulate fraction of the seston was analysed for nutrient (C:N:P) and fatty acid composition. For seston nutrient analyses, 0.3-1.0L water was pre-filtered over a 100μm gauze before being collected on pre-combusted, and for phosphorus, acid washed, GF/F glass fibre micro filters and dried overnight at 60°C. Total nitrogen and carbon was measured using a FISONS® NA2000 elemental analyser, total phosphorus was determined by an ammonium-molybdate method by measurements on a spectrometer at 720nm. The fatty acid spectra were established for the seston in the mesocosms. We pre-filtered 2-5L over 250μm gauze, before filtering on a GF/C filter that was subsequently stored under N_2 gas at -18°C until further processing. Fatty acids were extracted, esterified and analysed on a gas chromatograph according to Wiltshire et al. (2000), with the GC temperature settings of von Elert (2002). To quantify the fatty acid content we used an internal standard of heptadecanoic and tricosanoic fatty acid methyl esters. The nutritional status of *C. finmarchicus* was determined with RNA:DNA measurements, and quantified fluorimetrically by using ethidiumbromide and ribonuclease A (RNase) using a modification of the method of Clemmesen (1993). The response of the RNA:DNA ratios to changes in food availability and quality are dependent on the developmental stages of the copepods. (Wagner et al. 1998; Wagner et al. 2001). Hence, to get a good estimate of the nutritional status of *C. finmarchicus,* each copepodite stage had to be identified and to get a distinct RNA:DNA signal for each measurement 4-5 individuals of the same copepodite stage were pooled. A variability test was performed, in order to define the variability between aliquots of one homogenate. This test should be used as an indicator whether the method, normally used to study the nutritional condition of larval fish, also could be applied to copepodite stages. In this test we measured 15 parallel aliquots from the same homogenate using a pooled sample of mixed stages of *C. finmarchicus* copepodites. Moreover, we could use the test results in order to compare the methodical variation in RNA, DNA and RNA:DNA ratios compared to the experimentally induced variations.

Results

Variability test- We found a low variation when we measured the aliquots of the same homogenate of *C. finmarchicus* copepodites (Table II, 1) indicating a good reproducibility of the method applied. The coefficient of variation was lower than 4.4% (RNA:DNA), which for example is much lower than the coefficient of variation of 23.3% measured from all the C4 on the sixth day in both mesocosms. The small variation in the variability

Table II, 1. Mean DNA and RNA contents and RNA:DNA ratios from 15 parallel determinations of the same homogenate of pooled *Calanus* copepodites. ±SD are given in brackets. Coefficient of variability (CV) is given in percent.

Parameters	µg DNA	µg RNA	RNA:DNA
Conc.	2.40(0.06)	5.57(0.21)	2.32(0.10)
CV(%)	2.7	3.7	4.4

test samples compared to the experimental samples, indicates that the measured RNA:DNA ratios accomplished with our experimental protocol were larger than the methodological variations.

Laboratory study- In the laboratory study conducted over 5 days, we recorded differences in RNA:DNA ratios between copepodite stage (2-way ANOVA; stage, P <0.001) and the interaction between copepodite stage and the treatments (2-way ANOVA; stage × treatment, P <0.001), the treatments alone did not induce significant differences upon RNA:DNA ratios (2-way ANOVA; treatment, P = 0.20) (Fig. II, 1). The interaction between stage and treatment shows that the copepodite stages reacted differently to the
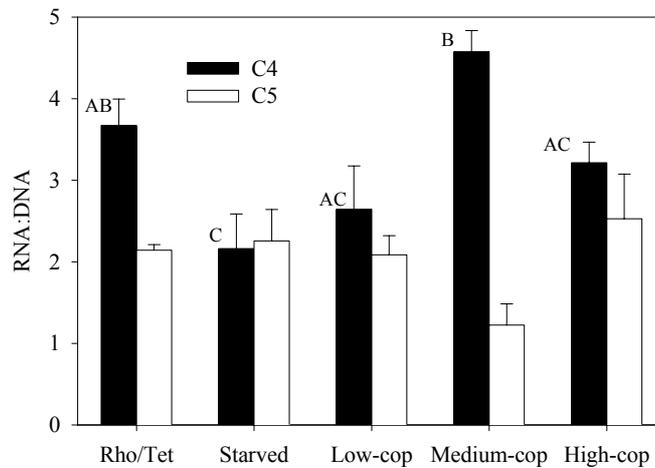

Fig. II, 1. RNA:DNA ratios of *C. finmarchicus* copepodites C4 and C5 cultivated in the laboratory for 5 days. Mean and standard error of 3 measurements are given. Treatments are: Rho/Tet is enrichment of *Rhodomonas* sp./*Tetraselmis* sp; Starved, particle free water; Low-cop - water from control mesocosms (without meso-zooplankton); Medium-cop – pre-handled by low densities of copepod grazers; High-cop – pre-handled by high densities of copepods. Identical letters identify non-significant differences for the C4 (Duncan's multiple range test)

experimental treatments. In a separate analysis of the different stages we found that the RNA:DNA ratios of the *C. finmarchicus* copepodite stage 4 (C4) were reflecting the different food treatments (ANOVA; P = 0.015). The RNA:DNA ratios for C4 followed more or less an expected pattern: the copepods that were starved and offered the High-cop treatment (high copepod pre-handling), had low ratios compared to the richer food

treatments (Rho/Tet and Medium-cop.). However, the RNA:DNA ratio of the C4 in the Low-cop treatment (not manipulated by copepod grazers) acted differently and closely resembled the Starved and the High-cop treatments. Contrary to the C4 the *C. finmarchicus* copepodite stage 5 (C5) was not affected by the different treatments (ANOVA; *P* = 0.16) (Fig. II, 1). This suggests that the RNA:DNA ratios measurements are not reflecting the food conditions for the C5s in our laboratory study.

Mesocosms- We found that different food quantity indicators showed different patterns. Chlorophyll *a* from the first mesocosm experiment suggested higher food abundance with increasing copepod density. The same pattern was also found in the second experiment but was measured with another technique (HPLC) (Feuchtmayr,
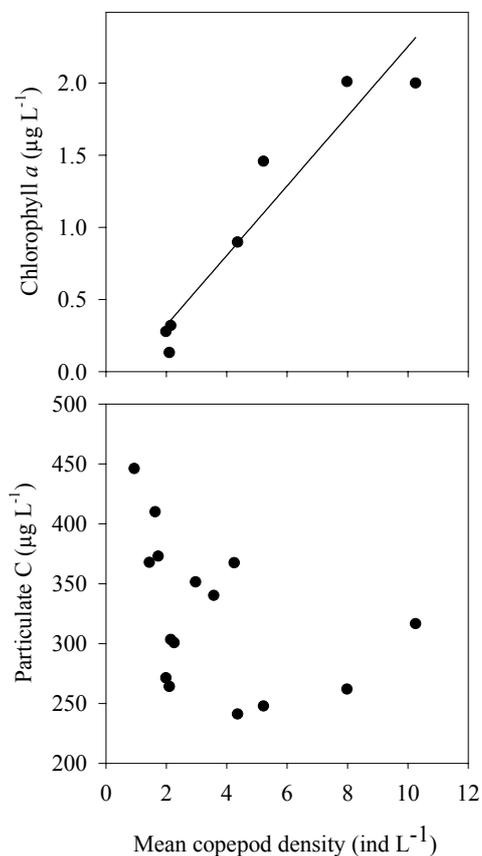

Fig. II, 2. Different indicators used for food quantity estimations, in relation to copepod densities from day 6 in the mesocosms. For Chlorophyll *a* only the first mesocosm experiment is presented while for Particulate C both mesocosm experiments are presented. Each data point represents one mesocosm. Equations are: Chlorophyll *a*; n = 7; y = 0.24x – 0.16; r^2 = 0.91; *P* <0.001 and Particulate C; n = 15; y = -9.87 + 358; r^2 = 0.17; *P* =0.12).
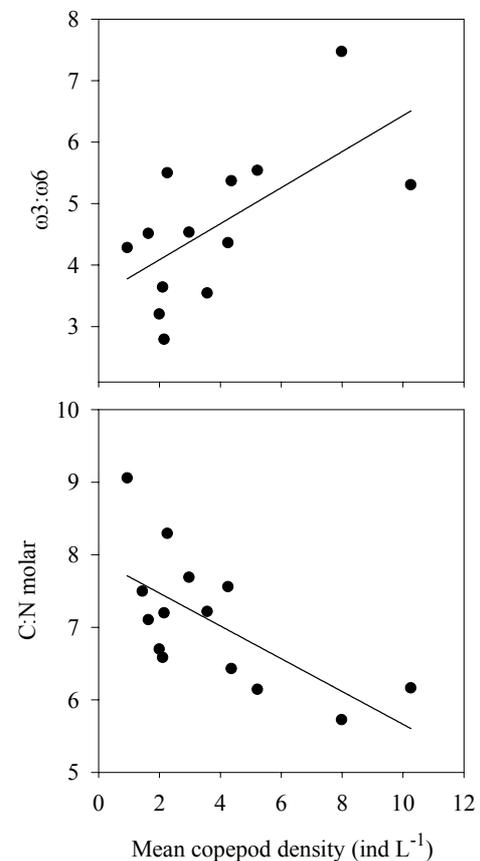
Fig. II, 3. Mean copepod densities against food quality parameters (ω3:ω6 ratio and C:N molar ratio) from two mesocosm experiments after six days of grazing. Each data point represents one mesocosm. The regression equations are: ω3:ω6; n = 13; y = 0.29x + 3.50; r^2 = 0.40; *P* =0.019 and C:N molar; n = 14; y = -0.23x + 7.9; r^2 = 0.44; *P* =0.0091.

Table II, 2. The actual copepod density in the mesocosms, n = number of observations, mean RNA:DNA ratios and in brackets ±SD for copepodite stage 4 (C4) from the 6th day of 2 consecutive mesocosms experiments.

	Experiment 1.			Experiment 2.	
Copepod density	n	RNA:DNA	Copepod density	n	RNA:DNA
2.06	2	2.89 (0.17)	1.34	3	3.19 (0.21)
1.98	1	2.49 (0.00)	1.54	3	2.71 (0.06)
4.74	2	2.27 (0.23)	2.64	3	2.64 (0.17)
8.81	2	1.26 (0.37)	3.91	4	2.26 (0.29)

unpublished) and therefore cannot directly be compared. On the other hand, the particulate C content from both experiments was not significantly affected by higher numbers of copepods (Fig. II, 2). At the same time, ω3:ω6 and C:N molar ratios, indicated increasing food qualities with increasing copepod densities (Fig. II, 3). These parameters are usually used as indicators of food quality, where lower C:N ratios, and higher ω3:ω6 ratios indicate higher food quality. In the mesocosms we aimed to produce a density gradient of copepods from 0-8 (Exp. 1) and 0-10 (Exp. 2) copepods per litre. In the first mesocosm experiment this was achieved, however, in the second experiment the gradient covered a smaller density range (Table II, 2) (from Saage 2003). Nevertheless, the gradients were in both trials strong enough to induce differences on the grazing zooplankton community.

Based on the results presented above, with increasing chlorophyll content (as a measure of living algae), and increasing quality parameters over the range of copepod densities, one would expect that the condition of the copepods in the enclosures would correlate positively with the copepod densities. However, we observed a completely different pattern, with after 6 days a negative correlation between RNA:DNA ratios of the animals with copepod density, although the RNA:DNA ratios varied with grazing time (Fig. II, 4). Initially, at the experimental start the RNA:DNA ratios were relatively high and we recorded ratios of approximately 4.7 for both mesocosms experiments. At the first sampling, after 3 days of grazing, the RNA:DNA ratios were still relatively high and there was no apparent negative effect of the different densities. In fact, we even found a positive relationship with RNA:DNA and the copepod density (C3-C5 grouped) in the first experiment (Fig. II, 4). In the second experiment, after 3 days, there was no significant relationship between the copepod density and RNA:DNA ratios ($r^2 = 0.07$; $P = 0.19$). After 6 days of grazing, however, the *C. finmarchicus* RNA:DNA ratios decreased with increasing copepod density in both mesocosm studies (Fig. II, 4). Between the two mesocosm experiments, the copepodite stage distribution differed slightly, with mostly C3
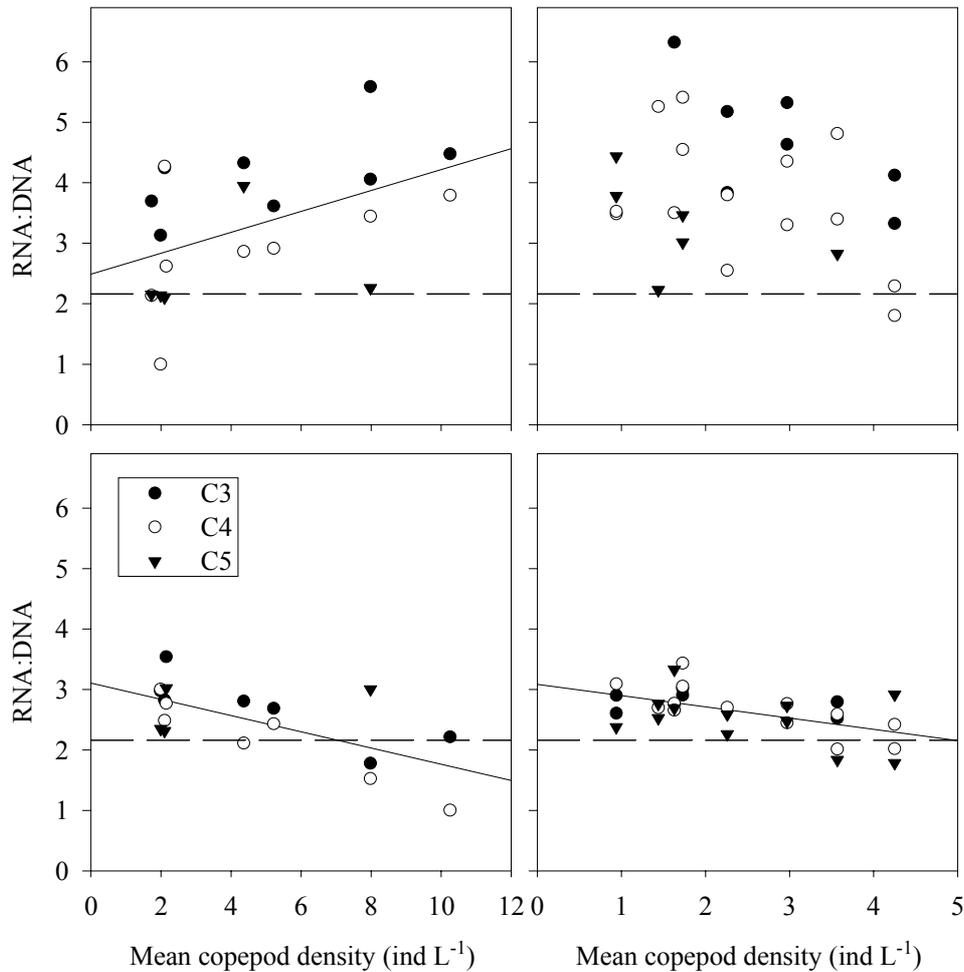
Fig. II, 4. Relation between *C. finmarchicus* RNA:DNA ratios and mean copepod density, after 3 and 6 days of grazing, in 2 consecutive mesocosm trials. The line through the data points represents the significant linear regressions for all C3-C5 copepodites grouped . The dashed horizontal lines denote the RNA:DNA ratio of C4 after 5 days of starvation from Fig. 1. Significant regressions are: Exp. 1 day 3; n = 22; y = 0.15x+2.63; r^2 = 0.18; *P* = 0.047; Exp. 1 day 6; n= 18; y = -0.13x+3.11; r^2 = 0.44; *P* = 0.003; Exp.2 day 6; n = 29; y = -0.20x + 3.12; r^2 = 0.31; *P* = 0.002.

and C4 in the first experiment and mainly C4 and C5 in the second experiment. Hence, over both mesocosm trials C4 was the single copepodite stage making the largest contribution. Thus, the only single stage available to test for differences in RNA:DNA ratios against different food parameters, parallel over both mesocosm studies. Moreover, this particular stage seemed to be the most sensitive copepodite stage to food changes (Fig. II, 1). Hence, we found that *C. finmarchicus* nutritional status was negatively linked to increasing concentrations of chlorophyll *a*, while the carbon content was not significantly imposing an effect on the *C. finmarchicus* RNA:DNA ratios (Table II, 3). The *C. finmarchicus* nutritional condition on the 6th day was significantly correlated to the ω3:ω6 and C:N molar ratios (Table II, 3), but surprisingly the direction of the correlation was such that the RNA:DNA ratio of the animals decreased with increasing food quality.

Table II, 3. Linear and non-linear regressions between seston food quantity/quality parameters and *C. finmarchicus* C4 RNA:DNA ratios after 6 days of grazing from 2 consecutive mesocosm studies. The food parameters are: Particulate carbon, total biovolume given as the sum of plankton particles smaller and larger than $1000\mu m^3$, plankton particles smaller and larger than $1000\mu m^3$, seston C:N and $\omega 3{:}\omega\,6$ ratios. The linear regression equation is $y = ax + b$ and the non-linear equation for $>1000\mu m^3$ is: $y = ax^b$.

Parameters	n	a	b	r^2	P
Particulate-C	20	3.75×10^{-3}	1.24	0.15	0.09
Chlorophyll *a*	7	-0.75	2.95	0.75	0.0125
Tot. biovol.	20	2.05×10^{6}	0.19	0.32	0.0103
$>1000\mu m^3$	20	0.87	0.087	0.79	<0.001
$<1000\mu m^3$	20	-1.32×10^{-6}	3.22	0.52	<0.001
C:N	19	0.39	-0.37	0.35	0.0085
$\omega 3{:}\omega 6$	17	-0.26	3.56	0.28	0.0277

The explanation of the patterns describe above lies in the size distributions of the algal particles in the mesocosms. After six days of copepod grazing in both mesocosm experiments, distinct changes in size distribution of the plankton was found. Increasing copepod densities had an impact on the total biovolumes in the mesocosms (Fig. II, 5). However, this decrease was mostly due to high biovolumes in a few low copepod density
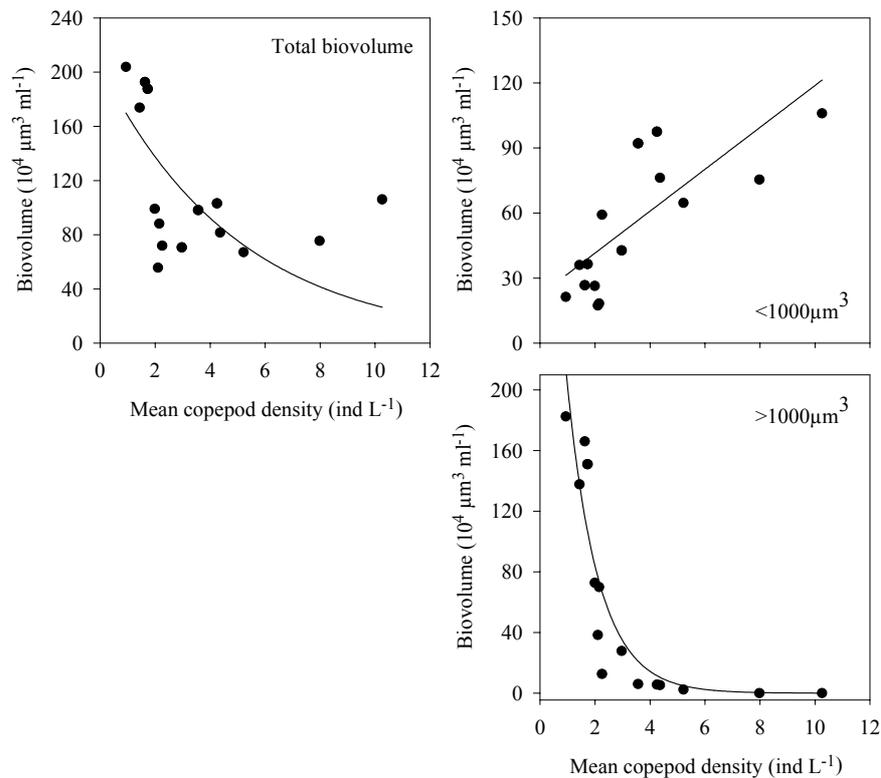

Fig. II, 5. The biovolumes of phytoplankton and microplankton, in relation to the mean copepod densities in the mesocosms. Biovolumes are the total and the summarised fraction of smaller and larger than $1000\mu m^3$, in the mesocosms. Each data point represents one mesocosm on the 6th day for both consecutive experiments. Equations are: (Total biovolume; n = 15; y = $2.05\times10^6 e^{-0.20x}$; $r^2 = 0.32$; P <0.0103), ($<1000\mu m^3$; n = 15; y = $2.11\times10^5 + 9.06 \times 10^4$; $r^2 = 0.60$; P <0.001) and ($>1000\mu m^3$; n = 15; y = $4.89\times10^6 e^{-0.88x}$; $r^2 = 0.83$; P <0.001).
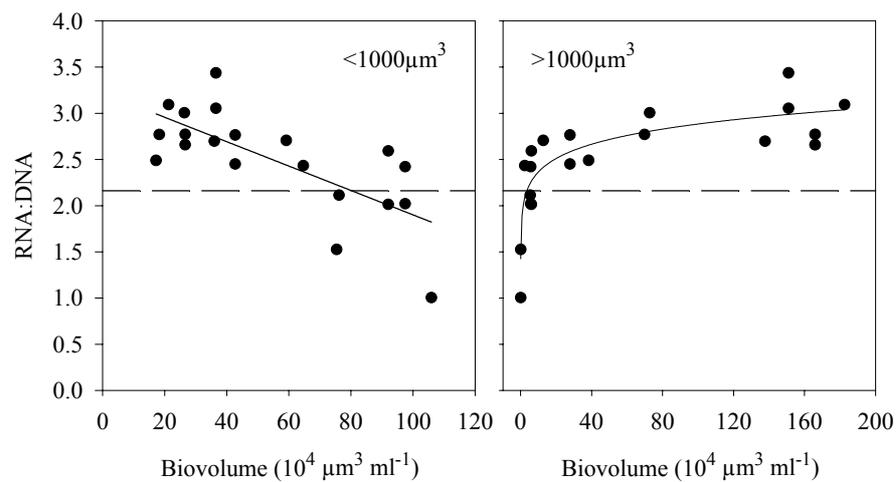
Fig. II, 6. The relationship between *C. finmarchicus* RNA:DNA ratios (C4) and the biovolumes of phytoplankton, ciliates and HNF, grouped as biovolumes consisting of larger and smaller than 1000μm^3 after six days for two consecutive experiments. Each data point represents 1 replicate sample, from each mesocosm. The dashed horizontal lines denote the RNA:DNA ratio of C4 after 5 days of starvation from Fig. 1. For regression equations see Table 3.

mesocosms, and otherwise the total biovolumes were not showing a strong effect with the increasing copepod densities (Fig. II, 5). On the other hand, with the increasing copepod densities the biovolumes of larger cells (>1000μm^3) decreased and the opposite was found for smaller plankton particles (<1000μm^3) (Fig. II, 5). Moreover, the RNA:DNA ratios of *C. finmarchicus* were closely related to biovolumes of summarised size fractions of the plankton (either <1000 or >1000μm^3) (Fig. II, 6). Where the increasing biovolumes of larger particles positively affected the *C. finmarchicus* RNA:DNA ratios (Fig. II, 6; Table II, 3), the increasing biovolumes of smaller particles had a negative impact on the *C. finmarchicus* RNA:DNA ratios (Fig. II, 6; Table II, 3).

