

**Fatty acids distribution in marine, brackish and freshwater  
plankton during mesocosm experiments**

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# **C H A P T E R 1**

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## Chapter 1

### General Introduction

#### 1.1. The role of fatty acids in aquatic food webs

Energy transfer from one trophic level to another is the basic process in food web dynamics (Lindeman, 1942). In the pelagic environment, all organisms mainly depend upon phytoplankton (primary producers), which use light energy in the process of photosynthesis to convert carbon dioxide and water into sugars and other essential compounds (amino acids, fatty acids, etc.).

Lipids constitute a significant part of carbon flux through trophic levels (Lee *et al.*, 1971; Sargeant *et al.*, 1977) and are the major constituents of living organic matter, involved in a variety of cellular functions including membrane structure (phospholipids and glycolipids) and energy storage (triacylglycerols and wax esters) (Vance & Vance, 1985; Arts & Wainman, 1998). These natural compounds can provide a record of biological activity in the upper water column and of processes occurring during sedimentation (Pinturier-Geiss *et al.*, 2001).

Herbivorous zooplankton are considered to play a key role in material and energy transfer between primary producers and higher trophic levels in aquatic environment (Cottonnec *et al.*, 2001). Reproductive success of zooplankton is related to the quantity and quality of food (Turner *et al.*, 2001; Turner *et al.* 2002). Changes in food quality can influence the assimilation efficiency (Angel, 1984), which in turn, may affect energy allocation for metabolism, somatic growth and reproduction (Pond *et al.*, 1996, Wacker & von Elert, 2003). The quantity and quality of food is highly variable (Pond *et al.*, 1993). This temporal and spatial variability is conditioned by the availability of phosphorus, nitrogen, silica and iron, light (Brett & Müller-Navarra, 1997), temperature (Waimann & Smith 1997, Brett & Müller-Navarra 1997) and differential grazing (Kilham *et al.* 1997; Gulati & Demott 1997).

Poor quality food is determined by the presence of deficiencies in the biochemical composition of the food relative to the consumer's requirements. A poor quality diet may result in food limitation even when food concentrations are high (Durbin *et al.*, 1983; Klepel & Burkart, 1995).

Fatty acids constitute the main part of the lipids in aquatic organisms, and many of the universally important fatty acids, like for instance the long-chain polyunsaturated fatty acids, are only synthesised *de novo* by phytoplankton (Pohl and Zurheide, 1979; Sargent and Whittle, 1981; Desvillettes *et al.*, 1997; Napolitano *et al.*, 1997). Because the transfer of tracer fatty acids is conservative, the use of fatty acids composition as a reliable method for tracing the food source through multiple food web linkages is possible and already established (Lee *et al.* 1977). According to Pond *et al.* (1996) fatty acid measurements are a reliable and accurate estimate of food availability and quality.

Most changes in the fatty acid composition of photosynthetic organisms reflect the composition in type of lipid. This in turn may in some instances be profoundly affected by specific growth conditions (Ackman & Tocher, 1968). The fatty acids composition of zooplankton represents the time-integrated dietary intake (Bourdier & Amblard, 1989).

### **1.1.1. Kinds of fatty acids**

From the perspective of animal nutrition, fatty acids may be grouped in two classes: the first group contains those fatty acids that cannot be synthesized *de novo* but are essential for animal growth and development and must be supplied by the food, while the second one contains those that can be synthesized (nonessential) (Vance & Vance, 1985; Sargent, Bell & Henderson, 1995).

In the case of aquatic organisms it is useful to group the essential fatty acids (EFA) in two groups, the linoleic acid (18:2 $\omega$ 6) family and the linolenic acid (18:3 $\omega$ 3) family (Olsen, 1998). The phototrophic organisms synthesize linoleic and linolenic acid *de novo* from stearic acid (18:0). Animals are unable to synthesize linoleic and linolenic acids, but exhibit specific capacities of bioconversion these EFA in polyunsaturated fatty acids (PUFA) (Gurr & Harwood, 1991).

Fatty acids without double bonds in the chain are called saturated (SAFA) and those with double bonds unsaturated (UFA). An UFA is synthesized from a SAFA introducing double bonds by enzymes called desaturases between the carboxyl end of the fatty acid molecule and the 9<sup>th</sup> carbon atom of the fatty acid molecule

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(Cook,1985). Monounsaturated fatty acids (MUFA) carry only one double bond. Those with more than two double bonds are called polyunsaturated (PUFA) and those with more than four double bonds highunsaturated fatty acids (HUFA).

### 1.1.2. Functions of selected fatty acids

#### **SAFA and MUFA**

efficient storage lipids (Roessler 1990)

#### **PUFA**

essential for maintaining membrane fluidity in phytoplankton (Hamm & Rousseau 2003); play an important role in cell membrane activity as precursors of prostaglandins, oocyte maturation and egg production in invertebrates (Broglio *et al.* 2003)

#### **HUFA**

play an important role in a wide range of physiological processes in invertebrates and are suggested to control the growth of zooplankton in natural communities (Ahlgren *et al.* 1997, Brett & Müller-Navarra 1997)

#### **18:3 $\omega$ 3**

primary precursor molecule for the  $\omega$ 3-family of fatty acids. Essential fatty acid for all animals

#### **18:4 $\omega$ 3 and 20:4 $\omega$ 3**

precursors for elongation to form 20:5 $\omega$ 3 and 22:6 $\omega$ 3 (Ballantyne *et al.* 2003, Hazzard & Kleppel 2003)

#### **20:5 $\omega$ 3 (EPA) and 22:6 $\omega$ 3 (DHA)**

fatty acid involved in membrane structure and function (Pond & Harris1996), with potentially strong influence of the fecundity (Jónasdóttir *et al.*1995) and growth in zooplankton (Fraser *et al.* 1989), important for the development and normal physiological activities of brain and eye tissues (Brett & Müller-Navarra 1997)

#### **18:2 $\omega$ 6**

primary precursor molecule for the  $\omega$ 6-family of fatty acids. Essential fatty acid for all animals

#### **20:1 $\omega$ 6 and 22:1 $\omega$ 11**

important for gametogenesis (Pond *et al.*1996)



**20:4 $\omega$ 6 (AA)**

one of the principal precursor of eicosanoids (prostaglandin) implicated in the viability and functioning of spermatozoa of marine organisms (Pond *et al.* 1996)

**1.1.3. Nomenclature**

The morphology of fatty acids can be characterised by the shorthand X:Y $\omega$ Z, where X equals the number of carbon atoms, Y equals the numbers of double bonds, and Z equals the position of the first double bond counting from the methyl end, the  $\omega$ 3 and  $\omega$ 6 designations. Fatty acids are characterized by having a carbonyl group at one end of the aliphatic chain, and a methyl group on the opposite end (Vance & Vance, 1985; Sargent *et al.*, 1987).

In the list below the most frequently mentioned fatty acids in this study are specified.

**Table 1-1:** Nomenclature of long-chain fatty acids

Systematic name	Trivial name	Shorthand designation
tridecanoic acid		13:0
Tetradecanoic acid	myristic acid	14:0
Pentadecanoic acid		15:0
Hexadecanoic acid	palmitic acid	16:0
Octadecanoic acid	stearic acid	18:0
Eicosanoic acid	arachidic acid	20:0
Docosanoic acid	behenic acid	22:0
Tetracosanoic acid	lignoceric	24:0
9-heyadecenoic acid	palmitoleic acid	14:1 $\omega$ 5
11-octadecenoic acid	vaccenic acid	16:1 $\omega$ 7
9-hexadecenoic acid	palmitoleic acid	18:1 $\omega$ 7
9-octadenoic acid	oleic acid	18:1 $\omega$ 9
9-eicosenoic	gadoleic	20:1 $\omega$ 7
Eicosenoic acid	gondoic acid	20:1 $\omega$ 9
Hexadecadienoic		16:2 $\omega$ 4
Octadecadienoic Acid		18:2 $\omega$ 6
9,12,15 Octadecatrienoic Acid	Linoelaidic Acid	18:3 $\omega$ 3
6,9,12- Octadecatrienoic Acid	Linolenic Acid	18:3 $\omega$ 6
Octadecatetraenoic Acid	Moroctic Acid	18:4 $\omega$ 3
Timnodonic acid	eicosapentaenoic acid (EPA)	20:5 $\omega$ 3
Eicosatetraonic acid	arachidonic acid (AA)	20:4 $\omega$ 6
Cervonic acid	docosahexaenoic acid (DHA)	22:6 $\omega$ 3