We investigated if the induced size distribution of the plankton affected the plankton food quality. Hence, we studied if the C:N and the ω3:ω6 ratios were correlated with the plankton size fractions (smaller and larger than 1000μm^3). We found that with increasing biovolumes of smaller plankton particles, the food quality increased (Fig. II, 7). The increase of smaller plankton particles was dominated by increases of *Teleaulax* sp., *Skeletonema* sp. and nanoflagellates. On the other hand, we found no relationship between C:N and ω3:ω6 ratios and the changes in the biovolume of the larger fraction (C:N; $r^2 = 0.14$; $P = 0.11$ and ω3:ω6; $r^2 = 0.09$; $P = 0.70$). Moreover, in a backward stepwise multiple linear regression including common food quality parameters, the biovolumes of smaller particles (<1000μm^3) was the only significant regression explaining 52% of the variation in *C. finmarchicus* RNA:DNA ratios.
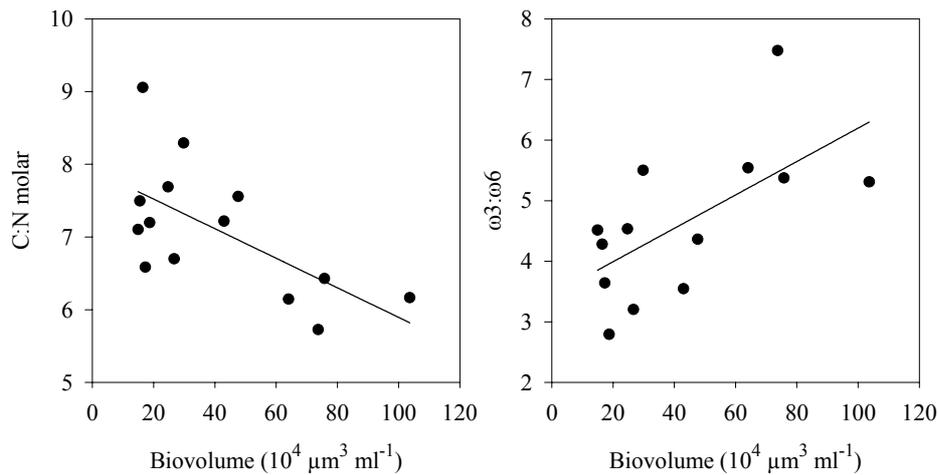
Fig. II, 7. The relation between the C:N molar ratio and the ω3:ω6 ratio and the biovolumes of phytoplankton smaller than $1000\mu m^3$. The regression equations are: ω3:ω6; n = 13; y = 2.76×10^{-6}x + 3.43; r^2 = 0.40; P =0.021 and C:N molar; n = 14; y = -2.03×10^{-6}x + 7.93; r^2 = 0.41; P =0.014.

Discussion

We investigated the impact of the copepods on the plankton community and also the resulting effects on the copepod grazer. The nutritional status of copepods can be established through various methods (e.g. egg production, life-history studies or RNA:DNA measurements). Our studies were performed in spring, when the copepod reproduction was low. In fact, we only caught few of adult *Calanus finmarchicus*, which made an egg production study unfeasible. Moreover, in the mesocosms, the plankton showed a rapid response to the various copepod densities, which made a life-history study unsuitable. Hence, the only workable determinant of nutritional status of *C. finmarchicus* was the RNA:DNA measurements, which has developed into a useful tool for copepods both in field and laboratory studies (e.g. Saiz et al. 1998; Wagner et al. 1998; Wagner et al. 2001).

Laboratory study- In our laboratory experiment, we found that *C. finmarchicus* copepodite stage 4 (C4) was affected by the different food treatments (Fig. II, 1). The RNA:DNA ratio of the C4 followed more or less an expected pattern: the highest ratios were found in the enriched *Rhodomonas* sp. / *Tetraselmis* sp. (Rho/Tet) and the Medium-cop (low pre-handling by copepods) treatments. The starved animals had the lowest ratios and slightly higher we found the Low-cop (no pre-handling) and the High-cop (Fig. II, 1). On the other hand, the C5 acted differently and we did not see any effect of the different food treatments (Fig. II, 1). Previous studies have described increasing RNA:DNA ratios

and food density relationships with increasing copepodite stages of *C. finmarchicus* (Wagner et al. 1998; Wagner et al. 2001). In these studies, the pattern was consistent at several food concentrations, but was less pronounces at the lower food concentrations. In our study this pattern was not found, instead C5 had the same or even slightly lower ratios compared to C4 (Fig. II, 1). These differences are difficult to explain, however, since C4 clearly responded to the various treatments, we conclude that the food was not limiting in all treatments. On the other hand, the indifferent variations in RNA:DNA ratios for C5 could potentially be explained with size differences. The C5 stage is larger than C4 and thus could have a slightly longer time to respond to food quality changes. Yet another explanation could be that for *C. finmarchicus* the main overwintering stage is the copepodite stage 5, and during overwintering this specific stage, compared to the other stages, contains high storage of wax esters. During spring the copepodites are preparing for the overwintering with an increased storage of lipids, during this phase the copepodites have an arrested development (reviewed in Hirche 1996). Potentially the C5s had already reached the preparatory stage preceding the overwintering resting stage. On the Norwegian coast this phase is, however, normally initiated in May-June (e.g. Marker et al. 2003), which is about one month later than our study. However, the temperatures in April and May 2002 exceeded the monthly mean temperatures by around 4°C compared to other years, which might have moved the accumulation process forward in time, and thus we would expect no food effect on RNA:DNA ratios.

Mesocosms- the food quantity, estimated as particulate carbon, was not correlated to the copepod densities or to the *C. finmarchicus* RNA:DNA ratios. Hence, the grazing copepods were not limited in terms of particulate C (Fig. II, 2; Table II, 3). Another food abundance indicator, the chlorophyll *a* content, was even positively affected by higher copepod grazing (Fig. II, 2), suggesting that the copepod grazing in fact stimulated food abundance. On the other hand, the RNA:DNA ratios of *C. finmarchicus* did not reflect increased food abundance, on the contrary, we found decreasing RNA:DNA ratios with this apparent food quantity increase (Table II, 3). Common food quality indicators, C:N and ω3:ω6 ratios, showed an increasing food quality with higher copepod densities (Fig. II, 3). These food quality parameters were correlated to the nutritional condition of *C. finmarchicus* (Table II, 3). However, the regressions were inverted to what we prior the experiment expected, suggesting that the copepods had a lower nutritional status when these two food quality parameters indicated a higher quality (Table II, 3). This is

contradictory to earlier studies where egg production was correlated to increasing ω3:ω6 ratios of the plankton (e.g. Jónasdóttir et al. 1995).

During the course of the experiments, we found that in all copepod densities, over time the RNA:DNA ratios decreased (Fig. II, 4). However, the copepod densities did not have a negative impact on the RNA:DNA ratios of the *C. finmarchicus*, until after 6 days of grazing (Fig. II, 4). In contrast to our laboratory experiment, this decrease appeared to be similar over all copepodite stages. Nevertheless, the RNA:DNA ratios were not elevated with increasing copepodite stages, which was consistent with the results from the laboratory experiment.

The copepod grazing had a strong impact on the plankton size distribution in the mesocosms (Fig. II, 5). Low copepod densities grazed the larger plankton particles down and the contrary was found for the smaller plankton that increased. In fact the larger plankton were almost completely removed in the higher copepod densities (Fig. II, 5). We observed the same effect in both mesocosm studies, even though the mean copepod density was lower than intended in the second trial (Table II, 2). This was consistent with previous studies that described similar effects in ecosystems dominated by copepods (Rothhaupt 1997; Sommer et al. 2001). The decrease of larger particles is explained with the selective grazing pressure that the copepods induce on the plankton community (Frost 1972). The copepods selective feeding is sensitive and copepods can even distinguish between particles of different taste and nutritional composition (Butler et al. 1989; Cowles et al. 1988; DeMott 1986; Paffenhöfer and Van Sant 1985). Hence, when copepods are the main dominating grazer they induce specific grazing pressures that influence the composition of the plankton community (Fig. II, 5). The increase of smaller particles can be explained in several ways: During copepod grazing the copepods fractionate larger particles (mostly chains) into smaller (O'Connors et al. 1976). On the other hand, the copepods remove the larger particles, which leave more nutrients available for the smaller particles to utilize, smaller cells being more effective in taking up nutrients, which origins from their shorter generation times and larger surface to volume ratios. Another potential explanation is that the increased predation pressure of the copepods on the micro-zooplankton decreases the pressure on the smaller particles (Zöllner et al. 2003).

When food quantity was classified regarding to favourable particle sizes there was a clear pattern on *C. finmarchicus* RNA:DNA status. This was, with increasing biovolumes of larger particles ($>1000\mu m^3$) *C. finmarchicus* RNA:DNA ratios were elevated (Fig. II, 6; Table II, 3). On the contrary when the biovolumes of the smaller

particles ($<1000\mu m^3$) increased, the RNA:DNA ratios decreased (Fig. II, 6; Table II, 3). This can be interpreted in two ways: Either, the decrease of larger particles was the limiting factor and the *C. finmarchicus* could not find enough suitable food particles that had a negative effect on the RNA:DNA ratios (Fig. II, 6). On the other hand, the increasing volumes of smaller particles impeded the foraging of the less abundant larger particles (Fig. II, 6). The increase in food quality in the mesocosm was closely related to the increase of smaller plankton particles ($<1000\mu m^3$) (Fig. II, 7). The increased food quality can be linked to the increases in small diatoms, cryptophyceans and nanoflagellates, where at least diatoms and cryptophyceans are rich in various ω3 fatty acids (Olsen 1999). Moreover, the increases of the small plankton particles also explain the increase of chlorophyll *a* content that we found. This shows that during periods of copepod dominance, and their effects on the plankton community, the chlorophyll *a* content should not be used as an indicator of food abundance. We suggest that during similar conditions the particle size availability is a far better indicator for food availability.

The removal of large particles and the consequential food quality increase could be beneficial for non-selective filter feeders that can utilize the smaller particles. This has previously been acknowledged in freshwater ecosystems where copepod grazing positively affected *Daphnia* growth (Becker et al. in press). In marine systems this role could be played by ciliates, appendicularians (Bedo et al. 1993) or by cladocerans. For example *Podon* sp. can ingest small centric diatoms (from $4\mu m$) such as *Skeletonema costatum* (Kim et al. 1989). On the other hand, calanoid copepods have been suggested to suppress earlier appendicularian stages (eggs and juveniles) (Lopez-Urrutia et al. 2004; Sommer et al. 2003a). This suppression could probably also be the case for the marine cladocerans, and has been shown for ciliates (Zöllner et al. 2003). Thus, the eventual food benefits caused by calanoid copepod grazing are most likely unused when the predation pressure on earlier stages of organisms that could use these resources is high. Nevertheless, this suggests that when copepods decrease in numbers, thus lowering the suppression on the earlier life stages of those organisms that could consume the smaller algal cells, there is a high density of high quality food available for the opportunistic grazer to utilise.

Since we could not detect a relationship with the particulate carbon content and the RNA:DNA ratio, we conclude that in our mesocosms the *C. finmarchicus* were food limited by the low abundance of favourable sized food particles, especially in the higher copepod densities. The size distribution differences of the food were originating from the

selective feeding behaviour of the copepod grazers. This indicates that the selective copepods in higher densities were not only forming the plankton size distribution, but also that the selective feeding increased the *C. finmarchicus* competition over food particles. Thus, we suggest that the selective feeding indirectly resulted in lower RNA:DNA ratios of the *C. finmarchicus*. The copepod densities in the Hopavågen fjord were in the same order of magnitude as in the highest copepod density we used in the mesocosm experiments. Therefore, we suggest that the changes in the plankton community and the resulting effects on the plankton grazer are of ecological relevance.

Chapter III

Resource quality effects on life histories of *Daphnia*

Abstract

In this study we investigated the changes in life histories imposed on the water flea, *Daphnia magna* due to biochemical and mineral limitations. Phosphorus deficient *Scenedesmus obliquus* were incubated with or without a single essential fatty acid, eicosapentaenoic acid (EPA). Additionally, the algae were spiked with dissolved phosphorus to create a range of C:P ratios from 600-200. This procedure created the possibility to study the importance of different essential resources. We found that somatic growth is retarded until a C:P ratio (molar) of around 350 is reached. Adding more phosphorus did not further increase growth. At the same time, at high C:P ratios the addition of EPA did not make a difference in growth, whereas below the nutrient threshold (C:P 350) the fatty acid had a strong positive impact on growth.

      In a second experiment we studied how the food conditions (with regard to EPA) affected the growth and investment in reproduction and secondly, if this effect was passed on to the next generation. We found that animals fed EPA made an earlier and larger investment in reproduction. In addition, the EPA enriched animals had a higher mortality. The juveniles from mothers fed EPA enriched algae had a higher growth rate than neonates from control mothers.

Introduction

In recent years, numerous studies on the effect of food quality differences on herbivorous zooplankton have appeared (e.g. Boersma 2000; Brett et al. 2000; DeMott 1998; Elser et al. 2000; Hessen 1992; Müller-Navarra 1995b; Sterner et al. 1993; Urabe and Sterner 2001). Despite the considerable interest in this subject, the increase in our knowledge on the factors that determine the quality of the food for herbivorous zooplankton has been relatively slow. Obviously, the important characteristics determining the quality of the food have been recognized some time ago, but the importance of these factors relative to one another has not yet been clarified. On the one hand this is probably caused by the fact that different characteristics of the food play a dominant role in different systems, but more importantly that different researchers have focused mainly on different aspects of food quality. As a result, different schools exist advocating different 'most-important' factors.

Four factors determining the nutritional quality of seston for zooplankters have been identified. First of all size and morphology of the algae is of importance. If the food particles are too small (Brendelberger 1991), or too large (Bern 1994), or if the cells have protective structures, such has thick cell walls (van Donk et al. 1997), or gelatinous sheaths (Porter 1976) the quality of the algae as a food source is low DeMott and Tessier (2002). Secondly, certain compounds of algae may be actually toxic for zooplankters (Turner and Tester 1997). Thirdly, the mineral composition of the algae/seston can influence its nutritional quality. If the phosphorus concentration of algae is low, nutritional quality of the food is low (DeMott 1998; Urabe et al. 1997), and lastly, biochemical features of the food, such as the fatty acid content determine the quality of the food. Especially highly unsaturated fatty acids, such as eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) are essential for many consumers, and several studies have indicated the importance of these fatty acids for herbivorous zooplankton (Brett and Müller-Navarra 1997; Müller-Navarra 1995a; Müller-Navarra 1995b; Sundbom and Vrede 1997; Wacker and von Elert 2001; Weers and Gulati 1997).

Not only have most of the studies cited above concentrated on one aspect of the food, they have also done so using a variety of different techniques. Whereas the students advocating mineral limitation mainly used experimental evidence to substantiate their claim, much of the evidence from those supporting biochemical limitations as the main factor of importance comes from field- and correlative evidence (but see von Elert and Wolffrom 2001). In previous studies on the effect of different feeding conditions of

Daphnia, and more specifically on the effect of differences in phosphorus availability, the algae were grown under different phosphorus conditions (DeMott 1998; Sterner et al. 1993). Unfortunately, this cultivation procedure not only affects the phosphorus content of the algae, but also their morphology and biochemical composition. The cell wall becomes thicker (Tillberg et al. 1984a) thus making the algae harder to digest for the zooplankton (van Donk et al. 1997). Previous work has shown that P-deficient algae have a higher total content of fatty acids (Boersma 2000; Müller-Navarra 1995a), but that the content of highly unsaturated fatty acids is lower. Therefore, it is unclear whether the decrease in growth of *Daphnia* when fed P-limited algae is in fact the direct consequence of phosphorus limitation, or of concurrent changes in the algae. Several experimental approaches have in the meant time been developed to overcome the problems mentioned above. Urabe et al. (1997) observed that Daphniids can take up dissolved nutrients from the surrounding water. DeMott (1998) worked with variable mixtures of P-deficient *Scenedesmus* and P-rich *Synechococcus*, and (Boersma 2000; DeMott 1998; Elser et al. 2001; Plath and Boersma 2001) used the fact that algae rapidly can take up dissolved phosphorus from the water, resulting in a direct change of algal phosphorus content without changes in morphology or biochemistry.

Only fairly recently there have been some attempts to study more than one aspect of factors determining food quality simultaneously (Boersma 2000; DeMott 2003; Elser et al. 2001). Elser et al. (2001) observed that in a lake with a very high C:P ratio, adding phosphorus to the seston improved the quality as food for *Daphnia*. This suggests that when phosphorus content of the food is very low this is the primary factor determining food quality. Boersma (2000) came to the same conclusion using laboratory cultured algae with roughly the same C:P ratio as the seston of the field study by Elser and co-workers. Plath and Boersma (2001) studied a range of different C:P ratios of the food and combined these with different fatty acid treatments. They concluded that when daphniids were fed severely limited algae the animals seemed to be co-limited by both biochemical as well as mineral factors, and explained this by changes in feeding activity. Plath and Boersma argued that the fatty acids might not be used primarily as a non-substitutable resource, but more as an extra energy source (but see DeMott 2003). Although this result is intriguing, the weakness of the study of Plath and Boersma (2001) is that they supplied EPA in emulsions with many saturated fatty acids present, and hence could not distinguish between the energy effect of the emulsion additions and the effect of EPA as an essential resource. Hence, in this study we set out to investigate whether EPA and phosphorus can

really co-limit growth of *Daphnia magna* individuals by supplying the animals with EPA only, using the method described by von Elert and Stampfl (2000).

Even though it has been suggested that different factors might be important during different life-phases, or more specifically that during the juvenile phase phosphorus limitations are more important, whereas after maturity other factors should become the quality determining factors (Urabe and Sterner 2001), most of the research to date has focused almost exclusively on somatic growth (but see Brett 1993; Sterner and Schulz 1998). Only recently, Urabe and Sterner (2001) included reproduction in a study on dietary effects. Hence, the second aim of this study was to investigate the effects of different dietary components on growth and reproduction, also taking into account the fate of juveniles produced under different conditions.

Material and methods

The *Daphnia magna* clone used in this study was originally collected from a pond in Frankfurt, Germany and had been kept in the laboratory for many years. Phosphorus-free medium was used (ADaM) (Klüttgen et al. 1994) in all experiments. Juvenile animals were collected from a stock culture and placed individually in 200ml jars. They were fed phosphorus sufficient *Scenedesmus obliquus* ($1mgC L^{-1}$ at 18°C) every day and the medium was renewed twice per week. Third brood juveniles from these animals were used as experimental animals. All experiments were carried out at constant dimmed light at 18°C.

A semi-continuous culture of *S. obliquus* was used as experimental food source. The algae were grown in Z/4 medium (Zehnder and Gorham 1960) with reduced phosphorus content (from $1.391mgP L^{-1}$ to $83.5\mu gP L^{-1}$). The algae were cultivated in a 10 litre bottle with an exchange rate of 1.8 litre Z/4 P- medium d^{-1}. An aliquot equivalent to 60mg carbon was harvested each day. Two fatty acid treatments were prepared by dividing the harvested algae over two bottles and supplementing one of the algae suspensions with eicosapentaenoic acid (EPA), dissolved in ethanol (von Elert and Stampfl 2000; Williams et al. 1990), such that the total EPA content in the incubation was 5% of the algal dry weight. Thereafter, the algal suspensions were incubated in the light for 3 hours. The incubation was ended with centrifugation (4000rpm, 5min) and the pellets were re-suspended in ADaM to achieve an algal concentration of $1mgC L^{-1}$. This resulted in an increase of EPA in (or on) the algae from below the detection limit to $7.6\mu g mgC^{-1}$, whereas there was no significant change in any of the other fatty acids (see for analytical

methods of fatty acid analysis Wiltshire et al. 2000). To investigate potential P-limitation the algal suspensions were subsequently enriched with 5 different amounts of a K_2HPO_4 solution (Plath and Boersma 2001). The additions were 0, 2.5, 5, 10, $20\mu gP\ L^{-1}$ which gave a range of C:P ratios from 600–230 (molar). For comparison we also had one treatment where we fed P sufficient algae to the animals. This gives a total of 11 different treatments. All treatments were carried out in quintuplicate, thus yielding a total of 55 experimental vessels. The food suspensions were renewed daily and kept in the dark in 5 litre bottles and the suspensions were gently stirred to prevent sedimentation.

The experimental animals were collected within 24 hours of birth and randomly divided in 120ml flow-through vessels, with a flow rate of $1L\ d^{-1}$. Every vessel contained four individuals. Six randomly chosen individuals were put together in an aluminium weighing boat to determine initial dry mass. After three days, two animals were harvested from each experimental vessel, and put together in aluminium weighing boats. These were then dried at 60°C for 24 hours and stored in an exicator until weighed on an electronic microbalance to the nearest $0.1\mu g$. Somatic growth rates were computed. Once the animals reached maturity, another animal was harvested to determine the weight at first reproduction, and the somatic growth rate over the whole juvenile period was computed (Lampert et al. 1994). The one remaining animal was followed until it reached the third adult instar, the number of viable offspring was established for the first and second adult instar, and the average mass of these offspring was measured.

To investigate if the effect of an essential fatty acid is passed on to the next generation we carried out a second experiment, using the same set-up as above, but with only one P-addition of $5\mu gP\ L^{-1}$. This gives a C:P molar ratio of around 350, and the algae should only differ in the EPA content. Third brood *D. magna* juveniles, less than 24 hours old, were used as experimental mothers. They were kept solitary in 200ml jars. Every day the animals were transferred into new glasses with fresh medium. From these animals, time of reproduction and the size of the successive broods were determined. The animals were cultivated until death or until they hatched their third brood of juveniles. Third brood juveniles of these experimental mothers were cross-tested for the different growth conditions. For comparison we also added third brood juveniles from P-sufficient mothers. We added a starvation treatment (fed only ADaM), in order to study how growth rate declines. The experimental set-up was identical to the one described above for the first experiment, with five individuals in each flow-through vessel, but all of them were harvested after 3 days, and somatic growth rates determined.