## 1.2. The role of zooplankton in aquatic food webs

The major components of the mesozooplankton (0.2 to 2 mm) community in lakes as well as in the sea are copepods and cladocerans. While cladocerans are typically more abundant in freshwater, copepods dominate in the marine environment. There are important differences between both zooplankton guilds, especially regarding their impact on the lower trophic levels, either directly via feeding or indirectly by influencing nutrient cycling (Lampert & Sommer 1997, Brendelberger 1991, Maier 1993, Hessen & Lyche 1991, DeMott 1995).

Cladocerans – hereafter referred to as *Daphnia* – are very efficient filter feeders. Although regarded as unselective feeders their filtering apparatus determines the maximum size of ingestible particles (1 to 30  $\mu\text{m}$ ) (Gliwicz & Siedlar 1980, Geller & Müller 1981, Gophen & Geller 1984). In contrast copepods actively select and catch their food particles (DeMott 1986). They are known to prefer larger particles over small ones, but an overlap between copepods and cladocerans in the food size spectra is known (10-30  $\mu\text{m}$ ) (Adrian & Scheiner-Olt 1999). These different grazing behaviours of cladocerans and copepods can have important and contrasting impacts on the fatty acid profile of the mesozooplankton itself as well as of the phytoplankton community. At last, this changes in the fatty acid profile can affect the zooplankton growth.

Early studies (Lampert 1977, Berggreen *et al.* 1988, Kiørboe *et al.* 1985) on zooplankton growth concentrated on the influence of food quantity measured as biovolume, chlorophyll a or carbon. Recently the determination of food quality became of increasing interest in aquatic science (Müller-Navarra & Lampert 1996, von Elert & Stampfl 2000, Jónastóttir 1994, Klein Breteler *et al.* 1999). Elemental composition (Jones *et al.* 2002) and changes in protein, carbohydrate, lipid and vitamin content (Sterner & Hessen, 1994) were studied in relation to zooplankton growth. The fatty acid composition seems to play a key role in the understanding of seston food quality. Especially the concentration of essential polyunsaturated fatty acids can limited zooplankton growth (Müller-Navarra 1995, Pond *et al.* 1996).

Zooplankton growth or nutritional status can additionally be determined by measurements of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The ratio of the two (RNA:DNA) has proven to be a useful tool in determination of nutritional

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status of various zooplankton organisms (Vrede *et al.* 2002, Wagner *et al.* 1998). An increased RNA:DNA ratio indicates a higher growth potential.

### 1.3. Approach

This studies was part of a project to examine the impact of major zooplankton taxa on natural phytoplankton assemblages in respect to changes in species composition, size structure, nutrient dynamics and food quality. Additionally the cascading effect on the microbial food web was investigated. In order to examine the quality of mesozooplankton food through the fatty acid composition of seston (particulate organic matter excluding zooplankton) and to quantify seasonal specific trends concerning polyunsaturated fatty acids (PUFA) profiles, we performed mesocosm experiments in one marine, one brackish and one freshwater system during two different seasons (summer, spring). In these mesocosm studies, it was hypothesised that:

1. the composition of the phytoplankton fatty acids change with season and during individuals blooms
2. the phytoplankton fatty acids content (food quality) depends on the availability of P and N in the water
3. the grazing impact of zooplankton on the phytoplankton is able to modify the seston fatty acid profile
4. some of the variability in zooplankton growth results from variations in food quantity and quality

Therefore we experimentally manipulated the zooplankton community in order to obtain a strong dominance of copepods or cladocerans at 4-5 different population densities.

# **C H A P T E R 2**

## Chapter 2

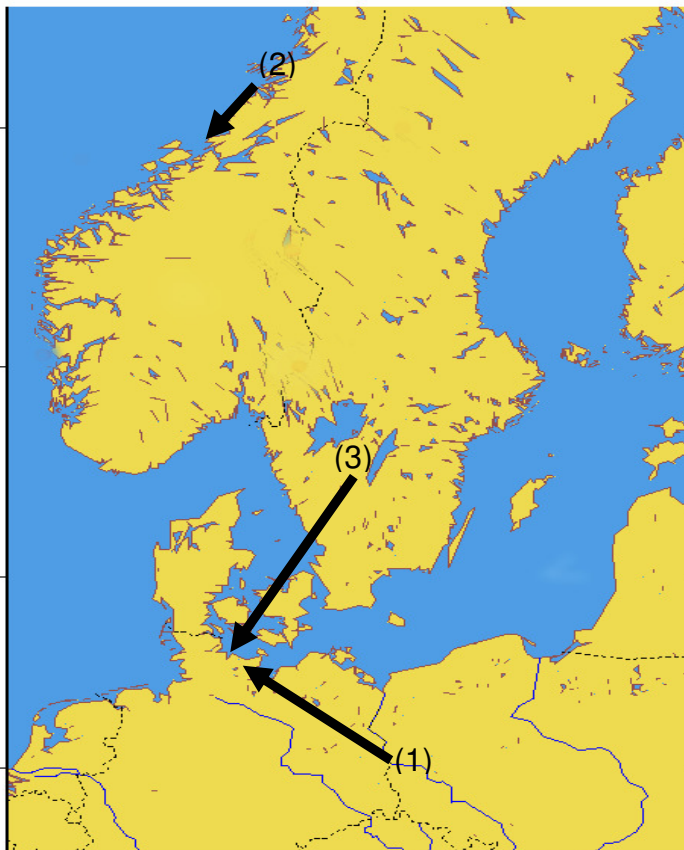
### Material and Methods

This study was performed as a part of a larger project where we aimed at determining the impact of different zooplankton organisms on freshwater, brackish and marine food webs.

#### 2.1. Study sites

The mesocosm experiments were conducted at three different sites between August 2000 and April 2003:

- (1) in Lake Schöhsee: a mesotrophic lake in Plön, Northern Germany; mean depth 13 m, maximal depth 30 m; area 82 ha. (**Fig. 2-1**)
- (2) in Hopavågen lagoon: a sheltered semi-enclosed marine lagoon situated on the outlet of the Trondheim Fjord, Norway; maximal depth 32 m; area 27 ha. (**Fig. 2-1**)
- (3) in Kiel Fjord: a shallow bight situated of the western Baltic Sea; mean depth 12-14 m; area 714 km<sup>2</sup>. (**Fig. 2-1**)