Results

Juvenile growth rates were positively affected by both the addition of phosphorus as well as the addition of EPA (Table III, 1). The interaction between the two factors was marginally significant. Animals fed P-limited *Scenedesmus* spiked with a range of P additions grew considerably better at the higher P concentrations compared to the lower ones (Fig. III, 1). Duncan's multiple range tests showed that the difference between
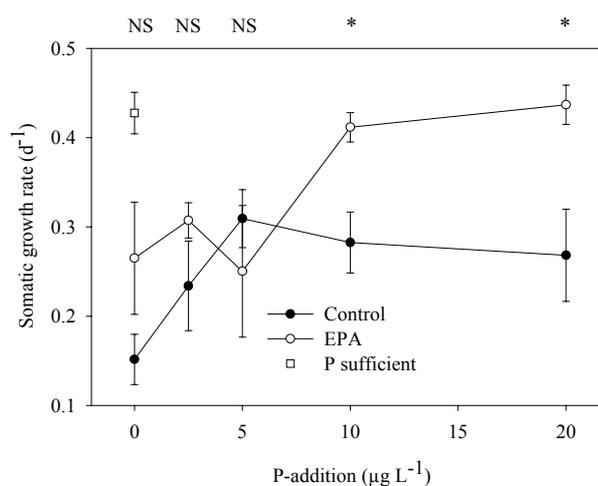

Fig. III, 1. Somatic growth rate over the first three days for *Daphnia magna* fed either P-sufficient or P-limited *Scenedesmus*. The phosphorus-limited algae were supplemented with a range of P-additions and with or without addition of EPA. Error bars indicate ±SE. NS (not significant), * (p <0.05), ** (p <0.01) and *** (p <0.001) indicate the significance of the differences between the control and the EPA treatment (Duncan's multiple range test).

Fig. III, 2. Somatic growth rates of the entire juvenile growth phase of *Daphnia magna* fed either P-sufficient *Scenedesmus* or P-limited *Scenedesmus* with different P-additions, with and without addition of EPA. Error bars denote ± SE for 5 replicates. NS (not significant), * (p <0.05), ** (p <0.01) and *** (p <0.001) indicate the significance of the differences between the control and the EPA treatment (Duncan's multiple range test).
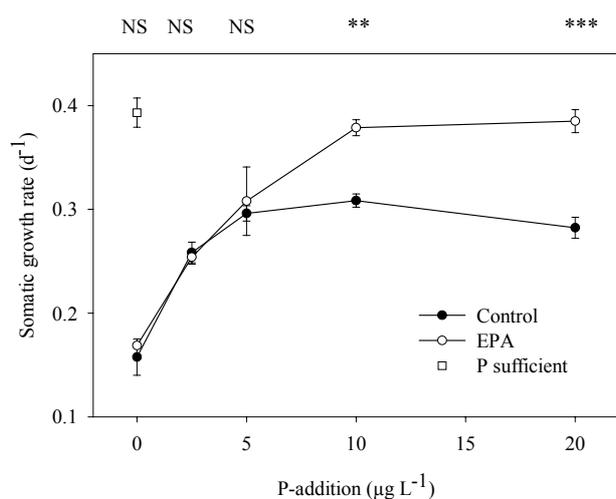
59

control and EPA addition was significant at the two highest P-additions only (10 and 20µgP L^{-1}).

We observed a similar pattern in the somatic growth rates until maturity. As was the case with the three-day growth rates, both the addition of phosphorus as well as the addition of EPA had a positive impact on the growth rates. However, also the interaction between these two factors explained a significant part of the variation in the growth rates

Table III, 1. Summary table of differences in life-history traits due to phosphorus and EPA additions, *P* values of two-way ANOVAs.

Trait	P-treatment	EPA-treatment	Interaction
Somatic growth rate (3 days)	0.005	0.0024	0.09
Somatic growth rate until maturity	<0.0001	0.0015	0.0044
Reproduction investment	<0.0001	0.0048	0.2971

(Table III, 1; Fig. III, 2). Nevertheless, as was the case with the growth rates over the first three days, the differences between the controls and the EPA-additions were significant for the two highest P-additions only (Duncan's multiple range test). Hence, we conclude that when the algae are severely P-limited, this is the main food quality-determining factor: adding P increased growth rates, adding EPA did not. After P-addition, the addition of the EPA increased growth further. This switch from P-limitation to EPA limitation happens around the addition of 5µg P L^{-1}, which corresponds to a C:P ratio of around 350.

The investment in reproduction, measured as number of offspring in the first clutch, was larger with added phosphorus (Table III, 1). The EPA addition also enhanced
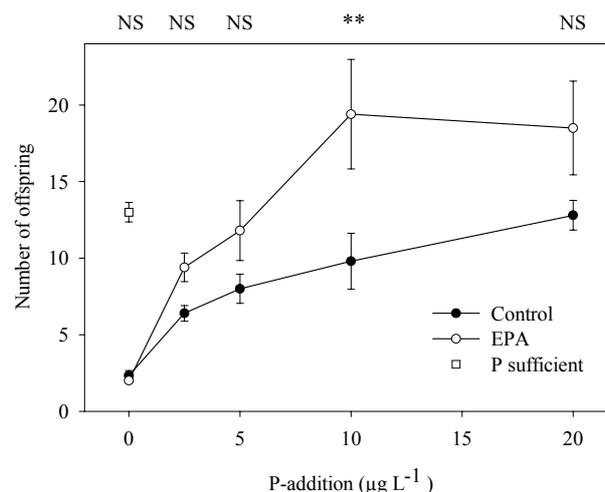
Fig. III, 3. Number of offspring in the first clutch of *Daphnia magna* fed either P-sufficient or P-limited *Scenedesmus*. The P-limited algae were spiked with a range of P-additions and also supplemented with or without EPA. Error bars denote ±SE NS (not significant), * (*p* <0.05), ** (*p* <0.01) and *** (*p* <0.001) indicate the significance of the differences between the control and the EPA treatment (Duncan's multiple range test).

the investment in reproduction over all P-additions. (Table III, 1; Fig. III, 3). The increase
in the number of viable offspring produced after EPA addition seemed more pronounced
than the increase in somatic growth rates (somatic growth rates at the highest two P-
additions only increased by 23% and 36%, whereas the number of produced juveniles
increased by 97% and 45%). The weight of the offspring was significantly affected only
by instar number of the mother (first *versus* second brood; repeated measurement analysis,
$F_{1,28} = 10.3$; $P = 0.003$), and by the three-way interaction between instar number, P and
Fatty acid treatment. The effect of phosphorus was marginally significant ($F_{3,28} = 2.7$; $P = 0.06$), with a tendency of animals that received more phosphorus producing offspring of a
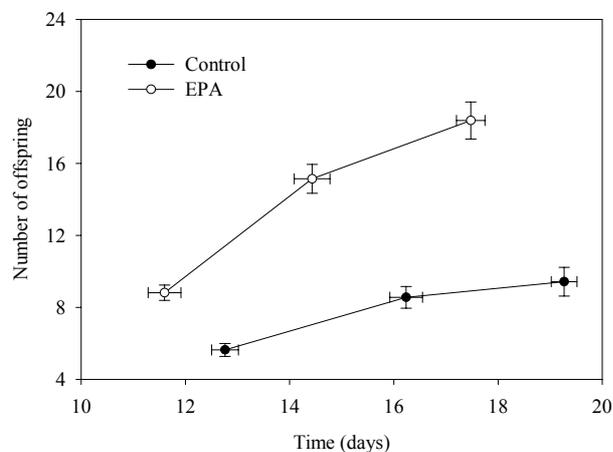

Fig. III, 4. Time and size of reproduction for *Daphnia magna* fed on phosphorus limited
Scenedesmus spiked with 5μgP L^{-1} (C:P 350) and also supplemented with or without EPA.
Error bars are ± SE.

higher mass.

In the second experiment, we found that daphniids that were fed moderately P-
limited *Scenedesmus* (C:P = 350) enriched with EPA made a significantly earlier
(ANOVA; $P < 0.005$) and larger (ANOVA; $P = 0.002$) investment in reproduction (Fig.
III, 4), which suggests better feeding conditions for the daphniids when EPA was added.
However, survival of the animals fed EPA-supplemented algae was significantly lower
than the survival of the animals that were fed control algae (Gehan's Wilcoxon test $P = 0.02$; Fig. III, 5). To test if the population growth rate differed between the two treatments,
we computed the intrinsic rate of population increase, *r*, using the Euler equation. The
animals from each treatment were randomly divided into three replicate populations. This
procedure ensured the variation needed to test significance. We found that even though
mortality is much higher in the EPA treatment the *r* for this treatment was 0.192 d^{-1} (SE ±
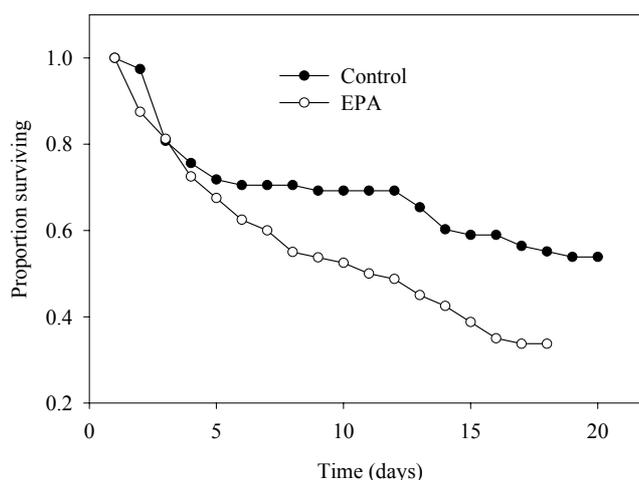
Fig. III, 5. Survival of *Daphnia magna* fed P-limited *Scenedesmus* spiked with 5µgP L^{-1} (C:P 350) and also supplemented with or without EPA.

0.009), which is significantly higher (*t*-test; $P = 0.036$) compared to the control 0.162 d^{-1} (SE ± 0.002).

Mothers that had been fed different foods produced offspring of different quality (Fig. III, 6), even though the maternal growth conditions did not have a significant effect on the initial neonate size (ANOVA; $P = 0.085$). However, the maternal growth condition did have an impact on the growth rate of the juveniles (2-way ANOVA; $P <0.001$) (Fig. III, 6). The neonates from EPA enriched mothers grew considerably better than juveniles from both of the other two treatments (Control and P-sufficient mothers), but only when the neonates were fed. Interestingly, no significant difference was found between the

Fig. III, 6. Somatic growth rate for *Daphnia magna* over three days dependent on the maternal growth conditions. The juveniles were eithe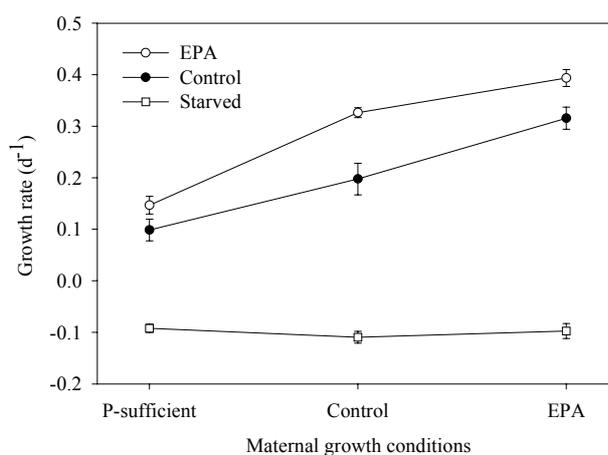r starved or fed P-limited *Scenedesmus* enriched with 5µgP L^{-1} (C:P 350), and either supplemented with or without EPA. The mothers had previously been fed P-sufficient or P-limited Scenedesmus enriched in the same way as the juveniles. Error bars are ± SE.

growth rates in animals that were born in EPA rich medium and then transplanted to EPA-poor medium, and those that were born in EPA poor medium and were transplanted to EPA-rich medium.

Discussion

Liebig's law of minimum (von Liebig 1855), states that at any one point only one factor can be actually limiting growth or reproduction. This translates in the prediction that increases in growth rate should level off with increasing phosphorus concentrations, if phosphorus is the limiting nutrient. Above the concentration of phosphorus where growth no longer increases, another resource should become the limiting factor. Addition of this resource should enhance growth further. Our results are consistent with this prediction. P-deficient algae are a poor food source for daphniids, resulting in low somatic growth rates. When we added phosphorus to these algae the growth rates increased, until reaching a plateau around $5\mu g \ P \ L^{-1}$. Above this concentration, supplementation with a highly unsaturated fatty acid (EPA) increased growth further. Both the somatic growth rates over the first three days as well as those for the whole juvenile period showed these patterns, even though there seems to be some (non-significant) tendency that in the three-day measurements also at the lower P-concentrations the EPA additions had a positive effect on the growth of the animals. This could have been a result of the maternal growth conditions, since the experimental animals all were born from mothers fed P-sufficient *Scenedesmus*, but this is not very likely as DeMott (2003) showed that the depletion of the phosphorus stores is very rapid. Plath and Boersma (2001) observed that when using emulsions of fatty acids instead of single fatty acids as was done here, these additions had a positive affect on growth of the animals even when phosphorus was limiting. They argued that at the lower phosphorus levels the emulsions might have acted as a rapidly degradable energy source, enabling higher uptake rates of the liming nutrient. As we did not observe significantly higher growth rates at the lower P-additions, and we fed EPA only, the explanation of Plath and Boersma might be correct, but a more parsimonious explanation might be that as from their second lowest P-treatment (adding $4\mu gP \ L^{-1}$ resulting in a C:P ratio of the algae of around 350) P was actually no longer limiting *Daphnia* growth, thus creating other limitations.

We conclude that above a C:P ratio of around 350 mineral phosphorus is really limiting growth of the daphniids, and adding phosphorus directly increases the growth rates of the animals. When the C:P ratios were lower, phosphorus seemed to be present in

ample supply for somatic growth and in our experiments the essential fatty acid EPA became the limiting factor for growth. The C:P threshold of 350 for *Daphnia* growth is in the same range as (Brett et al. 2000) found in the field, but higher than what (Vrede et al. 2002) showed in their laboratory study. However, the exact level of the threshold is probably very dependent on the kind of food that is used and also animal species could play a role. DeMott and Tessier (2002) argue that digestion resistance might play a more important role than previously accepted, as they observed no significant increases in growth when adding phosphorus or essential fatty acids to natural seston from different lakes in southern Michigan, USA, but they observed a strong increase in growth rates when adding a readily digestible alga, even though the C:P ratio in some of the lakes should indicate phosphorus limitation. In contrast, Elser et al. (2001) observed very strong responses to the addition of phosphorus to high C:P seston of a set of different lakes, whereas Boersma et al. (2001) observed weak responses for seston with a C:P ratio of around 300. Most likely, even severely phosphorus-limited *Scenedesmus* are probably not so digestion resistant that this plays a major role in this model system (Boersma and Kreutzer 2002), but in systems that are dominated by algae that are difficult to digest, the digestibility of the food is probably the primary determinant of food quality, followed by phosphorus content of the food as long as this is below the threshold, and only then the biochemical content.

Thus far, studies on the effect of different quality factors in the food have almost exclusively studied somatic growth (e. g. Elser et al. 2001; Urabe et al. 1997). Only recently Urabe and Sterner (2001) included reproductive traits. They suggested that the suite of elements or biochemicals necessary for somatic growth of young individuals differs from the suite needed by adult individuals for reproduction. They argued that N and P content relative to C in body tissues are higher for smaller *Daphnia* individuals (DeMott et al. 1998), whereas the opposite is the case for eggs (Sterner and Schulz 1998), as yolk is the energy source for embryonic development. This difference in C : nutrient ratio between eggs and postembryonic individuals should imply that young individuals require more N and P relative to C for their growth, while less N and P relative to C is required for the development of eggs. More generally this would imply that nutrients are more likely to limit somatic growth, whereas other factors should be more important for reproduction. Our results tend to support this. The increase in somatic growth rates after EPA addition was less pronounced than the increase in the number of viable offspring (somatic growth rates at the highest two P-additions only increased by 23% and 36%,

whereas the number of produced juveniles increased by 97% and 45%), thus suggesting a higher importance of EPA for reproduction. At the same time, adding phosphorus increased the somatic mass by 119μg (difference in weight at maturity between the lowest and highest addition), whereas the reproductive output (difference in egg number times average egg weight) increased by 40μg. Even though we observed that most of the variance for both somatic growth and reproduction was explained by the phosphorus content of the algae, this is most likely at least partly explained by the higher number of treatments. We also observed that the variance explained by the fatty acid treatments was more than double for reproduction as for somatic growth (3% *versus* 7%), thus suggesting a higher importance of the biochemical limitation for reproduction.

In their study on the effect of different nutritional limitations on the size of the offspring produced, Urabe and Sterner (2001) observed that daphniids fed on P-limited algae produced offspring that were smaller than when fed with P-sufficient algae. In this study we find a similar tendency, although here this result is only marginally significant. In contrast, Boersma and Kreutzer (2002) observed no significant effects in offspring weight between offspring born from mothers grown on P-limited and P-sufficient algae. The stronger effect observed by Urabe and Sterner might be a result of the fact that they did not directly measure weight of the eggs, but established the size of the eggs. If the quality of the eggs changes with food quality for the mother (DeMott et al. 1998), this would not be the most accurate of measures, especially since Boersma and Kreutzer observed that the amount of visible lipid droplets is actually larger in offspring born from P-limited mothers. Nevertheless, we conclude that food quality of the mother affects the quality of the offspring, and these quality differences had obvious effects when we studied the fate of the different neonates under different conditions (Fig. III, 6). Mothers that had been supplied with EPA produced neonates, which grew faster than neonates from the controls. Moreover, when control juveniles were fed EPA enriched food they increased growth to similar levels as the EPA enriched neonates. On the other hand, there was only a small effect of the EPA enrichment on neonates from P-sufficiently fed mothers. These results suggest that internal EPA pools in animals are quickly refilled, even though P-sufficient algae contain no or only traces of EPA, and is most likely explained by the observations of von Elert (2002), who found that animals fed algae lacking EPA still had a an internal pool of this fatty acid, thus suggesting synthesis from other fatty acids, e.g. linolenic acid.

We observed that mothers with access to EPA made a larger and earlier investment in reproduction. At the same time they had a lower survival. One could speculate that this lower survival is a cost of reproduction, which is a generally accepted phenomenon when comparing a range of different species (e.g. Bell 1984). Species that put more energy in reproduction tend to have a lower life expectancy. However, in many of the studies that investigated within species variation, correlations tended to be non-significant or even positive (see for a review Roff 1992). This is normally explained by correlations between female quality in general and reproduction and survival. With clonal organisms these correlations are not possible obviously, but Bell (1984) also observed these positive correlations in daphniids and rotifers. Moreover, the observation that daphniids kept under limiting conditions have a higher life-expectancy has been made several times (Ingle et al. 1937; Martínez-Jerónimo et al. 1994; Rose et al. 2000), but none of these authors linked this causally to lower reproductive rates. Lynch and Ennis (1983) did make this link, but they found no negative correlation between longevity and reproduction, stating that their results were inconsistent with the 'cost of reproduction hypothesis' (Williams 1966) or with the 'rate of living' hypothesis. In contrast we found this negative correlation, suggesting that indeed the animals that reproduce more at an earlier age trade this off against a decreased survival. It remains to be seen, however, whether this trade-off is the result of an evolutionary link between fast growth and high potential mortality later in life as a result of predation, or the result of constraints within the animal allowing it to spend its energy only once.

In summary, we observed that different aspects of food quality not only changed in importance depending on the severity of the limitations present, but also that they might play a role in different phases of an animal's life. We conclude that phosphorus limitation overrides biochemical limitations if the C:P ratio of the food is higher than 350, and that only when phosphorus is present in ample supply other factors such as the content of fatty acids will become important (see also Boersma 2000). Ontogenetic differences in food preferences are probably not very important in daphniids, and one would expect such changes in limiting factors to be more important in for example copepods, where food spectra and prey size can change considerable during life (e.g. Hart and Santer 1994; Villar-Argaiz et al. 2002). Nevertheless, our results suggest that even in daphniids such shifts in importance of different aspects of food quality could occur. Hence, the step that is needed next in the research on the effects of essential components of the food are more

detailed investigations where which factor is limiting production of the zooplankton rather than a continued discussion on the importance of single factors.

Chapter IV

Differential impacts of phosphorus and fatty acids on *Daphnia* growth and reproduction

Abstract

We investigated the impacts of various mineral and biochemical limitations on *Daphnia magna*. We found that daphniids have low saturation thresholds for growth for the polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and arachidonic acid (ARA). These growth saturation thresholds were much lower than previously described. Moreover, the daphniids were able to store considerable amounts of EPA. Around the double concentration of EPA was found in the daphniids compared to the food. We also found that different fatty acids are handled differently by the zooplankton. While the saturated fatty acid (20:0) was synthesized, the polyunsaturated fatty acids were preferably stored. There were also differences among the polyunsaturated fatty acids: EPA was found in higher concentrations in the eggs compared to ARA. On the other hand, the phosphorus content in the females varied with the phosphorus concentration of the food. However, independent of these changes, P was always equally allocated to the eggs with a specific P content of 1.4% of dry-weight (P%). Moreover, we found that storage of EPA, but not P, could fully compensate growth during periods of bad food quality. We found that *Daphnia* egg production is a major drain of fatty acids from the females.

Introduction

Food provides the consumer with essential nutrients, which cannot be synthesised by the animal. Essential nutrients can be divided in four classes: Amino acids are necessary in the protein synthesis. Fatty acids are important in membranes and also as precursors for immune responses. Vitamins play variable roles, and are common in catalytic reactions as coenzymes and for maintenance functions. Inorganic minerals are important for many functions: bone formation, oxygen transport, body water balance and constituents of enzymes. Phosphorus for example is necessary for growth and maintenance of skeletal tissue and teeth. It also plays important roles in energy rich compounds (ATP) and as precursors for phospholipids and DNA.

Some fatty acids are essential to many consumers and cannot be synthesized *de novo* by animals. This is because most animals lack the specific desaturases to insert double bonds between the methyl end of the fatty acid molecule and the 9th carbon atom of the fatty acid molecule (Cook 1985). Effectively, this translates in the fact that animals can synthesize ω9 fatty acids *de novo*, but are depending on satisfactory intake with the food of ω7, ω6, ω4 or ω3 fatty acids. Moreover, the polyunsaturated fatty acids, arachidonic acid (ARA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are precursors for prostaglandins, leukotrienes and tromboxanes, which are involved in inflammatory and immune responses. The compounds synthesized from EPA and DHA are less biologically active compared to the analogues synthesized from ARA. Elevated EPA and DHA concentrations in the diet resulted in a less reactive immune response, which could be beneficial for the consumer (Calder 2001). Polyunsaturated fatty acids are also necessary for the cell membrane fluidity (Rock and Cronan 1985). In general, fatty acids are effective sources to store energy for animals. Saturated and unsaturated fatty acids are oxidized differently, where the saturated fatty acids (SAFA) directly can be oxidised, the monounsaturated fatty acids (MUFA) and the polyunsaturated (PUFA) needs 2 additional enzymes to work before oxidation is possible (Mathews and van Holde 1996; Schulz 1985). Hence, the energy yield is slightly lower for unsaturated fatty acids compared to the SAFAs.