**Figure 2-1:** Maps of the Mesocosmos location

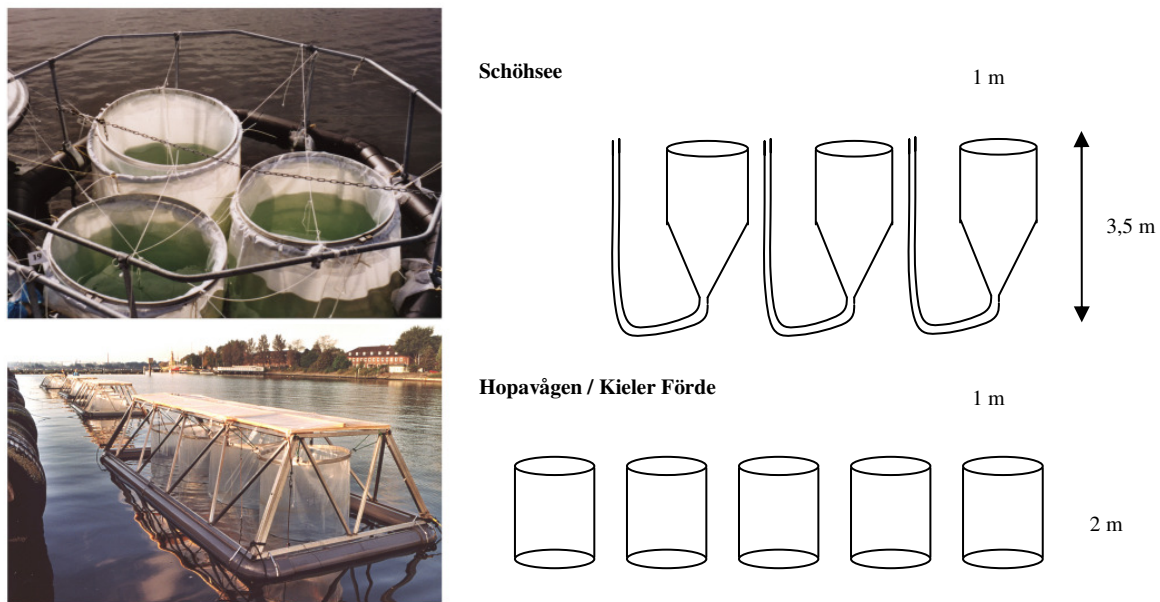
## 2.2. Experimental design

**Mesocosms** – The mesocosms consisted of 24 transparent polyethylene bags (Trikoron, BP chemicals), suspended in several floats (**Fig.2-2**). All enclosure bags were 1m in diameter and open at the top. In the first experiment (Schöhsee), the top 2 m of the mesocosm bags were cylindrical, whereas the bottom part was conically shaped and ended in a tube used to remove accumulated particulate matter. In this experiment, the bags were ~3.2 m deep, and comprised ~3,4 m<sup>3</sup> lake water. As zooplankton tended to accumulate within the lower part of the bags, at times many zooplankton were removed with the sedimented particulate matter. Therefore, in the following experiments, bags were simply sealed at the bottom. In the marine experiments weights were attached to the bottom end to keep the bags in an upright position. The resulting shape was approximately cylindrical, 2.5 m deep and contained 1.5 m<sup>3</sup> water.

The mesocosm bags were filled with lake water (50 µm pre-screened) pumped from 1 m depth (Schöhsee), or by hauling the submerged bags from ~3 m depth to the surface (marine experiments). Therefore, zooplankton that had entered the bags during the filling procedure in the marine experiments, had to be removed from within the bags by means of several net hauls (250 µm mesh size, 0.8 m diameter). In the first freshwater experiment, mesocosm bags were fertilised with inorganic phosphate (106 mg P bag<sup>1</sup>) after filling them in order to obtain a more balanced nutrient ratio closer to Redfield (N:P = 16:1). This was based on measurements of total nitrogen (TN) and total phosphorus (TP) prior to fertilisation indicating P limitation (molar TN:TP = 54:1). In the others experiments, fertilisation was not performed.

**Zooplankton** - In the lake Schöhsee copepods were collected with a 250 µm mesh size plankton net, bags of the cladocerans treatment were inoculated with laboratory-reared *Daphnia hyaline x galeata* obtained from the Max-Planck-Institut of Limnology in Plön. In the marine and brackish experiments, copepods were collected by means of horizontal tows with a plankton net (500 µm and 250 µm mesh size). In Norway, towing was performed at ~2 m depth within the Hopavågen lagoon, whereas Baltic copepods were collected at ~4 m depth at a deep (~15 m) site close to Laboe in Kiel Fjord. In the Schöhsee and Norway experiments the copepods were first collected in barrels (300 L) and submitted to bubbling with air to remove the cladocerans. Dead

and injured individuals were allowed to sink to the bottom of the barrels, from where they were removed prior to their addition to the mesocosms bags.



**Figure 2-2:** Pictures of the mesocosm arrangements (left) and simplified scheme of the bags used for the experiments (right). In the Hopavågen and Kiel Fjord experiments, the bags were covered with transparent foil mounted in frames to protect bag contents from bird faeces.

**Treatments** - Each treatment consisted of a gradient of zooplankton densities. The numerical scaling of copepods in the copepod treatment was four-fold that of *Daphnia* in the cladoceran treatment. This was done in order to achieve a comparable zooplankton biomass gradient, based on the assumption that *Daphnia* biomass ( $\sim 17 \mu\text{g}$  dry weight  $\text{ind}^{-1}$  for *D. hyaline*; Santer, 1990) was approximately four times that of the calanoid *Eudiaptomus* ( $4 \mu\text{g}$  dry weight  $\text{ind}^{-1}$  *Eudiaptomus*; Botrel *et al.*, 1976). The density gradient (**Tab. 2-1**) were scaled logarithmically and was established by adding an increasing amount of zooplankton to the mesocosm bags. However, since water volumes in the mesocosm bags were only approximate, actual initial zooplankton densities differed in some cases from intended densities. Highest zooplankton treatment densities exceeded naturally occurring maximum abundances, maximally two-fold. Each treatment density, except for the lowest density in Schöhsee experiment, was replicated twice. In all experiments, two bags without zooplankton served as control treatments. In control bags of the Hopavågen mesocosm, zooplankton that recruited from nauplii or early copepodite stages were removed daily by means of vertical net hauls ( $250 \mu\text{m}$  mesh size). In the Schöhsee and Baltic experiments, this procedure was not performed due to the abundance of large phytoplankton which would have been partially removed by net hauls.

**Table 2-1:** Treatments at the three study locations (size classes 250 to 500 $\mu\text{m}$  = small copepods (S), size class 500  $\mu\text{m}$  = large copepods (L)).

Study site	Treatment	N° bags	Seeding densities (ind L <sup>-1</sup> )
Schöhsee	cladocerans	11	1.25, 2.5, 5, 10, 20, 40
	copepods	11	5, 10, 20, 40, 80
Hopavågen	copepods	10	5, 10, 20, 40, 80
	copepods (S)	8	1, 3, 9, 27
	copepods (L)	8	0.3, 0.9, 2.7, 8.1
Kiel Fjord	copepods	10	5, 10, 20, 40, 80

**Sampling details** – All bags were sampled in 3 to 4 day-intervals. Before sampling, the entire enclosed water body was mixed with a Secchi-disk, in order to avoid sampling errors due to aggregations or sedimentation. At each sampling, 10 litres of water were collected, from which several plankton parameters were monitored: 1) composition of phytoplankton, 2) composition of zooplankton, 3) mineral composition of particulate matter (C, N, P) and 3) fatty acids.

Zooplankton samples were taken with vertical tows through the entire water column of the mesocosm bags with a plankton-net (55  $\mu\text{m}$  mesh size).

### 2.3. Variables and calculations

**Nutrients** – Samples for the analysis of seston C, seston N and seston P content were filtered onto precombusted (55°C, 24 h), acid- washed (10% HCl) Whatmann GF/F filters. After drying (~24 h), samples for seston C and N analysis were stored in a desiccator until combustion in a CHN-analyser (Fisons, 1500N). Samples for particulate P analysis were measured as orthophosphate according Grasshoff *et al.* (1999) after oxidative digestion.

**Fatty acid analyses** - Samples for the seston fatty acids analyses were pre-filtered over 250  $\mu\text{m}$  gauze, before filtering on a GF/C filter that was subsequently stored under N<sub>2</sub> gas at -18°C until further processing. Start samples for the analyses of copepods fatty acids were taken from the barrels in which zooplankton tows were initially concentrated. Final samples were collected with a zooplankton-net (41 $\mu\text{m}$



mesh size) from each bag at the end of the experiments and stored (N<sub>2</sub> gas at -18 °C). Fatty acids were extracted, esterified and analysed on a gas chromatograph (Hewlett Packard 5890 Series II.) according to Wiltshire *et al.* (2000), using the GC temperature settings of von Ellert (2002). To quantify the fatty acid content an internal standard of heptadecanoic (17:0) and tricosanoic fatty (23:0) acid methyl esters was used.

**RNA:DNA ratio** - The nutritional status of copepod *Calanus finmarchicus* was determined with RNA:DNA measurements. RNA and DNA were quantified fluorimetrically with ethidium bromide and ribonuclease A using a modification of the method of Clemmesen (1993). To get a distinct RNA:DNA signal four to five individuals of the same copepodite stage were pooled for each measurement.