The interaction between zooplankton and phytoplankton is very well suited to investigate questions on nutritional quality and essential components of the food. Reactions of most zooplankters are swift, both the consumers and their food are easy to culture. Moreover, cladoceran zooplankters, such as *Daphnia,* are cyclical parthenogens, and using one clone reduces variation in experimental studies. Although some studies

have focused on effects of amino acids (Guisande et al. 2000) and vitamins (Poulet et al. 1989; Viehoever and Cohen 1938) in the food of zooplankters, the main interest has revolved around minerals (especially phosphorus, but also nitrogen and selenium) and fatty acids. It has been found that algae with low concentrations of phosphorus are poor food for daphniids (Becker and Boersma 2003; DeMott et al. 1998; Sterner et al. 1993; Urabe et al. 1997; Weers and Gulati 1997). Moreover, the quantities of polyunsaturated fatty acids have shown to be of importance for *Daphnia* growth (Becker and Boersma 2003; Brett and Müller-Navarra 1997; DeMott and Müller-Navarra 1997; Park et al. 2002; Ravet et al. 2003; von Elert and Stampfl 2000; Wacker and von Elert 2001) (but see von Elert and Wolffrom 2001).

Zooplankton can accumulate considerable amounts of lipids, which can contribute up to 30 to 70% of the total zooplankton dry-weight (Arts et al. 1993; Cavaletto et al. 1989; Goulden and Place 1990). This accumulation process is tightly coupled to egg production where each cycle is preceded by a lipid increase (Tessier and Goulden 1982). About 98% of the zooplankton lipids are derived from the diet and the animals only synthesize a minor fraction *de novo* (Goulden and Place 1993). The storage lipids, triacylglycerols (TAG) and wax esters, are used as energy reserves and vary in concentration dependent on the nutritional status of the zooplankters. On the other hand, structural lipids, phospholipids (PLs) and sterols fluctuate less in the daphniids (Goulden et al. 1999). Due to variable enzymatic reactions polyunsaturated fatty acids are found in higher concentrations in the PLs compared to the TAGs. The polyunsaturated fatty acids of the PLs are especially important as precursors in the prostaglandin synthesis (Olsen 1999).

Compared to the lipids, storage and reallocation of phosphorus is less understood in *Daphnia*. In general daphniids have high requirements for phosphorus and are believed to be homeostatic consumers (Sterner 1990), meaning constant somatic C:P molar ratios, independent of the C:P ratio in their food. To remain homeostatic, the daphniids have to make adjustments when the food quality (P contents) changes. For example, daphniids increase their excretion and respiration rate when the food becomes P deficient (Darchambeau et al. 2003). The increased respiration rate could be due to an increased *Daphnia* feeding-appendage beat rate (Plath and Boersma 2001). Homeostasis implies that daphniids should not have luxury consumption of phosphorus, since a stored pool of P would alter the somatic C:P ratio. The largest compartments of phosphorus in daphniids are nucleic acids and phospholipids, making up 40-60% and around 20% of the total P

content respectively (Vrede et al. 1999). Moreover, daphniids with low amounts of RNA enhanced the P content of "other" P compounds, and Vrede et al. suggested that these compounds were free nucleotides enabling the daphniids to retain homeostasis. However, several studies have in fact shown that homeostasis for phosphorus is not as strict as previously believed (DeMott 2003; DeMott et al. 1998; Plath and Boersma 2001). Moreover, Sterner and Schwalbach (2001) showed that daphniids could compensate for periods of low P by reallocation of storage.

In this study we set out to investigate the effect of different nutritional limitations on growth and reproduction of *Daphnia*, contrasting phosphorus with its limited buffer within the animals with polyunsaturated fatty acids which we expect to be stored in substantial amounts. We addressed the following questions: 1) How do daphniids cope with excess amounts of essential components of the food, are they stored, and if so reallocated during periods of bad food quality? 2) How do daphniids divide up essential nutrients between somatic growth and reproduction, are there differences between P and HUFAs?

To answer these questions we performed several experiments studying: 1) Fatty acids saturation thresholds for *Daphnia* growth using gradients of fatty acid additions. 2) Storage of EPA by *Daphnia*. 3) How the specific phosphorus content of females and eggs change, when fed a food ranging in molar C:P ratios. 4) How different fatty acids are stored and allocated for reproduction. 5) If EPA and phosphorus can be reallocated from storage to balance periods of poor food quality. 6) The allocation of EPA to reproduction.

Material and methods

Algae and animals- We used a clone of *Daphnia magna* previously isolated from a pond in Frankfurt and kept at the Max Planck Institute for limnology for many years. All experiments were conducted at 20°C at constant, dim light. In all experiments, less than 24 hours old neonates were collected from third brood females fed phosphorus sufficient *Scenedesmus obliquus* in ample supply (1mgC L^{-1}) during their whole life. The neonates were pooled together and randomly divided over the different treatments. In order to reduce the impact of uncontrolled phosphorus addition all daphniids were kept in phosphorus free growth medium (ADaM) (Klüttgen et al. 1994). We used 2 different food sources, Phosphorus sufficient *Scenedesmus obliquus*, from a continuous culture and semi-continuously cultured P limited *S. obliquus*. In the cultures, we used Z/4 medium with and without reduced phosphorus content (from 1.39 mg P L^{-1} to 83.5 µg P L^{-1})

(Zehnder and Gorham 1960). All algae were obtained from stock cultures of the Max Planck Institute for Limnology, Germany. During all experiments, daphniids were transferred daily into fresh food suspensions or kept in a flow-through system.

General experimental procedure- To investigate how different essential compounds affect the fitness and the stoichiometry of *D. magna* we performed a range of experiments. We had two different set-ups: Firstly, we investigated how *Daphnia* perform in different food conditions. These experiments were conducted in a flow-through set-up. Pooled newborn neonates were divided into the flow-through vessels filled with 120ml of the different experimental foods. The food suspension had a flow rate of 1L per day to ensure constant food concentrations. From a sub sample of the pooled daphniids the initial dry weight was determined and the final dry weight was established from the experimental daphniids. At each sampling, 3-6 neonates were transferred to pre-weighed aluminium boats, dried at 60°C over night and weighed to the nearest 0.1µg on a Sartorius microbalance. From the dry weights the growth rates were computed and from these we calculated the growth differentials (DeMott et al. 2001; DeMott and Tessier 2002). Secondly, we investigated how nutrients are incorporated into *Daphnia* tissue and thus affect the stoichiometry of the *Daphnia*. These experiments were all conducted in a batch set-up, in 1L jars. This set-up was necessary to express different food variables on a sufficient number of individuals, for the consecutive analysis. All daphniids were pooled prior to the experiments and randomly divided over the different treatments. Before sampling, the daphniids were transferred to a suspension of control algae for 45 minutes to minimise the impact on ingested but not yet incorporated nutrients.

Nutrient enrichment techniques- We enriched various conditioned *Scenedesmus* with three different fatty acids, eicosanoic acid (20:0, saturated), 5,8,11,14,17-eicosapentaenoic acid (20:5ω3, EPA) and 5,8,11,14-eicosatetraenoic acid (20:4ω6, Arachidonic acid, ARA). We also had a control treatment that was incubated as the other treatments, only differing in the fatty acid addition. The enrichments were conducted according to von Elert (2002). The single fatty acids, dissolved in ethanol, were mixed with bovine serum albumin (BSA) dissolved in distilled water, to which *Scenedesmus* was added, and diluted with the growth medium. We incubated the fatty acids suspensions on a rotatory table in light for 13 hours, which is in the range of time for the algae to reach saturation (von Elert 2002). The incubation was ended with centrifugation (3,400 g for 5 min) the pellet was rinsed twice with 35ml ADaM and centrifuged to remove BSA and excess fatty acids. The food suspensions were diluted with ADaM to acquire food

concentrations of 1mgC L^{-1}. The different phosphorus treatments were obtained according to Plath and Boersma (2001) by enriching P limited *Scenedesmus* with various pulses of K$_2$HPO$_4$.

Analyses- Algal samples for carbon, phosphorus and fatty acid analysis were sampled by filtration at three dates during the enrichment phase. The samples for carbon and nitrogen were dried at 60°C over night and stored in an exicator until analysis using a FISONS® NA2000 elemental analyser. The particulate phosphorus samples were analysed directly with the ammonium-molybdate method by measurements on a spectrophotometer at 720nm. Samples for fatty acid analysis were stored at –18°C in Eppendorf vials under N$_2$-gas until analysis. The fatty acids were extracted and esterified according to Wiltshire et al. (2000). The samples were analysed by gas chromatography with the same configurations as von Elert (2002). The FAMEs were identified by comparison of retention times of known reference compounds. We used heptadecanoic acid methyl-ester and tricosanoic acid methyl-ester as internal standard.

Fatty acid demand and stoichiometry- To investigate in which amounts fatty acids are important for *Daphnia* growth we performed an experiment with variable fatty acid concentrations. We had an array of EPA, ARA and 20:0 (saturated) fatty acids concentrations in the food, these were obtained through mixing control algae with enriched algae in different proportions: 1.0, 0.5, 0.25, 0.125 and 0 of enriched algae. We used a logarithmic gradient since we expected the largest differences in the lower range of the fatty acid gradient. The juvenile growth was determined over three days in quintuplicates and the growth differentials were calculated as the difference compared to the control. This experiment was followed up to confirm our results on growth rates and also to determine how the daphniids take up and incorporate fatty acids when they are above the fatty acid saturation threshold. With the same set-up as above but only with the EPA gradient, neonates were cultivated in 1L jars on daily-renewed food suspensions in quadruplicates. The experiment was terminated after 6 days.

Female and eggs- We studied how *Daphnia* allocate essential nutrients between females and eggs, alternating both phosphorus conditions and availability of fatty acids in the food. Several of the food sources used in this study do not support juvenile growth, and hence, do not allow studies on reproduction. Therefore, to study allocations between eggs and adults we worked with adult daphniids, brought up on P sufficient *Scenedesmus* for 10 days. This age corresponds to the time of first reproduction (first clutch of eggs in the brood pouch). For the effects of phosphorus, we performed two consecutive

experiments studying the effect of alternating C:P molar ratios using P limited *Scenedesmus* enriched with phosphorus (Plath and Boersma 2001). In the first experiment, the C:P ranged between 433-93 and in the second between 848-477. For the fatty acids, we studied how the daphniids incorporated different fatty acids when fed P sufficient *Scenedesmus* enriched with 20:0, EPA, ARA and a unenriched control. After 7 days of feeding on these food sources the experiment was terminated. For the sampling, we selected females with eggs in their spherical phase, to ensure that the eggs were in similar stages, which during favourable food conditions corresponds to about 30 hours old eggs (Sobral et al. 2001). Eggs from the brood pouch of these females were removed with a gentle current of a small syringe. From each replicate we concentrated the eggs in Eppendorf tubes. The samples for P analysis were transferred to pre-weighed silver capsules in a drop of ADaM, dried at 60°C over night before the dry weights were established. Then the samples were ashed at 550°C and analysed for phosphorus content. The specific P content was calculated in percent phosphorus per dry weight.

Table IV, 1. Schedule of food regimes. The 6 different treatment identifications denote timing of food regimes, where numbers indicate time (in days) and letter availability of enriched (E) or control (C) food. Over the 6-day period all treatments received an equal total amount of enrichments (except treatment 6C). The schedule describes the timing and the proportion (in percent) of the enrichments the daphniids received each day.

Treatments	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
1E:1C	33	0	33	0	33	0
1E:2C	50	0	0	50	0	0
3E:3C	33	33	33	0	0	0
3C:3E	0	0	0	33	33	33
6E	17	17	17	17	17	17
6C	0	0	0	0	0	0

Alternating food conditions- To determine whether daphniids during bad food conditions can use stored fatty acids and phosphorus for consecutive growth, we performed two parallel experiments, in which we experimentally alternated the availability of P and EPA over a six-day growth period in flow-through. In both experiments, we used P limited *Scenedesmus* enriched with P and EPA using the methods of Plath and Boersma (2001) and von Elert (2002). We used P limited *Scenedesmus* also in the EPA experiment, as they have low content of EPA (Boersma 2000), which limits *Daphnia* growth (Becker and Boersma 2003). To ensure that the EPA treatments were not phosphorus limited they were all enriched with phosphorus. In total, we had six different treatments in a quintuplicate set-up adding up to 30 flow-through vessels per enrichment (EPA and P).

During the growth phase, the neonate daphniids were fed enriched and un-enriched food at various times and in different concentrations (Table IV, 1). The reason to use various concentrations was to ensure that all daphniids (except treatment 6C, fed only control algae) had the same availability of each addition over the total experimental period. Growth rates were determined over 6 days. In the EPA experiment, we found unexpectedly high variation in growth within each treatment. Potentially the reason could have been due to the low EPA saturation threshold for *Daphnia* growth (see Table IV, 3) in combination with the flow-through set-up. In the flow-through the daphniids would experience gradients of enrichments whenever a new food suspension was inserted. Hence, the daphniids might only have experienced short periods of actual EPA limitation. Therefore, it was necessary to clarify our results and to redo this experiment. This time we used a batch set-up, and transferred the daphniids each day into the new food suspensions, using a pipette.

EPA and egg production – To determine how important EPA is for egg production we enriched already adult females with EPA as above for 7 days. Thereafter, the females were transferred to control *Scenedesmus* and cultured on this food for 6 days. Samples for fatty acid analysis of females and their eggs were taken after 45 minutes, after 3 days and after 6 days of feeding. The interval between samplings corresponds roughly to one egg production cycle of *D. magna*. Moreover, to estimate how important egg production is for EPA losses in the daphniids, we calculated the predicted total EPA content for the females and the released broods (e.g. female day 3 + brood day 0) and compared these with the initial amounts in the females.

Results

Enrichment of algae- We enriched P sufficient *Scenedesmus* with a total of 3 different fatty acids. This enrichment technique is relatively new and allows adding a single fatty acid to the algae without affecting the others (Table IV, 2). Nevertheless, in our study we found a slight increase of the closest similar fatty acid, e.g. enrichment with 20:4ω6 also affected the content of 20:3ω6 (Table IV, 2).

Fatty acid demand and stoichiometry- We investigated how *Daphnia* growth is dependent on fatty acid concentrations of three single fatty acids. We found that the enrichment of 20:0 did not have an effect on the growth differentials that remained around zero indifferent from the control. On the other hand, already the lowest addition of

Table IV, 2. Fatty acid concentrations of *Scenedesmus obliquus* (µg mgC^{-1}) enriched with 3 different fatty acids used as food source for *Daphnia*. The values are averages from 3 measurements in brackets ±SE are given. Fatty acids marked with letters in superscript represent an overall significant difference between the additions (ANOVA) where treatments marked with identical letters are not significantly different (Newman-Keuls test).

Parameters	Control	Saturated	EPA	ARA
14:0	0.54 (0.02)	0.55 (0.04)	0.45 (0.01)	0.47 (0.06)
16:0	49.23 (3.01)	47.94 (5.30)	38.75 (3.53)	39.76 (3.79)
16:1ω7	0.36 (0.04)	0.35 (0.02)	0.27 (0.02)	0.27 (0.01)
18:0	7.95 (0.45) [A]	8.11 (0.48) [AC]	6.75 (0.12) [BC]	6.32 (0.28) [B]
18:1ω12/ω9/ω7	87.29 (11.49)	72.02 (8.31)	73.54 (5.26)	69.21 (2.48)
18:2ω6	7.11 (1.35)	5.93 (2.27)	6.73 (0.57)	6.07 (1.95)
18:3ω6	0.84 (0.15)	0.89 (0.13)	0.54 (0.02)	0.67 (0.06)
18:3ω4	0.60 (0.24)	0.54 (0.17)	0.48 (0.19)	0.52 (0.17)
18:3ω3	4.97 (1.15)	4.37 (2.00)	5.18 (0.66)	4.65 (1.76)
18:4ω3	0.94 (0.25)	0.84 (0.41)	1.51 (0.21)	0.90 (0.36)
20:0	0.39 (0.05) [A]	14.11 (0.92) [B]	0.47 (0.04) [A]	0.55 (0.01) [A]
20:1ω9	0.62 (0.11)	0.49 (0.19)	0.72 (0.05)	0.97 (0.07)
20:2ω6	0.13 (0.02)	0.04 (0.04)	0.04 (0.03)	0.10 (0.00)
20:3ω6	0.01 (0.01) [A]	0.05 (0.01) [A]	0.02 (0.02) [A]	0.28 (0.10) [B]
20:4ω6	0.01 (0.01) [A]	0.04 (0.03) [A]	0.02 (0.01) [A]	3.85 (1.33) [B]
20:3ω3	0.03 (0.03)	0.04 (0.04)	0.06 (0.03)	0.11 (0.07)
20:4ω3	0.00 (0.00) [A]	0.00 (0.00) [A]	0.45 (0.01) [B]	0.01 (0.01) [A]
20:5ω3	0.00 (0.00) [A]	0.00 (0.00) [A]	4.47 (0.68) [B]	0.00 (0.00) [A]
22:0	0.77 (0.03)	0.79 (0.06)	0.43 (0.22)	0.65 (0.02)
22:5ω3	0.15 (0.07)	0.16 (0.02)	0.10 (0.05)	0.14 (0.01)
22:6ω3	0.14 (0.01) [A]	0.11 (0.02) [AB]	0.06 (0.01) [B]	0.10 (0.01) [AB]
24:0	0.29 (0.12)	0.34 (0.05)	0.31 (0.02)	0.30 (0.05)
24:1ω9	0.00 (0.00)	0.02 (0.02)	0.01 (0.01)	0.03 (0.01)
SAFA	59.17 (3.24) [AB]	71.84 (5.37) [A]	47.15 (3.42) [B]	48.06 (4.01) [B]
MUFA	88.27 (11.55)	72.88 (8.53)	74.54 (5.26)	70.48 (2.43)
PUFA	14.95 (3.04)	13.02 (4.76)	19.67 (2.18)	17.40 (5.55)
HUFA	1.24 (0.30) [A]	1.16 (0.44) [AB]	6.61 (0.92) [C]	4.99 (1.67) [BC]
ω3	6.24 (1.49)	5.53 (2.42)	11.83 (1.56)	5.91 (2.09)
ω6	8.11 (1.31)	6.95 (2.17)	7.37 (0.61)	10.97 (3.29)
ω3:ω6	0.75 (0.05) [A]	0.74 (0.09) [A]	1.59 (0.09) [B]	0.51 (0.05) [A]

polyunsaturated fatty acids, 0.06 and 0.02µg mgDW^{-1} for ARA and EPA respectively, increased the growth differentials considerably compared to 20:0 (ANOVA; *P* <0.001) (Table IV, 3). Moreover, neither of the fatty acid concentration gradients affected the growth differentials significantly (ANOVA; *P* = 0.32) (Table IV, 3). Nevertheless, due to unexpectedly low growth on the control *Scenedesmus* we repeated the EPA dilution

Table IV, 3. Growth differentials for various concentrations of fatty acids (20:0, ARA, EPA) and phosphorus. All concentrations are µg mgDW^{-1} and the growth differentials are d^{-1}. In brackets are ±SE for 5 replicates.

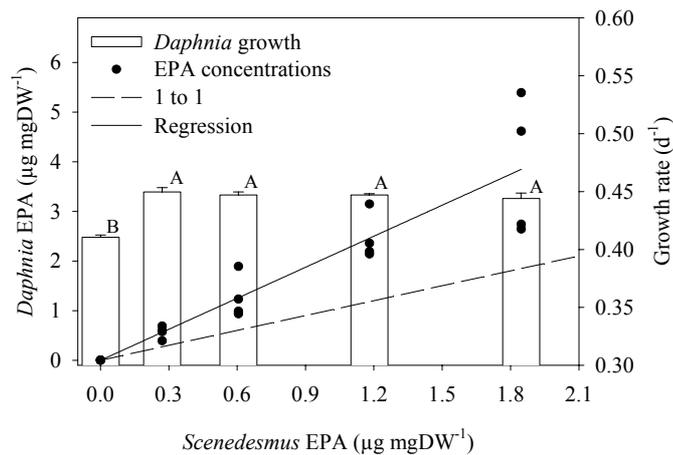| 20:0		ARA		EPA		Phosphorus	
Conc.	Growth diff.	Conc.	Growth diff.	Conc.	Growth diff.	Conc.	Growth diff.
0.84	0.03(0.05)	0.06	0.17(0.06)	0.02	0.23(0.04)	3.58	0.37(0.02)
1.22	0.05(0.02)	0.10	0.22(0.02)	0.25	0.38(0.01)	4.96	0.44(0.01)
2.34	-0.06(0.04)	0.63	0.25(0.02)	0.5	0.32(0.04)	7.28	0.44(0.00)
6.03	-0.01(0.04)	1.87	0.27(0.04)	2.01	0.28(0.06)	10.7	0.41(0.01)

Fig. IV, 1. *Daphnia* juvenile growth rates and incorporation of EPA over 6 days on 5 different concentrations of EPA. The regression equation is: $y = 2.08x - 4 \times 10^{-11}$; $r^2 = 0.84$; $P < 0.001$. Error bars are ±SE from 5 replicates. Bars marked with identical letters are not significantly different (Newman-Keuls test).

gradient. Moreover, in this set-up we also determined how fatty acids are incorporated in the *Daphnia* biomass. In this repetition, we came to the same conclusion concerning growth and EPA concentrations. Again, the lowest EPA concentration (0.27µgEPA mgDW^{-1}) supported higher growth compared to the control (Fig. IV, 1) (ANOVA; P <0.001). The gradient did not have any effect on growth when the control was omitted (ANOVA; $P = 0.68$). When we investigated how daphniids incorporate EPA from the food we found an overall increase in somatic EPA over all our treatments (ANOVA; P <0.001). Moreover, the changes in EPA of the food correspond well to the changes in the daphniids. However, the daphniids seemed to retain a higher proportion of the EPA, and we found around the double concentration of EPA in the daphniids (µg mgDW^{-1}) compared to the algae (Fig. IV, 1) ($y = 2.08x - 4 \times 10^{-11}$; $r^2 = 0.84$; P <0.001).

Stoichiometry of female tissues and eggs- We studied the impact on specific P content in females and their eggs in two experiments on variously phosphorus enriched *Scenedesmus*. Undoubtedly, the C:P molar ratio of the food had a strong effect on the specific phosphorus content of the daphniids in our experiment. Both experiments combined, we found a decrease in P content of the daphniids with elevated molar C:P

Table IV, 4. Summary table of ANOVA testing for differences in specific P content of *Daphnia magna* females and their eggs (stage). Experiment 1 was conducted with *Scenedesmus* with C:P ratios between 93-433 and experiment 2 between 477-848. P values of two-way ANOVAs.