**Zooplankton** - In the lake Schöhsee copepods were collected with a plankton net (250 µm mesh size), and consisted mainly of the calanoid species *Eudiaptomus gracilis*, and few copepodite stages of cyclopoid copepods (Sommer, 2003 and Feuchtmayr, 2004)

In the marine and brackish experiments, copepods were collected by means of horizontal tows with a plankton net (500 µm and 250 µm mesh size). In Norway, towing was performed at ~2 m depth within the Hopavågen lagoon, whereas Baltic copepods were collected at ~4 m depth at a deep (~15 m) site close to Laboe in Kiel Fjord. The Hopavågen copepods of the summer experiment consisted of a mixed assemblage of calanoids dominated by *Temora longicornis*, *Centropages hamatus*, *Centropages typicus* and *Pseudocalanus elongatus*, in spring it consisted of *Calanus finmarchicus* (copepodite stage 3-5), *Centropages hamatus* and *C. typicus* and *Oithona sp* (Saage, 2003). In contrast, copepods from Kiel Bight in summer were almost entirely composed of the calanoid *Acartia clausi* (Sommer, 2003) in spring they consisted mainly of *Centropages hamatus* and *Acartia clausi* (Feuchtmayr, 2004).

In the Schöhsee and Norway experiments the copepods were first collected in barrels (300 L) and submitted to bubbling with air to remove the cladocerans presence. In the Kiel Bight the water was screened with a 500 µm gauze to separate jellyfish from copepods. Dead and injured individuals were allowed to sink to the bottom of the barrels, from where they were removed prior to copepod addition to the mesocosms bags.

**Polyunsaturation Index of fatty acids** – a measure of the contribution of fatty acids with 16 carbon ( $C_{16}$ ) was calculated in order to understand the physiological state and/or the bloom status of the phytoplankton populations

$$\text{Polyunsaturation Index of fatty acids (C}_{16}\text{)} = \frac{C_{16}(\text{two or more double bounds})}{\text{Total fatty C}_{16}}$$

**Data analyses** – The relative fatty acid contributions (in percent of total fatty acids) were arcsine transformed to ensure an approximate normal distribution. In order to analyse the effects of food quality on the zooplankton regressions analysis were carried out using STATISTICA 6.0. A confidence interval of 95% was used.

**Biomarkers** – The list below shows fatty acids used as biomarkers for different plankton groups.

**Table 2-2:** Biomarkers used in this study (*A* Desvillettes *et al.* (1997), *B* Reuss & Poulsen (2002), *C* Sargent *et al.* (1987), *D* Hamm & Rousseau (2003), *E* Hygum *et al.* (2000), *F* Budge *et al.* (2001), *G* Claustre *et al.* (1989), *H* Budge *et al.* (2001), *I* White *et al.* (1980), *J* Pond *et al.* (1996))

Plankton	Biomarkers/characteristic FA	References
Chrysophyceae	18:4 $\omega$ 3	A, E
Cryptophyceae	18:4 $\omega$ 3, 18:1 $\omega$ 7	A, E
Diatoms	16:1 $\omega$ 7, 20:5 $\omega$ 3, high 16:1 $\omega$ 7/16:0	A, B, F
Dinoflagellates	18:1 $\omega$ 9	A, C, D
Flagellates in general	low 16:1 $\omega$ 7/16:0	G
Bacteria	13:0, 15:0	H, I
Flagellates	22:6 $\omega$ 3	J

# **C H A P T E R 3**

## Chapter 3

### Schöhsee Mesocosms: fatty acids in freshwater

The Schöhsee experiments were the longest mesocosm study and lasted for approximately three weeks (**Tab. 3-1**).