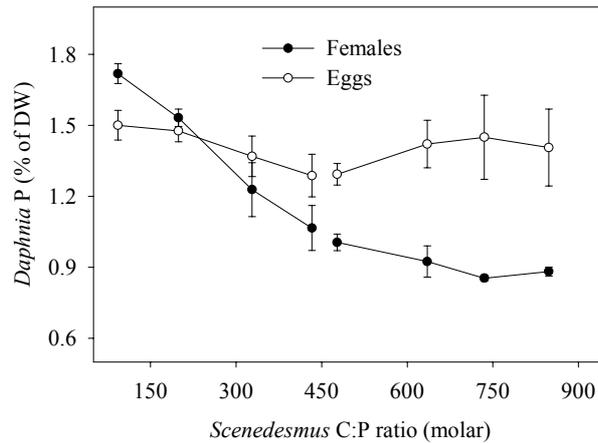| Experiment	C:P ratio	Stage	Interaction
1	<0.001	0.68	<0.001
2	0.99	<0.001	0.39
1 & 2	<0.001	<0.001	<0.001

Fig. IV, 2. *Daphnia magna* P content (% of dry-weight) of females and eggs after 7 days of feeding on *Scenedesmus* of various C:P molar ratios. Data from two consecutive experiments; Exp 1 conducted on the lower C:P ratios and Exp. 2 on the higher C:P ratios. Error bars denote ±SE for 5 replicates.

ratios (Table IV, 4). Moreover, the eggs had an overall higher P content compared to the females. The significant interaction of stage (female vs. egg) and C:P content of the food suggests that mainly the females were responsible for the decrease in specific P content (Table IV, 4; Fig. IV, 2). Overall, the females specific P content decreased from around 1.7% to 0.9% of dry-weight (P%) (ANOVA; $P < 0.001$), the eggs on the other hand remained stable around 1.4P% over all treatments (Fig. IV, 2) (ANOVA; $P = 0.75$). During unlimited conditions, below a C:P of around 350 (Becker and Boersma 2003; Brett et al. 2000), P was seemingly equally distributed between eggs and females (2-way ANOVA; between stage and C:P (93-328); $P = 0.45$). However, the females had a distinct decrease in P%, with elevated C:P molar ratios and this decrease was considerable already in the non-limiting C:P range (93-328; Fig. IV, 2) (ANOVA; $P < 0.01$). Moreover, at higher C:P ratios in experiment 2, the eggs had a considerably higher P% compared to the females (Table IV, 4).

Table IV, 5. Summary table of differences in various fatty acid contents of *Daphnia magna* females and their eggs (stage). *P* values of two-way ANOVAs; ns- denote non-significance.

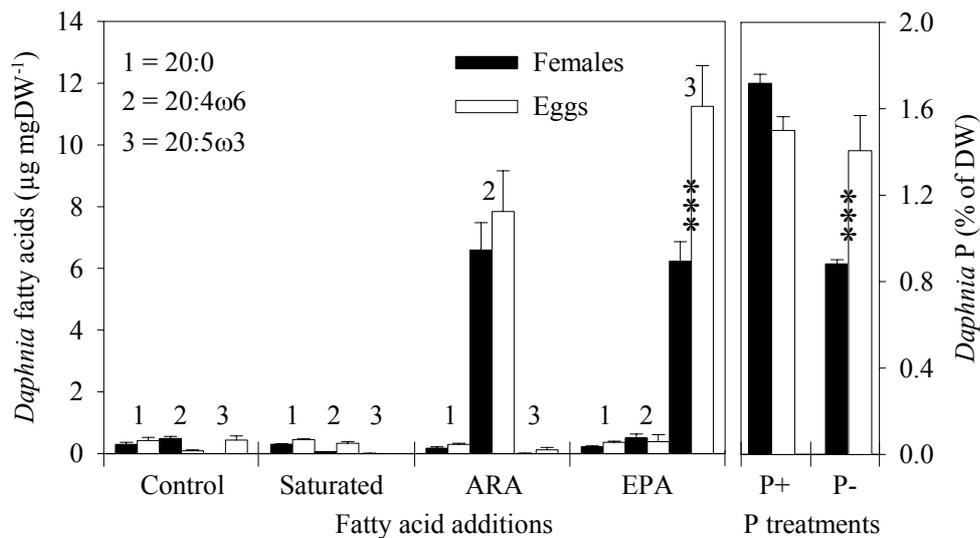| Parameters	Stage	FA addition	Interaction
18:3ω4	<0.001	ns	ns
20:1ω9	ns	ns	ns
20:0	<0.01	<0.05	ns
ARA, 20:4ω6	ns	<0.001	ns
EPA, 20:5ω3	<0.001	<0.001	<0.001

Fig. IV, 3. Fatty acid content and P content (% of dry-weight) of *Daphnia magna* females and their eggs. The daphniids were for 7 days fed either fatty acid enriched food or P-limited algae spiked with a pulse of P to achieve C:P: P+, 93 and P-, 848. The FA enrichments were: Control, Saturated (20:0), ARA (20:4ω6) and EPA (20:5ω3). The number 1-3 describes the content of each fatty acid. Asterisks denote significant differences between female and eggs (Newman-Keuls test). Error bars are ± SE for 4 (FA) and 5 replicates (P).

When the incorporation and allocation of fatty acids by *Daphnia* was investigated, we observed that different fatty acids, enriched in the food, were diversely incorporated in the adult daphniids. For comparison we included in these analyses two "neutral fatty acids", 18:3ω4 and 20:1ω9 that belong to other ω-families compared to ω3-EPA and should thereby not be affected by our experimental additions. Overall, there was a tendency that fatty acids were in higher concentrations, per dry weight, in eggs compared to the females (Table IV, 5). Neither ARA nor 20:1ω9 were preferably allocated in the eggs (Table IV, 5). All fatty acid additions had a significant impact on the somatic fatty acid content of the daphniids (Table IV, 5; Fig. IV, 3). Post-hoc comparisons suggested that only the addition of EPA improved the concentration within the eggs compared to the females (Newman-Keuls test; $P < 0.001$).

Alternating food conditions- We determined if daphniids could reallocate stored resources of phosphorus or EPA during periods of lower food quality. When EPA storage was considered, we found unusually high variation on *Daphnia* growth and hence, there was not an overall significant difference (Fig. IV, 4A). Therefore, we redid this experiment, this time daily transferring the daphniids into fresh food suspensions in a batch set-up. This procedure clearly improved the sensitivity of the experiment with smaller variations within each treatment. Furthermore, we found a positive effect of the
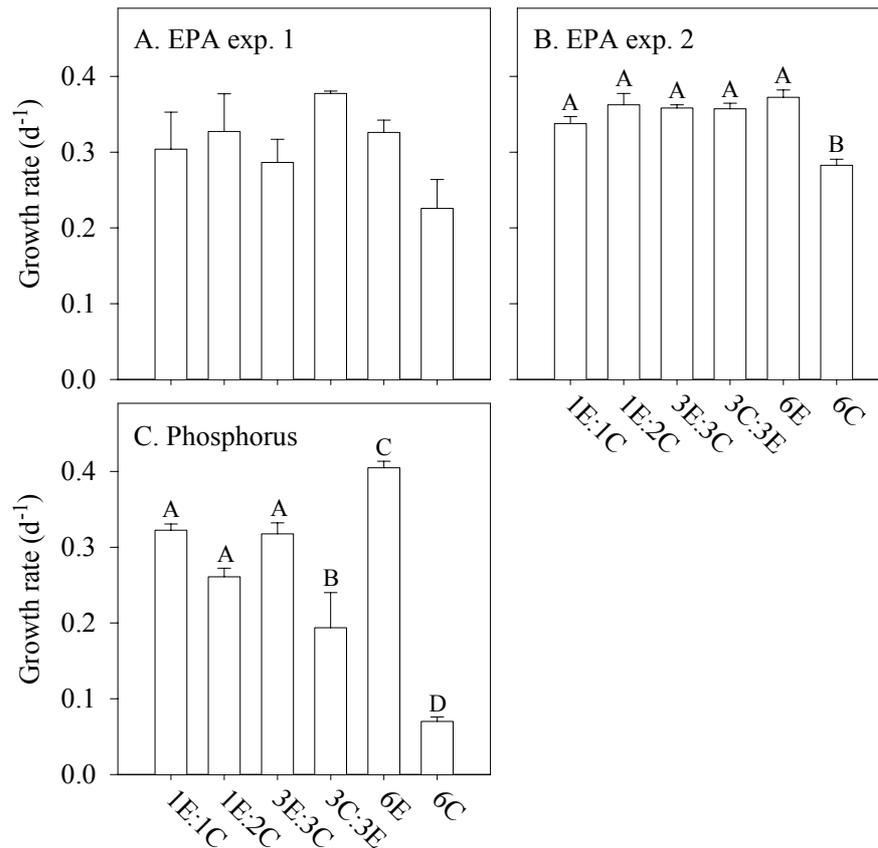
Fig. IV, 4. Effects on juvenile growth rate over 6 days on variable food conditions in regards to EPA and P content. The x-axes indicate the timing of food regimes where numbers indicate time (days) and letter enriched (E) or control (C) food availability for details see Table 1.. The experiment was terminated after 6 days. Error bars denote ±SE for 5 replicates. Bars marked with identical letter are not significantly different (Newman-Keuls test).

EPA additions (Fig. IV, 4B) (ANOVA; P <0.001). Post-hoc comparison showed that all treatments had higher growth rates compared to treatment 6C, and that there were no differences between the other treatments (Newman-Keuls test). When the P content was alternated, we found an overall significant difference between the treatments (Fig. IV, 4C) (ANOVA; P <0.001). All variable food conditions affected the *Daphnia* growth rates that decreased to intermediate levels between the two extremes, treatment 6E and 6C. Post-hoc comparisons indicated that all treatments with alternating food were significantly different

Table IV, 6. Summary table of differences in *Daphnia magna* total fatty acid contents per female and brood. F and P values of time versus stage (female vs. brood), repeated measurement analysis. ns- denotes non-significance.

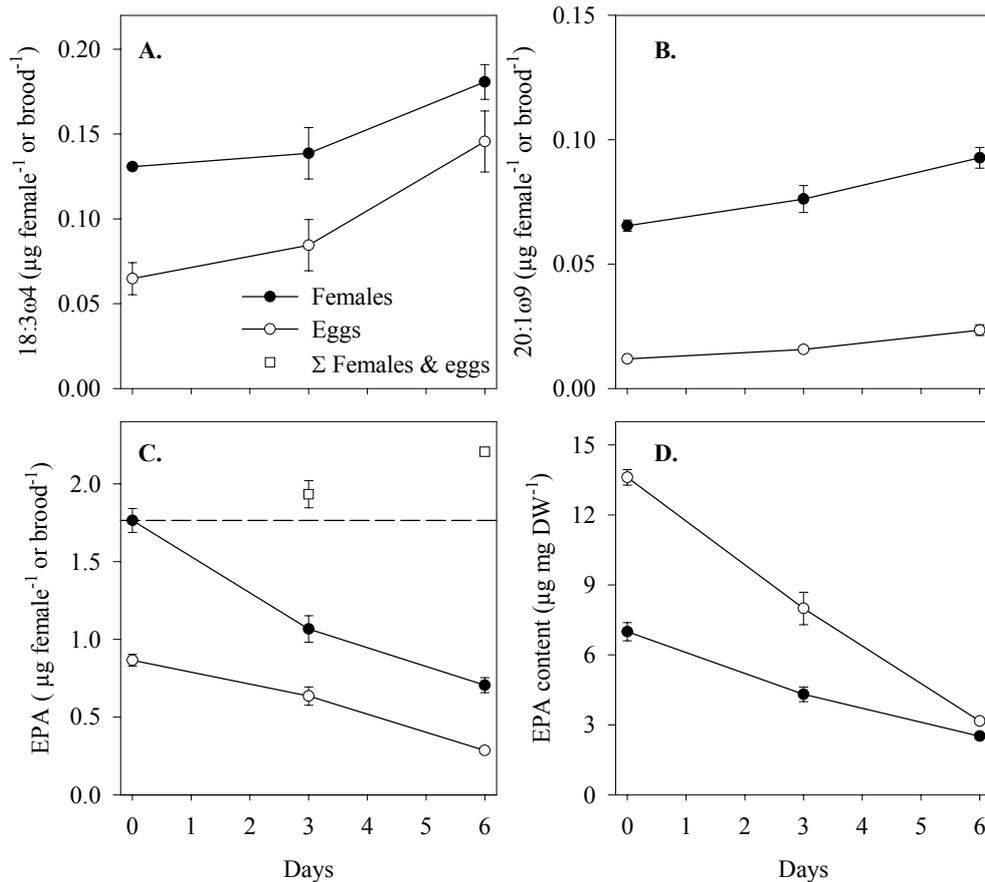| Parameters	Female vs. brood		Time		Interaction	
	$F_{1,4}$	P	$F_{2,8}$	P	$F_{2,8}$	P
18:3ω4	22.0	<0.01	15.6	<0.01	0.80	ns
20:1ω9	1233	<0.001	15.7	<0.01	2.5	ns
EPA, 20:5ω3	149.6	<0.001	99.2	<0.001	10.94	0.001

Fig. IV, 5. Development of the fatty acid pool within adult *Daphnia* and their eggs over six days. The daphniids were prior day 0 fed EPA enriched food for 6 days, after which, all daphniids were fed un-enriched *Scenedesmus*. **A**. The total content of 18:3ω4 female[-1] or brood[-1]. **B**. The total content of 20:1ω9 female[-1] or brood[-1]. **C**. The total content of EPA female[-1] and brood[-1]. Squares describe the sum fatty acid of females plus the previous brood or broods. **D**. The concentration of EPA mgDW[-1]. Error bars are ±SE from 3 replicates.

from the two constant treatments 6E and 6C (Newman-Keuls test).

EPA and egg production- In detail, we studied the allocation of EPA between females and eggs. To compare the impact on EPA, we used 18:3ω4 and 20:1ω9 which on the contrary to EPA, were continuously available in the food. At the start of the experiment the females had higher EPA content (EPA female[-1]) in absolute amounts compared to the broods (Fig. IV, 5C). We found a rapid decrease in EPA in absolute amount over time in both eggs and adults (Table IV, 6; Fig. IV, 5C). The adults showed a larger drop in EPA over the initial 3-day period (39% vs. 23% for the brood). On the other hand, over the whole 6-day period, there was an indifferent decrease between the females and the broods (60% vs. 67%). Also the amounts of 18:3ω4 and 20:1ω9 in the daphniids were affected over time; still, they were available in the food and increased both in the females and eggs over time (Table IV, 6; Fig. IV, 5A; Fig. IV, 5B). Moreover, to estimate how egg production affected the EPA content of the adults we computed the total EPA

content of the female plus the previous brood or broods. We found that both the sum of EPA content from day 3 and day 6 are higher than the content of the initial females (Fig. IV, 5C, open squares). This indicates an overall accumulation of EPA when the egg production was neglected. When only the concentrations of EPA in eggs and females were considered, we found the eggs to have higher concentrations of EPA ($\mu g \ mgC^{-1}$) over the whole experimental period compared to the adults (Fig. IV, 5D).

Discussion

Demand of nutrients- Many studies have linked different fatty acids as determinants for *Daphnia* growth and development in field (Brett and Müller-Navarra 1997; Müller-Navarra 1995b; Wacker and von Elert 2001) and in laboratory studies (Müller-Navarra 1995a; Park et al. 2002) using phytoplankton of various nutrient state thereby altering the fatty acid content. Several studies have used various techniques to enrich the *Daphnia* diet with different fatty acids, be it fatty acid emulsions, microencapsulated lipids or PUFA-rich algae (Plath and Boersma 2001; Sundbom and Vrede 1997; Weers and Gulati 1997). The disadvantage of these techniques is that it is difficult to attribute effects to a single fatty acid. However, various techniques to enrich phytoplankton with single fatty acids have recently been developed and used (Becker and Boersma 2003; Ravet et al. 2003; von Elert 2002; von Elert and Stampfl 2000; von Elert and Wolffrom 2001). Overall there is now a substantial amount of evidence indicating that various ω3 fatty acids in general and EPA in particular are important determinants for the food quality of daphniids (but see von Elert and Wolffrom 2001). However, the above studies only described the effect on growth by switching the availability of the fatty acids, between present or not present. Hence, it is not possible to assess the quantities of the compounds necessary for growth. This is a very important question, since establishing saturation thresholds would help assessing the likelihood of fatty acid limitations in the field. Moreover, it is important to determine if the amounts used in previously mentioned studies where in this range or considerably above the specific fatty acid saturation threshold, especially since some reports exist that free fatty acids can actually be deleterious (Reinikainen et al. 2001). Moreover, it is possible that fatty acids in great surplus could be used differently compared to when administrated in the "natural range". To investigate this we determined *Daphnia* growth on concentration gradients of fatty acids. This is common a technique previously used in phosphorus quality studies (e.g. Becker and Boersma 2003; DeMott 1998; Plath and Boersma 2001; Vrede et al. 2002), but no one has investigated this

relationship for single fatty acids under laboratory conditions. In the field (Müller-Navarra 1995b), a positive relationship between *Daphnia* somatic growth and the EPA concentration in natural lake seston up to 0.8μg EPA L^{-1} was found. Wacker and von Elert (2001) found a close relationship of *Daphnia* growth and the concentration of 18:3ω3 levelling off at around 5μg 18:3ω3 L^{-1}. Moreover, there are a few laboratory studies that investigated the assimilation of various seston fatty acids concentrations in animal tissues for daphniids (Weers et al. 1997) and rotifers (Olsen 1999).

We used *Scenedesmus* enriched with different fatty acids in a logarithmic dilution gradient of natural concentrations (Ahlgren et al. 1997; Müller-Navarra 1995b), concentrating on three fatty acids (20:0, ARA, EPA), which were supplemented in equal amounts. Nevertheless, after the incubation the actually measured amounts of the treatments differed slightly (Table IV, 2; Table IV, 3). The highest concentration for both PUFAs (EPA, ARA) was about 2μg fatty acids mgDW^{-1} (equals about 4μg fatty acids L^{-1}) and for the 20:0 three times higher, about 6μg fatty acids mgDW^{-1}. This pattern can be explained with that saturated fatty acids are less prone to be oxidized compared to the PUFAs. We found high growth differentials already with the lowest addition of EPA (0.02μg EPA mgDW^{-1}). Already with the lowest additions in both experiments the growth differentials and growth rate increased to high levels. Enrichments above these concentrations did not affect the growth differentials (Table IV, 3) or the growth rate further (Fig. IV, 1). This pattern is contradictory to the findings of Müller-Navarra (1995b) where the *Daphnia* growth rates levelled off above 0.8μg EPA L^{-1}. Nevertheless, our findings suggest a low EPA requirement for *Daphnia* growth, far less than their requirements for phosphorus (between 2 and 4μgP mgDW^{-1}) (Table IV, 3) (Plath and Boersma 2001). Moreover, 0.8μg EPA L^{-1} (Müller-Navarra 1995b) and 0.04μg EPA L^{-1} (this study) are considerably lower than the enrichments used in recent studies; about 8 μg EPA L^{-1} (Becker and Boersma 2003), 12 μg EPA L^{-1} (von Elert and Stampfl 2000), 105μg EPA L^{-1} (von Elert and Wolffrom 2001) and to 146μg EPA L^{-1} (von Elert 2002). We also found elevated growth with the addition of ARA, although compared to EPA the growth was on a lower level (Table IV, 3) (2-way ANOVA; $P = 0.018$). The elevated growth with ARA additions is not consistent with the findings of von Elert (2002), even though he also observed a slight but non-significant increase with this particular fatty acid compared to the control. Only the additions of 3 different ω3 fatty acids had a positive effect on *Daphnia* growth in his study. From these experiments together we conclude that PUFAs and EPA are important food quality determinants enhancing growth of daphniids.

However, the fatty acid requirements for growth were fulfilled already with minute additions and larger additions did not improve growth further.

Nutrient storage- In general the daphniids can allocate nutrients (energy) for different purposes e.g. reproduction, storage, somatic growth and maintenance. Of these, investments in egg production seem to be most sensitive to periods of starvation. The storage is mainly used for maintenance, however, storage can partly also be used for growth (Bradley et al. 1991). Daphniids can store lipids in a cyclic process tightly coupled to egg production (Tessier and Goulden 1982). Each egg production cycle is preceded by a visible accumulation of lipid reserves, which decrease with the actual production of eggs. The egg production is determined by the energy availability during the first part of each instar (Bradley et al. 1991). Thus, the adult females enrich their eggs with a pool of fat, which improves the neonates resistance to starvation.

For phosphorus, however, daphniids are believed to be homeostatic consumers and should thereby only have limited possibilities to store this element. On the other hand, the knowledge that up to 30-70% of the zooplankton dry-weight consist of (carbon rich) lipids (Arts et al. 1993; Cavaletto et al. 1989; Goulden and Place 1990), which fluctuate during each egg production cycles (Tessier and Goulden 1982) complicates the homeostasis theory. Hence, in order to maintain a constant C:P ratio over an egg production cycle P would also have to be incorporated by the female. Several recent studies have shown that the homeostasis is not as strict as previously assumed, and there are some variations in somatic C:P and specific P per dry weight with changes in C:P of the food (DeMott 2003; DeMott et al. 1998; Plath and Boersma 2001). One study even found a positive relationship between the specific P content of the daphniids and their growth rate (DeMott et al. 1998). However, this relation could not be reproduced by Sterner and Schwalbach (2001). Nevertheless, they showed that daphniids during alternating P food quality conditions could compensate for periods of low P. For example, daphniids that spent about 50% of the day in P- food and the remaining in P+ food had similar growth as when fed a 50% mixture of P- and P+ food. This compensation suggests that the daphniids can reallocate stored compounds for growth at least over short intervals.

When we investigated the allocation of different fatty acids and phosphorus we found that all fatty acid additions in the food enhanced the fatty acid content of the daphniids (Table IV, 5). Although significant, the net incorporation of 20:0 was much lower compared to the incorporation of ARA and EPA (Fig. IV, 3). These two compounds were preferably accumulated in the females and the eggs. EPA even had a considerably

higher concentration in the eggs compared to ARA (Table IV, 5). This indicates a diverse use of these fatty acids, which suggests a selective storage of PUFAs, and that the 20:0 is metabolised either for energy or into other compounds (Fig. IV, 3). The higher concentration in the eggs of the EPA enriched daphniids further suggests a potentially higher need for this fatty acid initially for neonatal development.

We showed that the EPA saturation threshold for daphniids is very low. This suggests that the daphniids often could face an environment above this threshold. We investigated how the daphniids handle this surplus and studied if daphniids incorporate EPA under different EPA availability. To our knowledge, only one study has investigated the *Daphnia* incorporation of fatty acids from a food source with variable fatty acid concentrations (Weers et al. 1997), where the daphniids were fed a diet of *Chlamydomonas* with emulsions differing mainly (but not solely) in DHA:EPA ratios. Weers et al. (1997) found that EPA in the diet was detected in similar proportions in the daphniids, independent of the proportion in the food. DHA on the other hand, was retained in much lower fractions. Olsen (1999) also described this relation between food and rotifers (*Brachionus plicatilis*), which initially incorporated similar proportions (about 1:1) of $\omega 3$ and $\omega 6$ fatty acids as in the food. This relation reached a plateau when the $\omega 3$ fatty acids in the food were in above 60% of total fatty acids. When we fed daphniids differently EPA-enriched *Scenedesmus* we found another pattern; the daphniids incorporated EPA from the food to about the double concentration ($\mu g\ mgDW^{-1}$) (Fig. IV, 1). Evidently daphniids can retain considerable amounts of EPA. However, it is unclear how and for what the storage can be reallocated.

When we studied the allocation of phosphorus, we found that the *Daphnia* females show a similar pattern as for EPA i.e., more P in the diet also enhanced the P content in the individuals where the females varied between 0.9-1.7P% (% of DW). When the P-limitation became severe the females stabilised at about 0.9P%, which could indicate the lowest possible P content for survival. This level is reached when fed a food of C:P 450 molar ratio. However, the females showed a considerable decrease in specific P content already on low (non-limiting) C:P ratios of the food. Phosphorus was always allocated in the same amounts to the eggs independent of P content in the food (Fig. IV, 2). Not even when P was in great surplus the females boosted their eggs with a higher concentration. The fatty acids were allocated differently, while ARA was allocated in the same concentration EPA was had considerably higher concentrations in the eggs (Fig. IV, 3 and Table IV, 5). This suggests a somewhat contrasting allocation for EPA and phosphorus

between females and eggs. When in surplus, the daphniids preferably retained both ARA and EPA, where EPA to a larger extent was allocated in the eggs. Phosphorus on the other hand was preferably allocated in the eggs only when P was limiting (Fig. IV, 2 and Fig. IV, 3). Faerovig and Hessen (2003) found a similar pattern, that daphniids allocate rather constant proportions of phosphorus to the eggs independent of the P concentration in the food.

Reallocation of storage- We investigated if daphniids can reallocate stored fatty acids and phosphorus to compensate growth during periods of poor food quality. This we performed by switching the availability of enriched and non-enriched foods over various periods in a flow-through set-up. In the EPA experiment, we did not find any overall significance (Fig. IV, 4A). This was due to unusually large variations probably caused by the low EPA saturation threshold for *Daphnia* growth ($<0.02\mu$gEPA mgDW^{-1}) combined with the cultivation in flow-through. This set-up might not have changed the food sources rapid enough to enforce EPA limitations. Thus, the daphniids might never have to utilise any storage (Fig. IV, 4A). Of course, the same reasoning could be used for the phosphorus experiment. However, the phosphorus saturation threshold is much higher compared to EPA (above 2μgP mgDW^{-1}) (Plath and Boersma 2001). Therefore, we argue that the flow-through experiment with regard to P could sufficiently exchange the food and induce specific limitation pressures. Hence, we also found strong effects of our P treatments.

Clearly there are differences in how phosphorus and EPA can be reallocated for growth. As expected, EPA that we previously found in variable concentrations within the daphniids (Fig. IV, 1), could be reallocated for growth to a much larger extent than P (Fig. IV, 4). Alternating the EPA availability in the food did not have a negative impact on *Daphnia* growth. The growth rates did not even decline when EPA enriched food was available only every three days (1E:2C). In contrast, already when the availability of P decreased to every second day the growth decreased considerably compared to the daphniids continuously fed enriched algae. Increasing this period lowered the growth slightly more. Moreover, we found differences depending on the timing of the enrichment, over the initial three days or the final three days. This timing did not have a negative impact on the EPA enriched daphniids. Apparently the initially EPA-starved daphniids could recover over the last 3-day period and the initially EPA fed daphniids could reallocate their storage. The phosphorus treatments acted differently and the timing of enrichment had an impact. The initially P-enriched daphniids (3E:3C) had a considerably higher growth rate compared to the initially P-starved daphniids (3C:3E). The differences

between these treatments could demonstrate a reallocation of stored P for growth supporting the findings of Sterner and Schwalbach (2001), where the daphniids could compensate periods of bad quality food. In our study, the previously P enriched daphniids could grow faster because of their internal P storage. The initially P-starved daphniids could not utilise any storage and could therefore only use the enriched food during the final period, which resulted in a lower total growth. Another explanation is that the initially P starved daphniids, after three days were in to bad a state from which they could not recover. However, these daphniids (3C:3E) grew faster than the control daphniids (6C), which indicate that the daphniids were not in too poor a condition. From these findings we conclude that the reallocation of phosphorus is far less efficient than the reallocation of EPA (Fig. IV, 4).

EPA and egg production- We studied how daphniids enriched with EPA choose to allocate this resource when transferred to unenriched food. After 6 days of feeding enriched food the daphniids and the eggs are rich in EPA, and the females had approximately twice as much EPA in absolute amounts compared to the brood (1.7μg female^{-1} compared to 0.8μg brood^{-1}) (Fig. IV, 5C). A similar pattern was also found with the absolute concentration of 18:3ω4 and 20:1ω9. However, when concentrations are considered the eggs had a considerably higher EPA content per dry weight compared to the females (Fig. IV, 5D). After this starting day the daphniids were cultivated on control P+ *Scenedesmus* and we monitored the fatty acid content every 3 days over two intervals. The fatty acids available in the food (18:3ω4 and 20:1ω9) increased slightly over the period. EPA on the contrary decreased drastically in the females and also in the broods (Table IV, 6). In order to determine in what range egg production is responsible for the maternal decrease we computed the predicted maternal EPA content. This is the female plus the previously released brood or broods (Fig. IV, 5C). This is possible as the interval of three days is about one egg production cycle for *Daphnia magna*. These results indicate that EPA is enriched in the system, since the sum of EPA is higher than the initial values of the female. This is a pattern earlier described by von Elert (2002) in which the daphniids elongated other ω3 fatty acids to EPA. Nevertheless, our results showed that the egg production and release of eggs is the major drain of EPA from the females.

From these studies we conclude that *Daphnia* have a low saturation threshold for fatty acids. On the other hand the storage capacity for these nutrients is large for the daphniids. In contrast, phosphorus requirements of *Daphnia* are high and they have only limited possibilities to utilise storage of this compound. The fatty acid storage can be

reallocated to support growth and are also very important for reproduction. Hence, we found that egg production is a major drain of EPA from the females. These results strongly suggest that daphniids are much more vulnerable to periods of P-limitation than to essential fatty acid limitation, and questions the interpretation of those studies which found strong correlations between EPA content and *Daphnia* growth above the very low concentrations for saturation observed in this study.

Discussion

Discussion

Secondary production in aquatic systems is generally constrained by food quality or quantity. Whereas the importance of food quantity limitation has been the subject of many studies over the past decades, research into the effects and implications of food quality on secondary production in natural systems is relatively new. Differential quality differences of food particles is of great importance in aquatic systems not only because it affects biological production, and ultimately the production of commercially important species such as fish, but also because events such as harmful algal blooms affect the quality of the aquatic environment as a whole. Hence, to further the field of food quality research the main topic of this thesis was the qualitative constraints of secondary production of zooplankton, focusing on several aspects of elemental and biochemical food limitation for different zooplankton species. Especially the role of essential fatty acids and the interaction with phosphorus was studied. Furthermore, I investigated the implications of differences in feeding behaviour of different zooplankton guilds for the phytoplankton community, and through this, for the feeding conditions for other zooplankton guilds.

Effects of zooplankton interactions- Various zooplankton guilds affect the phytoplankton community differently. Calanoid copepods with their selective feeding pattern show sensitive food selection, preferring larger and better-quality particles. Hence, these particles are grazed to low abundance when copepods are the prominent grazer. Conversely, in an ecosystem dominated by non-selective filter feeders such as *Daphnia*, the grazing pressure is higher on smaller particles, and large particles prevail since they are ingested to a lesser extent (Sommer et al. 2001). This suggests that one guild could benefit from the presence of the other. The separate niches are occupied by cladocerans (e.g. *Daphnia*) and calanoid copepods in fresh water. In marine ecosystems, occupation of these different feeding niches is less clear. Most likely, appendicularians and cladocerans are the main metazoans feeding on smaller particles, and the larger particle niche is occupied by calanoid copepods. Not only do different guilds have different selectivities, they also have different requirements for different nutrients. Hence, feeding of different guilds and the resulting retention of different elements in animal tissue could induce different limitations for phytoplankton. For example, cladocerans and copepods differ considerably in phosphorus requirements, whereby daphniids and the marine cladocerans *Podon* sp. and *Evadne* sp. have lower C:P ratios compared to the copepods (30-60 versus

130 respectively) (from Gismervik 1997; Hessen and Lyche 1991). This implies that daphniids have to retain a higher proportion of phosphorus from their food in order to maintain the nutritional requirements, resulting in phytoplankton P limitations upon *Daphnia* grazing (Rothhaupt 1997).

A question that arose from differences in feeding mode and nutrient requirements is whether grazing by one guild could actually be positive for another guild, and as such, is a mutualistic relationship between copepods and cladocerans/appendicularians expected? Combining the data from chapter I and II, it can be concluded that growth of the freshwater cladoceran *Daphnia* was indeed positively affected by copepod manipulated seston (Fig. I, 1). On the other hand, there was no discernible effect on the freshwater copepods from seston manipulated by daphniids. That no effects was detected may have been a function of chosen method lacking sophistication (i.e. development in copepodite stages), and using the RNA:DNA technique that was developed for copepods (Chapter II) would probably have yielded more informative results

Calanus finmarchicus exhibited lower growth potential on copepod-manipulated seston (Fig. II, 4). However, the biomass of potential marine filter feeders (*e.g.* cladocerans and appendicularians) was too low in the experiments and so I could neither assess the effect of copepods on the filter feeders nor could I establish the effects of filter feeders on copepods. Nevertheless, copepod grazing induced the same pattern in marine as in freshwater ecosystems, i.e. an increase in small plankton of high quality (Fig. II, 7), which most likely should have been beneficial for cladocerans or appendicularians. This quality increase induced by copepod grazing was mostly dependent on the considerable increase of small sized diatoms, cryptophyceans and nanoflagellates. These particles were apparently not a good food source for *Calanus finmarchicus* since the growth potential of this species decreased (Fig. II, 6). Possibly, other, smaller copepods could have benefited from this, but due to the short duration of the experiments I did not see a numerical response of smaller copepods as a result of an increase in smaller cells. Nor did I observe density increases in filter feeders (something observed in the limnic mesocosms (Feuchtmayer, unpublished)). Either the experimental seeding density of other copepod species was too low and thus they could not be sampled effectively, or as their developmental stages are within the size range of particles selected by larger copepods, that they were also preyed upon. Interestingly, the production of smaller high quality algal cells did not seem to be grazed in the marine mesocosms.

Whether copepods are beneficially affected by an interaction with filter feeders remains to be resolved. More research is needed, and until then I can only speculate. There are a couple of positive indications supporting beneficial effects on copepods due to manipulation of non-selective filter feeders. One line of evidence to suggest that prior manipulation of phytoplankton by non-selective filter feeders has beneficial effects for copepods is that the smallest particles are removed by filter feeders, whilst larger particles are rejected. The remaining phytoplankton cells are of size range more suitable for efficient copepod grazing. For this to perpetuate, the larger algal cells must be able to mutiply (i.e. remain outside the filter feeding range). The strongest evidence against a positive effect is the high P requirements that some common cladocerans have. This is the case for daphniids, as well as *Podon* sp. and *Evadne* sp. exhibit C:P ratios of 30, 34 and 59 respectively, whereas copepods (marine and freshwater) have higher C:P ratios (C:P = 130) (from Gismervik 1997; Hessen and Lyche 1991). Thus, cladocerans should retain P and induce nutrient limitation, thereby reducing the growth of larger particles. Hence, a beneficial effect of filter-feeders on copepods is expected only in systems, where and at times when nutrient limitation is not important.

Elemental and biochemical limitations- Different zooplankton guilds have various requirements for different elements (Hessen and Lyche 1991; Sterner and Schulz 1998). There is no doubt that *Daphnia* has high requirements for phosphorus, whereas copepods, *Bosmina* and *Diaphanosma* have lower requirements. Hence, the likelihood that these zooplankters are affected by phosphorus limitation is lower (but see Villar-Argaiz and Sterner 2002). For these zooplankters it has been suggested that nitrogen limitation could be more important (Hessen and Lyche 1991; Sterner and Schulz 1998).

Differences in requirement for fatty acids between zooplankters is less well studied, although some indications exist suggesting copepods have a higher affinity for ω6, and cladocerans for ω3 fatty acids (e.g. Fig I 4). Most studies investigated the effects of fatty acids on daphniids (e.g. Ravet et al. 2003; von Elert 2002) and there are relatively few for copepods (e.g. von Elert and Stampfl 2000), many of them involving correlative evidence (Jónasdóttir and Kiorboe 1996). One major problem in studying the effects of fatty acids on copepods is finding a suitable algal food source. *Cryptomonas* sp. and *Rhodomonas* sp. are rich in PUFAs and generally good food sources for copepods. Further, *Chlamydomonas reinhartii* supports naupliar growth, but is a bad food source for copepodite development (Santer 1994). Typically *C. reinhartii* has a low PUFA content, and von Elert and Stampfl (2000) investigated if the poor food quality of *C. reinhartii* to
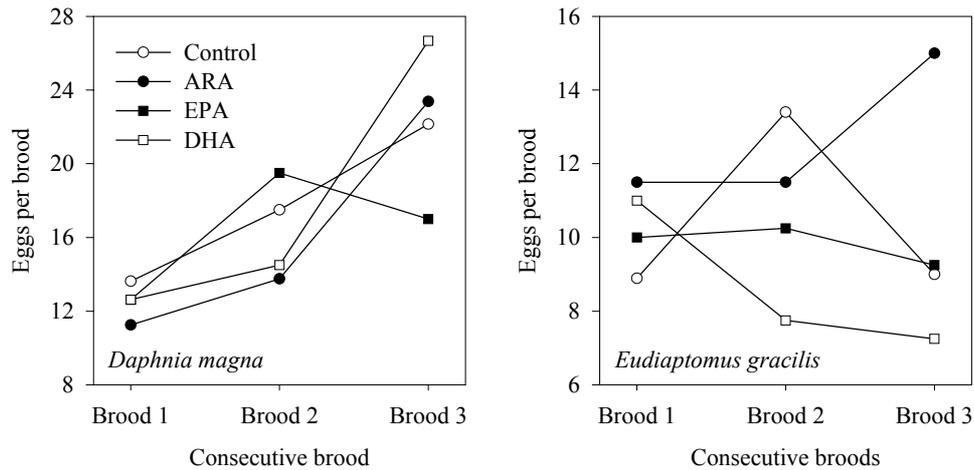
Fig. V, 1 Egg production of *Daphnia magna* and *Eudiaptomus gracilis* feeding on *Chlamydomonas reinhartii* with different additions of fatty acids. Control was un-enriched, in the other treatments ARA, EPA or DHA was added.

copepods was related to this deficiency. The ω3 fatty acid additions they used did not improve the food quality of *C. reinhartii* for copepods. In a similar set-up, I studied the effect of various fatty acids on *Eudiaptomus gracilis* and *D. magna* egg production and included a member of the ω6 fatty acid family, arachidonic acid (ARA). Overall I found no significant differences, however, there was a tendency that the ARA addition enhanced the copepod egg production in successive broods (Fig. V, 1). This pattern was not found with the other additions of fatty acids, and the broods remained stable or decreased. In contrast, no change over time was observed for daphniids, and there were no significant differences between the additions (Fig. V, 1). Although far from conclusive, this presents some evidence for differences between copepods and cladocerans in their requirement for different fatty acids.

For daphniids in general, it has been shown that especially ω3 fatty acids can influence growth (Fig. I, 5) (e.g. Müller-Navarra 1995b; von Elert 2002). Moreover, with a high requirement for phosphorus, daphniids are likely to encounter P, as well as, essential fatty acid (EFA) limitations. Plath and Boersma (2001) suggested that daphniids were co-limited by EFAs and P, yet in their study, the energy effect could not be ruled out. Conversely, in chapter III, I found that essential resources limited *Daphnia* growth sequentially, in concordance with von Liebig's law of the minimum. Severely P limited *Scenedesmus* was a poor food source either with or without the addition of EPA (Fig. III, 1; Fig. III, 2). Additions of P positively affected growth until the limitation shifted to EPA limitation, occurring at C:P of 350. Below this threshold EPA additions enhanced growth (Fig. III, 1; Fig. III, 2). Further, these two essential nutrients are used differently by

daphniids, with a much larger storage capacity for fatty acids than for phosphorus, and hence, daphniids accumulated excess EPA from the food (Fig. IV, 1). Such storage capability should be beneficial for daphniids, because it can be used as compensation during sub-optimal conditions. Indeed, I found that the storage of EPA was reallocated and could fully compensate growth during periods of poor food quality (Fig. IV, 4). Phosphorus on the other hand, was less efficiently reallocated, and growth decreased (Fig. IV, 4). Fatty acids are also important for reproduction, visualised as an accumulation of fat droplets preceding egg production (Tessier and Goulden 1982). I found that *Daphnia* females allocated high EPA concentrations to the eggs (Fig. IV, 3), and in fact it appears, that egg production is the major drain of EPA from females (Fig. IV, 5).

The above interactions of phosphorus and EPA provide strong laboratory evidence that only one resource at a time can limit *Daphnia* growth. It remains to be seen, how these results translate in natural conditions. For example, P limited algae have an altered morphology and biochemistry, such as a thicker cell wall (Tillberg et al. 1984b), and thus become less digestible for zooplankters (van Donk and Hessen 1993; van Donk et al. 1997). Besides this, several laboratory studies found that P limited algae increased in fatty acid content, but that the content of the EFAs decreased (Boersma 2000; Müller-Navarra 1995a). Similar effects were found in a field study, where various polyunsaturated fatty acids were positively correlated to particulate P content (Ahlgren et al. 1997), but see Müller-Navarra et al. (2004). Overall these results are very interesting, firstly because P and EFA limitation separately are suggested to be the "most limiting nutrients" in the freshwater food quality controversy (see introduction). Secondly, the possibility that the level of P limitation actually influences the proportion of EFAs in algal cells (Ahlgren et al. 1997; Boersma 2000; Müller-Navarra 1995a; Weers and Gulati 1997). Thus, P availability affects zooplankton food quality directly in the concentration of P, and indirectly, by increasing the digestion resistance, and decreasing the proportion of EFA. Only a few studies have acknowledged the potential importance of P to EFA interactions on zooplankton growth (Boersma 2000; Müller-Navarra 1995a; Park et al. 2002). These studies cultured various algae under different P conditions and studied effects on C:P and fatty acid content, and the effects on *Daphnia* growth. Increasing P limitation led to lower fractions of EPA in algae. However, this decrease only had minor impact on *Daphnia* growth, explained by the low EPA requirements *Daphnia* have for growth. The EPA saturation threshold was previously suggested to be $0.8 \mu g$ EPA L^{-1} (Müller-Navarra 1995b), yet, I found a considerably lower threshold of $0.04 \mu g$ EPA L^{-1} (Table IV, 3).

Hence, in the study of Park et al. (2002) only severely P limited *Scenedesmus* sp. and *Synechococcus* sp. should have been directly limited by EPA. Nevertheless, ω3-PUFAs and the algal species were good predictors for *Daphnia* food quality, and certainly better than P as a predictor. Daphniids storage capacity for EFAs and the low saturation threshold could indicate that EFA is only rarely limiting growth in the field.

    Conclusion and outlook – Various guilds of zooplankton have different feeding preferences that alter the phytoplankton community. I found that plankton manipulated by the feeding of calanoid copepods had a positive effect on *Daphnia* growth, but, the reciprocate effect on copepods was less clear. Phosphorus and EPA represent essential resources for *Daphnia*, as such, cannot be substituted for each other. Daphniids store essential fatty acids, and use them enhance the reproductive outcome or to compensate growth during sub-optimal food conditions.

    Several questions regarding the importance of food quality for zooplankton remain to be solved. One of the most important questions to be solved is whether laboratory results are applicable to field conditions. The knowledge of the importance of food quality comes mainly from laboratory studies, where extreme food qualities easily can be manipulated. Although the evidence are strong, it is still unclear whether the same results could be found in a natural environment, where variations are less extreme and more parameters are interacting (see above). To overcome this, laboratory techniques have to be applied to field experiments, e.g. in mesocosm experiments, by adding various nutrients and studying the effects on zooplankton growth. This would provide stronger evidence compared to the more common correlative indications. Some studies incorporated mesocosm techniques, and somewhat controversial results were found compared to laboratory studies (Boersma and Stelzer 2000). DeMott and Tessier (2002) described a technique to assess the resource limitation daphniids face in the field. Natural lake seston was enriched and manipulated, and the consequential impacts on *Daphnia* studied. With this method it is possible to determine whether energy, phosphorus, essential fatty acid or digestion resistance limit *Daphnia* growth. Hence, in order to solve the food quality controversy (see introduction), and to really understand which compounds are "most limiting" and when, studies should follow a similar protocol. Moreover, in future studies, not only the single limitations, but also the interactions of essential nutrients should be incorporated. For example phosphorus concentrations can affect the content of essential fatty acids in algae, which in turn suggest interesting patterns of direct and indirect P limitations.

It is also important to understand the impacts of food quality limitation throughout the food web. It has been shown that the abundance of planktivorous fish has a major impact on nutrient recycling in aquatic food webs, and can be explained with the variable predation pressures on *Daphnia*. Daphniids in high abundance, generally drive phytoplankton to P limitation. However, under high predation from planktivorous fish the recycling of phosphorus increases, leading to a shift in phytoplankton limitation from P to N (Elser et al. 1996). Further interactions are also possible, because predators and prey have different nutritional requirements, and these generally increase at higher trophic levels. Hence, for the fish to maintain this high requirement, a large proportion of nutrients has to be retained, which leads to a direct stoichiometric resource limitation on the ecosystem due to lower nutrient recycling. Studies in these directions will increase our understanding of how the effects of essential resources interact between and within trophic levels and are necessary steps to in understanding ecosystem interactions.

Summary

This thesis focuses on the effects of essential nutrients on various zooplankters. Essential nutrients are non-substitutable and zooplankters, like all other consumers, depend on a satisfactory intake with their food. Especially at the animal-plant interface the composition of the food and the nutritional requirements of the herbivores are often not optimally adjusted. Consequently zooplankton regularly faces food sources of sub-optimal quality, and life history constraints are imposed on the zooplankters. There are several aspects determining the food quality for zooplankters. In this thesis, effects of particle size, nitrogen (N), phosphorus (P) and essential fatty acids are discussed. This is done not only in the static sense, investigating the effects of different food sources on zooplankters, but also by investigating the effects zooplankters have on their food, and through that on the quality of what they are consuming. For example the ingestible size range differs between different species and developmental stages of zooplankters. Moreover, the nutritional preferences differ between zooplankters, which might alter the recycling of nutrients. Hence, differences between zooplankters induce different pressures on the phytoplankton community, which in turn affects the food quality for the zooplankters present in the system.

The first aim of this thesis was to investigate how various zooplankters (copepods and daphniids) affect the phytoplankton community, and the consequential impacts on zooplankton growth. Calanoid copepods exhibit an active food selection with a preference for larger particles. Conversely, the general filter feeder *Daphnia* is restricted to grazing on smaller particles. These dissimilar feeding patterns by these guilds, induce different grazing pressures on the various sizes of phytoplankton. Hence, under copepod dominance larger particles decrease and as a consequence smaller particles becomes more abundant, whereas the opposite is found when *Daphnia* is the main grazer. These feeding patterns indicate that the two zooplankton guilds could represent separate niches (or at least niches with minor overlap). I investigated the effects of the two feeding patterns in freshwater and marine mesocosm studies. The mesocosms were stocked with different densities of copepods and in the freshwater study with a *Daphnia* gradient. I found that, daphniids grew faster on seston previous manipulated by copepods compared to seston that was not manipulated by meso-zooplankton. On the other hand, seston previously manipulated by daphniids provided a bad food source for *Daphnia*. The calanoid copepods appeared not to be affected by previous zooplankton handling, which might only be a reflection of using

too blunt a method. However, by using a more sensitive technique (RNA:DNA) on marine copepods I found that the growth potential decreased with increasing copepod densities.

Various food quality parameters were correlated to zooplankton growth. *Daphnia* growth was strongly correlated to the concentration of 20:4ω3, an essential fatty acid. In the marine mesocosm, *Calanus finmarchicus* growth potentials were correlated to both ω3:ω6 and C:N molar ratios. With *C. finmarchicus* this relationship was however directly in contrast to our expectations, indicating a lower growth potential on higher quality food. However, this relationship was explained by the increase of smaller plankton induced by copepod grazing. Thus, the *C. finmarchicus* growth potentials were strongly correlated to altered size distribution of the plankton, and the abundance of suitable sized food particles.

The second aim of this thesis was to determine how zooplankters handle different essential resources and if resources are equally important through ontogeny. In laboratory studies, the interaction of eicosapentaenoic acid (EPA) and phosphorus limitations on *Daphnia magna* life history was investigated. For these studies *Daphnia* was chosen for its ease of handling, and the wealth of information that is available already for this species. Phosphorus deficient *Scenedesmus obliquus* enriched with or without a single essential fatty acid, were used as food source. To study the impacts of different essential resources over a shifting limitation pressure, the algae were spiked with dissolved P. On low concentrations of phosphorus, *Daphnia* growth was retarded. Adding phosphorus increased the growth rates, until a threshold concentration when the growth reached an asymptote. When *Daphnia* growth was limited by phosphorus, addition of EPA had no impact. Only after the daphniids had reached the P threshold, EPA addition increased growth.

The third objective was to study the effects of fatty acids and phosphorus on *Daphnia* stoichiometry. By using fatty acids concentration gradients I found that daphniids have very low saturation thresholds for polyunsaturated fatty acids (PUFA). Moreover, various fatty acids were handled differently by the daphniids and PUFAs were preferably stored. However, also among the PUFAs allocation differences were found, and especially EPA was allocated to the eggs, which should improve the reproductive output. This allocation pattern by *Daphnia* resulted in emptying the EPA storage. However, the phosphorus allocation to the eggs was not dependent on the P content of the females, which always allocated the same amount of P to the eggs regardless of her own P condition. Hence, daphniids are not able to enrich their eggs with phosphorus in order to improve their reproductive output. I found that storage of EPA, but not phosphorus, could

easily be reallocated for growth. Hence, daphniids rich in EPA could sustain poor food qualities over days without decreasing growth rates.

Zusammenfassung

Diese Arbeit behandelt die Auswirkungen wichtiger Nährstoffe auf verschiedene Zooplankter. Essentielle Nährstoffe sind nicht ersetzbar und Zooplankter sind, genauso wie alle anderen Konsumenten, von einer ausreichenden Aufnahme mit ihrem Futter abhängig. Vor allem bei der Tier-Pflanzen Schnittstelle ist die Zusammensetzung des Futters und die Nährstoffanforderungen der Herbivoren oft nicht optimal eingestellt. Deshalb stehen Zooplankter oft Nahrungsquellen von sub- optimaler Qualität gegenüber, die sie in ihrer Entwicklung beeinträchtigen. Es gibt einige Aspekte, die entscheidend sind für die Futterqualität des Zooplanktons. In dieser Arbeit werden Effekte von Partikelgröße, Stickstoff (N), Phosphor (P) und essentielle Fettsäuren diskutiert. Dies wurde nicht nur im statischen Sinn betrachtet, indem man die Effekte unterschiedlicher Futterquellen erforscht, sondern auch die Effekte des Zooplanktons auf Ihre Nahrung, und dadurch auf die Qualität der konsumierten Nahrung. Zum Beispiel unterscheiden sich verschiedene Arten und Entwicklungsstadien des Zooplanktons in den für sie ingestierbaren Futtergrößen. Außerdem unterscheiden sich Zooplankter in ihren Nährstoffpräferenzen, und können das Recycling der Nährstoffe ändern. Folglich üben Unterschiede im Zooplankton unterschiedlichen Druck auf die Phytoplanktongemeinschaft aus, während diese wiederum die Nahrungsqualität für die vorhandenen Zooplankter beeinflussen.

Das erste Ziel dieser dieser Arbeit war, herauszufinden wie verschiedene Zooplankter (Copepoden und Daphnien) die Phytoplanktongemeinschaft beeinflussen, sowie die daraus folgenden Einflüsse auf das Zooplanktonwachstum zu erforschen. Calanoide Copepoden führen eine aktive Futterselektion mit einer Präferenz für größere Partikel durch. Andererseits ist der Filtrierer *Daphnia* auf das Grazing kleinerer Teilchen begrenzt. Diese unterschiedlichen Mechanismen der Nahrungsaufnahme der verschiedenen Gilde verursachen unterschiedlichen Fraßdruck auf die verschiedenen Phytoplanktongrößen. Folglich werden große Partikel reduziert wenn Copepoden dominieren und kleine Partikel sind häufiger vorhanden, während das Gegenteil der Fall ist, wenn Daphnien als Hauptgrazer vorhanden sind. Dieses Fraßmuster lässt vermuten, dass die beiden Zooplankton Gilde verschiedene Nischen repräsentieren (oder zumindest Nischen mit geringer Überlappung). Ich habe die Effekte der beiden Fraßmuster in Mesokosmenstudien im Süß- und Salzwasser erforscht. Die Mesokosmen wurden mit unterschiedlichen Dichten von Copepoden und im Süßwasser zusätzlich mit einem

Daphniengradienten beimpft. Ich fand heraus, dass Daphnien schneller mit Seston wuchsen, die von Copepoden manipuliert wurden, als mit Partikeln die nicht durch Mesozooplankton manipuliert waren. Andererseits stellte Seston, das vorher von Daphnien manipuliert wurde, eine schlechte Futterquelle für Daphnien dar. Die calanoiden Copepoden schienen nicht durch die vorhergehende Behandlung des Futters durch Zooplankton beeinflußt zu werden, was auch durch die Anwendung einer zu ungenauen Methode verursacht worden sein könnte. Indem ich eine empfindlichere Technik (RNA:DNA) bei marinen Copepoden anwandte, fand ich heraus, dass das Wachstum potentiell mit zunehmenden Copepodendichten abnahm.

Verschiedene Futterqualitätsparameter wurden mit dem Wachstum des zooplanktons korreliert. Daphnienwachstum war stark abhängig von der Konzentration von 20:4ω3, einer essentiellen Fettsäure. Bei dem marinen Mesocosmenversuch wurde das Wachstumspotential von *Calanus finmarchicus* mit ω3:ω6 und C:N Verhältnissen (molar) korreliert. Dieses Verhältnis war jedoch genau umgekehrt zu unseren Erwartungen für *C. finmarchicus*, und zeigte ein niedrigeres Wachstumspotential bei höherer Futterqualität. Dieses Verhältnis konnte durch die von Copepoden induzierte Zunahme des kleinen Planktons erklärt werden. Daher waren die Wachstumspotentiale von *C. finmarchicus* stark korreliert mit der veränderten Größenverteilung des Planktons, und der Häufigkeit geeigneter Futterpartikel.

Im zweiten Teil der vorliegenden Arbeit wurde untersucht wie Zooplankter mit unterschiedlichen essentiellen Ressourcen umgehen und welchen Stellenwert diese Ressourcen während der Ontogenese haben. In Laborexperimenten wurde die Interaktion von Eicosapentaensäure (EPA) und Phosphorlimitation auf die Entwicklung von *Daphnia magna* untersucht. *Daphnia* wurde für diese Untersuchungen gewählt, weil diese Art einfach in der Handhabung ist und eine große Menge an Informationen über sie vorliegt. Als Futter dienten phosphor-limitierte *Scenedesmus obliquus*, von denen ein Teil mit einer essentiellen Fettsäure angereichert war. Um den Einfluss von verschiedenen essentiellen Ressourcen bei sich änderndem Limitationsdruck zu untersuchen, wurden Algen mit gelöstem Phosphor angereichert. Das Wachstum von *Daphnia* war bei niedrigen Phosphorkonzentrationen verringert. Die Zugabe von Phosphor erhöhte die Wachstumsraten bis zu einer Schwellenkonzentration, ab der das Wachstum asymptotisch verlief. Bei Phosphor-limitiertem Wachstum der Daphnien hatte eine Zugabe von EPA keinen Effekt. Die Zugabe von EPA führte nur dann zu einem erhöhten Wachstum, wenn die Daphnien zuvor die Phosphor-Schwellenkonzentration erreicht hatten.

Als dritter Aspekt dieser Arbeit wurden die Effekte von Fettsäuren und Phosphor auf die Stöchiometrie von Daphnien untersucht. Die Verwendung von Fettsäure-Konzentrationsgradienten zeigte, dass die Daphnien sehr niedrige Sättigungsgrenzen für mehrfach ungesättigte Fettsäuren hatten (polyunsaturated fatty acids = PUFA). Zudem verarbeiteten Daphnien verschiedene Fettsäuren unterschiedlich und speicherten vorzugsweise PUFAs. Auch in der Anreicherung von PUFAs konnten Unterschiede gefunden werden. Besonders EPA wurde in Eiern akkumuliert, was zu einer Steigerung des Reproduktionserfolges führen sollte. Diese Art der Anreicherung bei *Daphnia* führte zu einer Erschöpfung des EPA Vorrats. Im Gegensatz dazu war die Akkumulation von Phosphor in Eiern unabhängig vom P-Gehalt der Weibchen, die immer dieselbe Menge an Phosphor in den Eiern akkumulierten. Das zeigte, dass Daphnien nicht in der Lage sind ihre Eier mit Phosphor anzureichern, um den Reproduktionserfolg zu erhöhen. Ich konnte feststellen, dass gespeichertes EPA im Gegensatz zu Phosphor für das Wachstum leicht wieder zur Verfügung gestellt werden konnte. Daher konnten Daphnien, die reich an EPA sind, schlechte Futterbedingungen einige Tage überdauern, ohne dass sich die Wachstumsraten verringerten.

References

Ahlgren, G., W. Goedkoop, H. Markensten, L. Sonesten, and M. Boberg. 1997. Seasonal variations in food quality for pelagic and benthic invertebrates in lake Erken - the role of fatty acids. Freshwater Biology 38**:** 555-570.

Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson. 1994. The cell. Garland publishing, Inc.

Anderson, T. R., M. Boersma, and D. Raubenheimer. 2004. Stoichiometry: linking elements to biochemicals. Ecology *in press*.

Anderson, T. R., and D. W. Pond. 2000. Stoichiometric theory extended to micronutrients: Comparison of the roles of essential fatty acids, carbon, and nitrogen in the nutrition of marine copepods. Limnology and Oceanography 45**:** 1162-1167.

Arts, M. T., R. D. Robarts, and M. S. Evans. 1993. Energy reserve lipids of zooplanktonic crustaceans from on oligotrophic saline lake in relation to food resources and temperature. Canadian Journal of Fisheries & Aquatic Sciences 50**:** 2404-2420.

Båmstedt, U. 1983. RNA concentration in zooplankton - seasonal-variation in boreal species. Marine Ecology Progress Series 11**:** 291-297.

Båmstedt, U., and H. R. Skjoldal. 1980. RNA concentration of zooplankton - relationship with size and growth. Limnology and Oceanography 25**:** 304-316.

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

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

Bedo, A. W., J. L. Acuna, D. Robins, and R. P. Harris. 1993. Grazing in the micron and the submicron particle size range - the Case of *Oikopleura dioica* (Appendicularia). Bulletin of Marine Science 53**:** 2-14.

Bell, G. 1984. Measuring the cost of reproduction. 2. The correlation structure of the life tables of five freshwater invertebrates. Evolution 38**:** 314-326.

Bengtsson, J. 1986. Life histories and interspecific competition between three *Daphnia* species in rockpools. Journal of Animal Ecology 55**:** 641-655.

Bern, L. 1994. Particle selection over a broad size range by crustacean zooplankton. Freshwater Biology 32**:** 105-112.

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

Boersma, M. 1995. Competition in natural populations of *Daphnia*. Oecologia 103**:** 309-318.

Boersma, M. 2000. The nutritional quality of P-limited algae for *Daphnia*. Limnology and Oceanography 45**:** 1157-1161.

Boersma, M., and C. Kreutzer. 2002. Life at the edge: Is food quality really of minor importance at low quantities? Ecology 83**:** 2552-2561.

Boersma, M., C. Schöps, and E. McCauley. 2001. Nutritional quality of seston for the freshwater herbivore *Daphnia galeata x hyalina:* biochemical versus mineral limitations? Oecologia 129**:** 342-348.

Boersma, M., and C. P. Stelzer. 2000. Response of a zooplankton community to the addition of unsaturated fatty acids: an enclosure study. Freshwater Biology 45**:** 179-188.

Boersma, M., and J. Vijverberg. 1994. The use of carbon to length regressions as a tool to estimate the degree of food limitation in the field. Internationale Vereinigung für Theoretische und Angewandte Limnologie. Verhandlungen 25**:** 2392-2394.

Bradley, M. C., N. Perrin, and P. Calow. 1991. Energy allocation in the cladoceran *Daphnia magna* Straus, under starvation and refeeding. Oecologia 86**:** 414-418.

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

Brett, M. T. 1993. Resource quality effects on *Daphnia longispina* offspring fitness. Journal of Plankton Research 15**:** 403-412.

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

Brett, M. T., D. C. Müller-Navarra, and S. K. Park. 2000. Empirical analysis of the effect of phosphorus limitation on algal food quality for freshwater zooplankton. Limnology and Oceanography 45**:** 1564-1575.

Butler, N. M., C. A. Suttle, and W. E. Neill. 1989. Discrimination by freshwater zooplankton between single algal cells differing in nutritional status. Oecologia 78: 368-372.

Calder, P. C. 2001. N-3 polyunsaturated fatty acids, inflammation and immunity: pouring oil on troubled waters or another fishy tale? Nutrition Research 21: 309-341.

Cavaletto, J. F., H. A. Vanderploeg, and W. S. Gardner. 1989. Wax esters in 2 species of freshwater zooplankton. Limnology and Oceanography 34: 785-789.

Checkley, D. M., and L. C. Entzeroth. 1985. Elemental and isotopic fractionation of carbon and nitrogen by marine, planktonic copepods and implications to the marine nitrogen cycle. Journal of Plankton Research 7: 553-568.

Ciros-Peréz, J., M. J. Carmona, and M. Serra. 2001. Resource competition between sympatric sibling rotifer species. Limnology and Oceanography 46: 1511-1523.

Clemmesen, C. 1993. Improvements in the fluorometric determination of the RNA and DNA Content of Individual Marine Fish Larvae. Marine Ecology Progress Series 100: 177-183.

Clemmesen, C. 1994. The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae - laboratory calibration. Marine Biology 118: 377-382.

Clemmesen, C. 1996. Importance and limits of RNA/DNA ratios as a measure of nutritional condition in fish larvae, p. 67-82. *In* Y. Watanabe, Y. Yamashita and Y. Oozeki [eds.], Survival strategies in early life stages of marine resources. A.A. Balkema.

Clemmesen, C. and others 2003. Variability in condition and growth of Atlantic cod larvae and juveniles reared in mesocosms: environmental and maternal effects. Journal of Fish Biology 62: 706-723.

Collins, M. 1978. Algal toxins. Microbiological reviews 42: 725-746.

Cook, H. W. 1985. Fatty acid desaturation and chain elongation in eucaryotes, p. 181-212. *In* D. E. Vance and J. E. Vance [eds.], Biochemistry of lipids and membranes. The Benjamin/Cummings Publishing Company, Inc.

Cowles, T. J., R. J. Olson, and S. W. Chisholm. 1988. Food selection by copepods discrimination on the basis of food quality. Marine Biology 100: 41-50.

Cuddington, K. M., and E. McCauley. 1994. Food-dependent aggregation and mobility of the water fleas *Ceriodaphnia dubia* and *Daphnia pulex*. Canadian Journal of Zoology 72: 1217-1226.

Darchambeau, F., P. J. Faerovig, and D. O. Hessen. 2003. How *Daphnia* copes with excess carbon in its food. Oecologia 136: 336-346.

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

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

DeMott, W. R. 1995. The influence of prey hardness on *Daphnia's* selectivity for large prey. Hydrobiologia 307: 127-138.

DeMott, W. R. 1998. Utilization of a cyanobacterium and a phosphorus-deficient green alga as complementary resources by daphnids. Ecology 79: 2463-2481.

DeMott, W. R. 2003. Implications of element deficits for zooplankton growth. Hydrobiologia 491: 177-184.

DeMott, W. R., and R. D. Gulati. 1999. Phosphorus limitation in *Daphnia*: Evidence from a long term study of three hypereutrophic Dutch lakes. Limnology and Oceanography 44: 1557-1564.

DeMott, W. R., R. D. Gulati, and K. Siewertsen. 1998. Effects of phosphorus-deficient diets on the carbon and phosphorus balance of *Daphnia magna*. Limnology and Oceanography 43: 1147-1161.

DeMott, W. R., R. D. Guldati, and E. Van Donk. 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.

DeMott, W. R., and W. C. Kerfoot. 1982. Competition among cladocerans: nature of the interaction between *Bosmina* and *Daphnia*. Ecology 63: 1949-1966.

DeMott, W. R., and F. Moxter. 1991. Foraging on cyanobacteria by copepods - responses to chemical defenses and resource abundance. Ecology 72: 1820-1834.

DeMott, W. R., and D. C. Müller-Navarra. 1997. The importance of highly unsaturated fatty acids in zooplankton nutrition - evidence from experiments with *Daphnia*, a cyanobacterium and lipid emulsions. Freshwater Biology 38: 649-664.

DeMott, W. R., and A. J. Tessier. 2002. Stoichiometric constraints vs. algal defenses: Testing mechanisms of zooplankton food limitation. Ecology 83: 3426-3433.

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

Dussart, B. H., and D. Defaye. 2001. Introduction to the Copepoda, 2nd ed. Backhuys Publishers.

Elendt, B. P., and W. R. Bias. 1990. Trace nutrient deficiency in *Daphnia magna* cultured in standard medium for toxicity testing. Effects of the optimization of culture conditions on life history parameters of *D. magna*. Water Research 24: 1157-1167.

Elser, J. J., D. R. Dobberfuhl, N. A. Mackay, and J. H. Schampel. 1996. Organism size, life history, and N:P stoichiometry. BioScience 46**:** 674-684.

Elser, J. J. and others 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature 408**:** 578-580.

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

Elser, J. J., K. Hayakawa, and J. Urabe. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. Ecology 82**:** 898-903.

Faerovig, P. J., and D. O. Hessen. 2003. Allocation strategies in crustacean stoichiometry: the potential role of phosphorus in the limitation of reproduction. Freshwater Biology 48**:** 1782-1792.

Flöder, S., and U. Sommer. 1999. Diversity in planktonic communities: An experimental test of the intermediate disturbance hypothesis. Limnology and Oceanography 44**:** 1114-1119.

Frangoulos, M., C. Guisande, I. Maneiro, I. Riveiro, and J. Franco. 2000. Short-term and long-term effects of the toxic dinoflagellate *Alexandrium minutum* on the copepod *Acartia clausi*. Marine Ecology Progress Series 203**:** 161-169.

Frost, B. W. 1972. Effects of size and concentration of food particles on feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnology and Oceanography 17**:** 805-815.

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

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

Gliwicz, Z. M. 1994. Retarded growth of cladoceran zooplankton in the presence of a copepod predator. Oecologia 97**:** 458-461.

Gliwicz, Z. M., and G. Umana. 1994. Cladoceran body size and vulnerability to copepod predation. Limnology and Oceanography 39**:** 419-424.

Goulden, C. E., R. E. Moeller, J. N. McNair, and A. R. Place. 1999. Lipid dietary dependencies in zooplankton. *In* M. T. Arts and B. C. Wainman [eds.], Lipids in freshwater ecosystems. Springer.

Goulden, C. E., and A. R. Place. 1990. Fatty Acid Synthesis and Accumulation Rates in Daphniids. Journal of Experimental Zoology 256**:** 168-178.

Goulden, C. E., and A. R. Place. 1993. Lipid accumulation and allocation in daphnid cladocera. Bulletin of Marine Science 53**:** 106-114.

Grasshoff, K., M. Ehrhardt, and K. Kremling. 1983. Methods of seawater analysis, 2nd edition ed. Verlag Chemie.

Guisande, C., I. Maneiro, and I. Riveiro. 1999. Homeostasis in the essential amino acid composition of the marine copepod *Euterpina acutifrons*. Limnology and Oceanography 44**:** 691-696.

Guisande, C., I. Riveiro, and I. Maneiro. 2000. Comparisons among the amino acid composition of females, eggs and food to determine the relative importance of food quantity and food quality to copepod reproduction. Marine Ecology Progress Series 202**:** 135-142.

Hart, R. C., and B. Santer. 1994. Nutritional suitability of some uni-algal diets for freshwater calanoids: unexpected inadequacies of commonly used edible greens and others. Freshwater Biology 31**:** 109-116.

Hebert, P. D. N. 1977. Niche overlap among species in *Daphnia carinata* complex. Journal of Animal Ecology 46**:** 399-409.

Hessen, D. O. 1990. Niche overlap between herbivorous cladocerans; the role of food quality and habitat homogeneity. Hydrobiologia 190**:** 61-78.

Hessen, D. O. 1992. Nutrient Element Limitation of Zooplankton Production. American Naturalist 140**:** 799-814.

Hessen, D. O., N. E. W. Alstad, and L. Skardal. 2000. Calcium limitation in *Daphnia magna*. Journal of Plankton Research 22**:** 553-568.

Hessen, D. O., and A. Lyche. 1991. Interspecific and intraspecific variations in zooplankton element composition. Archiv für Hydrobiologie 121**:** 343-353.

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

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

Huisman, J., and F. J. Weissing. 1999. Biodiversity of plankton by species oscillations and chaos. Nature 402**:** 407-410.

Hutchinson, G. E. 1961. The paradox of the plankton. American Naturalist 95**:** 137-145.

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

Ingle, L., T. R. Wood, and A. M. Banta. 1937. A study of longevity, growth, reproduction and heart rate in *Daphnia longispina* as influenced by limitations in quantity of food. Journal of Experimental Zoology 76**:** 325-352.

Interlandi, S. J., and S. S. Kilham. 2001. Limiting resources and the regulation of diversity in phytoplankton communities. Ecology 82**:** 1270-1282.

Jang, M. H., K. Ha, G. J. Joo, and N. Takamura. 2003. Toxin production of cyanobacteria is increased by exposure to zooplankton. Freshwater Biology 48**:** 1540-1550.

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

Jónasdóttir, S. H. 1994. Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. Marine Biology 121**:** 67-81.

Jónasdóttir, S. H., D. Fields, and S. Pantoja. 1995. Copepod egg production in Long Island Sound, USA, as a function of the chemical composition of seston. Marine Ecology Progress Series 119**:** 87-98.

Jónasdóttir, S. H., and T. Kiorboe. 1996. Copepod recruitment and food composition - do diatoms affect hatching success. Marine Biology 125**:** 743-750.

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

Kaartvedt, S. 2000. Life history of *Calanus finmarchicus* in the Norwegian Sea in relation to planktivorous fish. ICES Journal of Marine Science 57**:** 1819-1824.

Kerfoot, W. C. 1977. Implications of Copepod Predation. Limnology and Oceanography 22**:** 316-325.

Kerfoot, W. C., and K. L. Kirk. 1991. Degree of taste discrimination among suspension-feeding cladocerans and copepods - Implications for detritivory and herbivory. Limnology and Oceanography 36**:** 1107-1123.

Kim, S. W., T. Onbe, and Y. H. Yoon. 1989. Feeding habits of marine cladocerans in the Inland Sea of Japan. Marine Biology**:** 313-318.

Kiorboe, T. 1989. Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. Marine Ecology Progress Series 55**:** 229-234.

Kleppel, G. S., and C. A. Burkart. 1995. Egg production and the nutritional environment of *Acartia tonsa*: The role of food quality in copepod nutrition. ICES Journal of Marine Science 52**:** 297-304.

Klüttgen, B., U. Dulmer, M. Engels, and H. T. Ratte. 1994. ADaM, an artificial freshwater for the culture of zooplankton. Water Research 28**:** 743-746.

Laabir, M., S. A. Poulet, R. P. Harris, D. W. Pond, A. Cueff, R. N. Head, and A. Ianora. 1998. Comparative study of the reproduction of *Calanus helgolandicus* in well-mixed and seasonally stratified coastal waters of the western English Channel. Journal of Plankton Research 20**:** 407-421.

Lampert, W. 1981. Inhibitory and toxic effects of blue-green algae on *Daphnia*. Internationale Revue der gesamten Hydrobiologie 66**:** 285-298.

Lampert, W. 1982. Further studies on the inhibitory effect of the toxic blue green *Microcystis aeruginosa* on the filtering rate of zooplankton. Archiv für Hydrobiologie 95**:** 207-220.

Lampert, W. 1987. Feeding and nutrition in *Daphnia*. Memorie dell'Istituto Italiano di Idrobiologia 45**:** 143-192.

Lampert, W., K. O. Rothhaupt, and E. von Elert. 1994. Chemical induction of colony formation in a green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). Limnology and Oceanography 39**:** 1543-1550.

Lampert, W., and U. Sommer. 1997. Limnoecology. Oxford University Press, Inc.

Lampert, W., and I. Trubetskova. 1996. Juvenile growth rate as a measure of fitness in *Daphnia*. Functional Ecology 10**:** 631-635.

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

Lynch, M., and R. Ennis. 1983. Resource availability, maternal effects, and longevity. Experimental Gerontology 18**:** 147-165.

Machlin, L. J. 1973. Phosphorus in human nutrition. *In* E. J. Griffith, A. Beeton, J. M. Spencer and D. T. Mitchell [eds.], Environmental phosphorus handbook. John Wiley & sons. Inc.

Marker, T., P. Andreassen, E. Arashkewich, and B. W. Hansen. 2003. Lipid deposition and sexual maturation in cohorts of *Calanus finmarchicus* (Gunnerus) originating from Bergen (60 degrees N) and Tromso (69 degrees N) reared in Tromso, Norway. Marine Biology 143**:** 283-296.

Martínez-Jerónimo, F., R. Villaseñor, G. Rios, and F. Espinosa. 1994. Effect of food type and concentration on the survival, longevity, and reproduction of *Daphnia magna*. Hydrobiologia 287**:** 207-214.

Mathews, C. K., and K. E. van Holde. 1996. Biochemistry, 2nd ed. Benjamin/Cummings Publishing company inc.

Matveev, V. F. 1983. Estimating competition in cladocerans using data on dynamics of clutch size and population density. Internationale Revue der Gesamten Hydrobiologie 68**:** 785-798.

McEvoy, L. A., T. Naess, J. G. Bell, and O. Lie. 1998. Lipid and fatty acid composition of normal and malpigmented atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. Aquaculture 163**:** 237-250.

Müller-Navarra, D. C. 1995a. Biochemical versus mineral limitation in *Daphnia*. Limnology and Oceanography 40**:** 1209-1214.

Müller-Navarra, D. C. 1995b. Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. Archiv für Hydrobiologie 132**:** 297-307.

Müller-Navarra, D. C., M. T. Brett, S. Park, S. Chandra, A. P. Ballantyne, E. Zorita, and C. R. Goldman. 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. Nature 427**:** 69-72.

Nakata, K. 1990. Abundance of nauplii and protein synthesis activity of adult female copepods in the Kuroshio front during the Japanese sardine spawning season. Journal of the Oceanogrphical Society of Japan 46**:** 219-229.

Nakata, K., H. Nakano, and H. Kikuchi. 1994. Relationship between egg productivity and RNA/DNA ratio in *Paracalanus* sp. in the frontal waters of the Kuroshio. Marine Biology 119**:** 591-596.

Nizan, S., C. Dimentman, and M. Shilo. 1986. Acute toxic effects of the cyanobacterium *Microcystis aeruginosa* on *Daphnia magna*. Limnology and Oceanography 31**:** 497-502.

O'Connors, H. B., L. F. Small, and P. L. Donaghay. 1976. Particle-size modification by 2 size classes of estuarine copepod *Acartia clausi*. Limnology and Oceanography 21**:** 300-308.

Olsen, Y. 1999. Lipids and essential fatty acids in aquatic food webs: what can freshwater ecologists learn from mariculture?, p. 161-202. *In* M. T. Arts and B. C. Wainman [eds.], Lipids in Freshwater Ecosystems.

Paffenhöfer, G. A. 2002. An assessment of the effects of diatoms on planktonic copepods. Marine Ecology Progress Series 227**:** 305-310.

Paffenhöfer, G. A., and K. B. Van Sant. 1985. The feeding response of a marine planktonic copepod to quantity and quality of particles. Marine Ecology Progress Series 27**:** 55-65.

Park, S., M. T. Brett, D. C. Müller-Navarra, and C. R. Goldman. 2002. Essential fatty acid content and the phosphorus to carbon ratio in cultured algae as indicators of food quality for *Daphnia*. Freshwater Biology 47**:** 1377-1390.

Plath, K., and M. Boersma. 2001. Mineral limitation of zooplankton: Stoichiometric constraints and optimal foraging. Ecology 82**:** 1260-1269.

Porter, K. G. 1976. Enhancement of algal growth and productivity by grazing zooplankton. Science 192**:** 1332-1334.

Poulet, S. A., A. M. Hapette, R. B. Cole, and J. C. Tabet. 1989. Vitamin C in marine copepods. Limnology and Oceanography 34**:** 1325-1331.

Ravet, J. L., M. T. Brett, and D. C. Müller-Navarra. 2003. A test of the role of polyunsaturated fatty acids in phytoplankton food quality for *Daphnia* using liposome supplementation. Limnology and Oceanography 48**:** 1938-1947.

Reinikainen, M., M. Ketola, and M. Walls. 1994. Effects of the concentrations of toxic *Microcystis aeruginosa* and an alternative food on the survival of *Daphnia pulex*. Limnology and Oceanography 39**:** 424-432.

Reinikainen, M., J. A. O. Meriluoto, L. Spoof, and K. Harada. 2001. The toxicities of a polyunsaturated fatty acid and a microcystin to *Daphnia magna*. Environmental Toxicology 16**:** 444-448.

Richardson, K., S. H. Jónasdóttir, S. J. Hay, and A. Christoffersen. 1999. *Calanus finmarchicus* egg production and food availability in the Faroe-Shetland Channel and northern North Sea: October-March. Fisheries Oceanography 8**:** 153-162.

Rock, C. O., and J. E. Cronan. 1985. Lipid metabolism in procaryotes, p. 73-115. *In* D. E. Vance and J. E. Vance [eds.], Biochemistry of lipids and membranes. The Benjamin/Cummings Publishing Company, Inc.

Roff, D. A. 1992. The evolution of life histories. Chapman & Hall, New York, NY.

Rose, R. M., M. S. Warne, and R. P. Lim. 2000. Life history responses of the cladoceran *Ceriodaphnia cf. dubia* to variation in food concentration. Hydrobiologia 427**:** 59-64.

Rothhaupt, K. O. 1995. Algal nutrient limitation affects rotifer growth rate but not ingestion rate. Limnology and Oceanography 40**:** 1201-1208.

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 Zooplankton in Mesokosmosexperimenten. Diplomarbeit Christian-Albrechts-Universität, Kiel, Germany.

Saiz, E., A. Calbet, A. Fara, and E. Berdalet. 1998. RNA content of copepods as a tool for determining adult growth rates in the field. Limnology and Oceanography 43**:** 465-470.

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., and F. van den Bosch. 1994. Herbivorous nutrition of *Cyclops vicinus*: the effect of a pure algal diet on feeding, development, reproduction and life-cycle. Journal of Plankton Research 16**:** 171-195.

Schmidt, K., M. Koski, J. Engström-Öst, and A. Atkinson. 2002. Development of Baltic Sea zooplankton in the presence of a toxic cyanobacterium: a mesocosm approach. Journal of Plankton Research 24**:** 979-992.

Schulz, H. 1985. Oxidation of fatty acids, p. 116-142. *In* D. E. Vance and J. E. Vance [eds.], Biochemistry of lipids and membranes. The Benjamin/Cummings Publishing Company, Inc.

Sobral, O., C. Chastinet, A. Nogueira, A. M. V. M. Soares, F. Goncalves, and R. Ribeiro. 2001. In vitro development of parthenogenetic eggs: A fast ecotoxicity test with *Daphnia magna*? Ecotoxicology & Environmental Safety 50**:** 174-179.

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

Sommer, U., Z. M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG model of seasonal succession of planktonic events in fresh waters. Archiv für Hydrobiologie 106**:** 433-471.

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

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

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford.

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

Sterner, R. W. 1993. *Daphnia* growth on varying quality of *scenedesmus*: mineral limitation of zooplankton. Ecology 74**:** 2351-2360.

Sterner, R. W., D. D. Hagemeier, W. L. Smith, and R. F. Smith. 1993. Phytoplankton nutrient limitation and food quality for *Daphnia*. Limnology and Oceanography 38**:** 857-871.

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

Sterner, R. W., and K. L. Schulz. 1998. Zooplankton nutrition: Recent progress and a reality check. Aquatic Ecology 32**:** 261-279.

Sterner, R. W., and M. S. Schwalbach. 2001. Diel integration of food quality by *Daphnia*: luxury consumption by a freshwater planktonic herbivore. Limnology and Oceanography 46**:** 410-416.

Sundbom, M., and T. Vrede. 1997. Effects of fatty acid and phosphorus content of food on the growth, survival and reproduction of *Daphnia*. Freshwater Biology 38**:** 665-674.

Tessier, A. J., and C. E. Goulden. 1982. Estimating food limitation in cladoceran populations. Limnology and Oceanography 27**:** 707-717.

Tillberg, J. E., T. Barnard, and J. R. Rowley. 1984a. Phosphorus status and cytoplasmic structures in *Scenedesmus* (Chlorophyceae) under different metabolic regimes. Journal of Phycology 20**:** 124-136.

Tillberg, J. E., T. Barnard, and J. R. Rowley. 1984b. Phosphorus Status and Cytoplasmic Structures in *Scenedesmus obtusiusculus* Chlorophyceae under Different Metabolic Regimes. Journal of Phycology 20**:** 124-136.

Tilman, D. 1982. Resource competition and community structure. Princton University Press.

Townsend, D. W., and N. R. Pettigrew. 1997. Nitrogen limitation of secondary production on Georges Bank. Journal of Plankton Research 19**:** 221-235.

Turner, J. T., J. A. Lincoln, and A. D. Cembella. 1998. Effects of toxic and non-toxic dinoflagellates on copepod grazing, egg production and egg hatching success. *In* B. Reguera, J. Blanco, M. L. Fernandez and T. Wyatt [eds.], Harmful Algae. Xunta de Galicia and Intergovernmental Oceanographic Commission of UNESCO.

Turner, J. T., and P. A. Tester. 1997. Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnology and Oceanography 42**:** 1203-1214.

Twombly, S., N. Clancy, and C. W. Burns. 1998. Life history consequences of food quality in the freshwater copepod *Boeckella triarticulata*. Ecology 79**:** 1711-1724.

Urabe, J., J. Clasen, and R. W. Sterner. 1997. Phosphorus limitation of *Daphnia* growth: is it real? Limnology and Oceanography 42**:** 1436-1443.

Urabe, J., and R. W. Sterner. 2001. Contrasting effects of different types of resource depletion on life-history traits in *Daphnia*. Functional Ecology 15**:** 165-174.

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

van Donk, E., and D. O. Hessen. 1993. Grazing resistance in nutrient-stressed phytoplankton. Oecologia 93**:** 508-511.

van Donk, E., M. Lürling, D. O. Hessen, and G. M. Lokhorst. 1997. Altered cell wall morphology in nutrient-deficient phytoplankton and its impact on grazers. Limnology and Oceanography 42**:** 357-364.

van Wazer, J. R. 1973. The compunds of phosphorus. *In* E. J. Griffith, A. Beeton, J. M. Spencer and D. T. Mitchell [eds.], Environmental phosphorus handbook. John Wiley & sons. Inc.

Vanni, M. J. 1986. Competition in zooplankton communities: suppression of small species by Daphnia pulex. Limnology & Oceanography 31**:** 1039-1056.

Viehoever, A., and I. Cohen. 1938. The responses of *Daphnia magna* to vitamin E. The American Journal of Pharmacy and the sciences supporting public health. 110**:** 297-315.

Villar-Argaiz, M., J. M. Medina-Sanchez, and P. Carrillo. 2002. Linking life history strategies and ontogeny in crustacean zooplankton: Implications for homeostasis. Ecology 83**:** 1899-1914.

Villar-Argaiz, M., and R. W. Sterner. 2002. Life history bottlenecks in *Diaptomus clavipes* induced by phosphorus-limited algae. Limnology and Oceanography 47**:** 1229-1233.

von Elert, E. 2002. Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. Limnology and Oceanography 47**:** 1764-1773.

von Elert, E., D. Martin-Creuzburg, and J. R. Le Coz. 2003. Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (*Daphnia galeata*). Proceedings of the Royal Society of London Series B-Biological Sciences 270**:** 1209-1214.

von Elert, E., and P. Stampfl. 2000. Food quality for *Eudiaptomus gracilis*: the importance of particular highly unsaturated fatty acids. Freshwater Biology 45**:** 189-200.

von Elert, E., and T. Wolffrom. 2001. Supplementation of cyanobacterial food with polyunsaturated fatty acids does not improve growth of *Daphnia*. Limnology and Oceanography 46**:** 1552-1558.

von Liebig, J. 1855. Die Grundsätze der Agrikulturchemie. Vieweg.

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

Vrede, T., J. Persson, and G. Aronsen. 2002. The influence of food quality (P:C ratio) on RNA : DNA ratio and somatic growth rate of *Daphnia*. Limnology and Oceanography 47**:** 487-494.

Wacker, A., and E. von Elert. 2001. Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. Ecology 82**:** 2507-2520.

Wagner, M., E. Durbin, and L. Buckley. 1998. RNA:DNA ratios as indicators of nutritional condition in the copepod *Calanus finmarchicus*. Marine Ecology Progress Series 162**:** 173-181.

Wagner, M. M., R. G. Campbell, C. A. Boudreau, and E. G. Durbin. 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., and U. Larsson. 1999. Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. Journal of Plankton Research 21**:** 2309-2321.

Weers, P. M. M., and R. D. Gulati. 1997. Growth and reproduction of *Daphnia galeata* in response to changes in fatty acids, phosphorus, and nitrogen in *Chlamydomonas reinhardtii*. Limnology and Oceanography 42**:** 1584-1589.

Weers, P. M. M., K. Siewertsen, and R. D. Gulati. 1997. Is the fatty acid composition of *Daphnia galeata* determined by the fatty acid composition of the ingested diet. Freshwater Biology 38**:** 731-738.

Williams, G. C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. American Naturalist 100**:** 687-690.

Williams, J. P., E. Maissan, K. Mitchell, and M. U. Khan. 1990. The manipulation of the fatty acid composition of glycerolipids in cyanobacteria using exogenous fatty acids. Plant & Cell Physiology 31**:** 495-504.

Wiltshire, K. H., M. Boersma, A. Möller, and H. Buhtz. 2000. Extraction of pigments and fatty acids from the green alga *Scenedesmus obliquus* (Chlorophyceae). Aquatic Ecology 34**:** 119-126.

Zehnder, A. A., and P. R. Gorham. 1960. Factor influencing the growth of *Microcystis aeruginosa* Kütz. emend. Elenk. Canadian Journal of Microbiology 6**:** 645-660.

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

Acknowledgements

First of all I thank my supervisor Maarten Boersma. You have the knowledge, the patience and the ability to inspire, and you were always there for discussions and to give comments. Winfried Lampert for accepting me as a PhD student and for continuous academic support.

This work would not have been possible without the help from the IfM-MPIL food-web-team, Ulrich Sommer, Maarten Boersma, Barbara Santer, Klaus Jürgens, Hans-Georg Hoppe, Klaus Gocke, Heidrun Feuchtmayr, Eckart Zöllner, Daniela Brepohl, Frank Sommer, Thomas Hansen, Regine Koppe, Julia Wohlers, Kerstin Maczassek and Andrea Saage. I also thank the Deutsche Forschungsgemeinschaft for financing my work (BO 1488/3-1), European Commission's programme (Improving Human Potential—Access to Research infrastructure) for the access to Trondheim Marine Systems Research Infrastructure in Hopavågen, Sletvik, Norway. Winfried Lampert, Jon Grey, Raquel Ortells, Karen Wiltshire, Lena Sivars Becker, Kirsten Kessler, Sonja Vernooij, Bernd Seidendorf, Bill DeMott, Petter Larsson and the *Daphnia*-club inspired stimulating discussions and gave comments on earlier versions of this thesis. I am alse very thankful to Heidrun Feuchtmayr and Martina Zeller who with short notice translated the summary into German.

Special thanks goes to Heinke Buhtz, for teaching me the fatty acid analysis procedure and Yvonne Funck, Dieter Albrecht, Sabine Flöder, Karin Wiedenhöft, Nadine Ryk, Heike Wardenga, Maren Volquardsen, Ilka Dankert and Anita Möller for laboratory assistance. Jon Grey, Heinke Buhtz and Anita Möller gave me the opportunity to roam in their laboratory and were always helpful. Martin Beutler and bbe-moldaenke for instructions in how to operate the FluoroProbe. Nils Tokle, Øystein Leiknes, Jon Værnes and Alexandra Neyts made our stay in Norway excellent. Thanks for assistance, administrative work, cooking skills, "gå på tur aldrig sur".

Heidrun Feuchtmayr has always been a great roommate, thanks for schooling me in Bavarian traditions. Eckart Zöllner, Kirsten Kessler, Isabel Klaiber and Heiko Petrich have introduced me to Schleswig-Holstein lifestyle. Thanks for all fun times and interesting cultural experiences. Ineke van der Veen opened the door to Germany and without you this journey and thesis would not have happened.

Thanks to all colleagues and friends - you've been great and you have made my stay in Plön a pleasure! Kajaking on the Trave, ice-creams at Cini, competitions for "free" coffee, PIMMS, Bohnen-Speck-Birnen, die Butter, Mischung impossible, Kartoffelkeller, Laphroaig, IKEA pencils and wine, Go-ke-tours, Tuesday friends and lomo, Ostsee-Therme, Berlin, LMNO, secchi-disc on the bottom of an enclosure, thunderstorm and copepods, Überhosen, Phoebe Buffay, the probe does not respond, BoKasper and "mit steht immer mit Dativ"!

I am especially grateful to my parents, my brother and my mother in law whom always been supportive and encouraging, and to my wife Lena for always being there.

Curriculum Vitae

Name: Claes Becker

Date of Birth: 11 of October 1970 in Skarpnäck Sweden

Nationality: Swedish

10/2000 – 05/2004 PhD-studies at the Max Planck Institute for Limnology, as a student of Christian-Albrechts-Universität, Kiel, Germany

09/1999 – 03/2000 Master of Science in Biology at Uppsala University, Sweden; Title: Effects of labile substrates on the utilisation and mineralisation of aquatic humic matter.

09/1995 – 09/1999 Studies in Biology at Uppsala University, Sweden in following topics: Limnology, Eco-toxicology, Ecology, Chemistry, Botany

Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation selbstständig verfasst 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 Prüfungsverfahrens vorgelegen hat.

The separate chapters are submitted for publication in international scientific journals.

Chapter 1   Becker, C., H. Feuchtmayr, D. Brepohl, B. Santer and M. Boersma. *in press*. Differential impacts of copepods and cladocerans on lake seston, and resulting effects on zooplankton growth. Hydrobiologia.

Chapter 2   Becker, C., D. Brepohl, H. Feuchtmayr, E. Zöllner, F. Sommer, C. Clemmesen, U. Sommer, and M. Boersma. *submitted*. Impacts of copepods on marine seston and resulting effects on *Calanus finmarchicus* RNA:DNA ratios.

Chapter 3   Becker, C., and M. Boersma. 2003. Resource quality effects on life histories of *Daphnia*. Limnology and Oceanography 48: 700-706.

Chapter 4   Becker, C., and M. Boersma. *submitted*. Differential impacts of phosphorus and fatty acids on *Daphnia* growth and reproduction.

Kiel, 17.03.2004 …………………………..……….