**Table 3-1:** General water parameters and weather conditions during the Schöhsee experimental periods

Study site	Period	Water temp (°C)	Salinity (PSU)	Weather conditions
Schöhsee Summer	09-28 August 2000	18.6 - 20.2	0	dry & sunny
Schöhsee Spring	04-25 May 2001	12°C-18°C	0	dry & sunny

In the summer experiment the fatty acid analyse of *Daphnia*, copepods and phytoplankton (seston) were performed up to August 17th. At this point, the bags of the copepod treatment still showed negligible contamination by *Daphnia* individuals (<2 ind L<sup>-1</sup>). During this time, calcite precipitated visibly in both the lake and mesocosm bags, a frequent phenomenon in lakes termed “whiting”. This event had a great impact on P availability as phosphate easily co-precipitates with calcite and thus makes P unavailable to phytoplankton (Kleiner, 1988). In the spring experiments the analyses were constrained to the first 14 days because biofilms growing on enclosure bag surfaces were observed and phytoplankton species, which were initially not detectable, increased in biomass and hence availability for zooplankton.

The zooplankton in both experiments consisted mainly of the calanoid species *Eudiaptomus gracilis*, and few copepodite stages of cyclopoid copepods. The cladocerans consisted of *Daphnia hyalina* x *galeata* (Sommer, 2003 and Feuchtmayr, 2004).

The zooplankton samples could only be taken at the end of the experiments in order not to change the grazing effect. Due to the problem with contamination described above, I decided afterwards to evaluate only the first two weeks of the experiments. Therefore no corresponding fatty acids data for the zooplankton were available and an estimation of the influence of seston fatty acids on animal fatty acid concentrations was not possible.

### 3.1. Results: fatty acids abundance and composition

#### 3.1.1. Fatty acids in the seston

##### Summer experiment, copepod treatments

The total FA concentrations (**Figure 3.1**) in the seston varied from 5.25 to 12.41  $\mu\text{g mg C}^{-1}$ .

**Table 3-2** summarises the fatty acids composition in the summer phytoplankton community. During the entire study, SAFA accounted for 29.1 - 47.4% of total FA. They consisted mostly of palmitic acid or 16:0 (15.5 – 25.3% of total FA) and, always in lower proportions, of myristic acid or 14:0 (5.9 – 14.6% of total FA) and of stearic acid or 18:0 (3.0 – 5.8% of total FA). Saturated acids with 20, 22 carbon atoms did not exceed 1.9% of total fatty acids and at with 24 carbon atoms were not present.

The MUFA accounted for 19.2 – 34.3% of total FA, the great majority consisting of palmitoleic acid or 16:1 $\omega$ 7 (5.7 – 9.7% of total FA), oleic acid or 18:1 $\omega$ 9 (6.5 – 20.2% of total FA) and vaccenic acid or 18:1  $\omega$ 7 (1.9 – 4.0% of total FA). 18:1 $\omega$ 9 concentrations increased with time (exception: treatment cop5). 14:1 $\omega$ 5 only accounted for a low proportion of total FA (<0.5%). Other MUFA, such as 20:1 $\omega$ 9 appeared in a more sporadic manner and only accounted for a low proportion of total FA (generally <0.5%), 20:1 $\omega$ 7 was not present.

The PUFA accounted for 28.9 – 46.0% of total FA. They mainly belonged to the linolenate series ( $\omega$ 3) (17.6 – 32.6% of total FA), and also to the linoleates ( $\omega$ 6), but in lower proportions (8.9 – 14.6% of total FA). PUFA with 16 carbon atoms and three or four double bonds were not present. Those with two double bonds (16:2 $\omega$ 4) were relatively constant during the experiment (on average 1.4% of total FA). PUFA with 18 carbon atoms, such as 18:2 $\omega$ 6, linolenic acid or 18:3 $\omega$ 3 and stearidonic acid or 18:4 $\omega$ 3 reached relatively high proportions (> 2.8%) and decreased in the summer experiment with time.

PUFA with 20 and 22 carbon atoms were mainly represented by eicosapentaenoic acid (EPA) or 20:5 $\omega$ 3 (7.8 – 14.8% of total FA), and arachidonic acid (AA) or 20:4 $\omega$ 3 (3.4 – 7.7% of total FA). In concentrations of EPA and AA increased with time.

Docosahexaenoic acid (DHA) or 22:6 $\omega$ 3 was not present.

### Spring experiment, copepod treatments

The total FA concentrations (**Figure 3-1**) in the seston varied from 2.72 to 12.91  $\mu\text{g mg C}^{-1}$

**Table 3-2** summarises the fatty acids composition in the spring phytoplankton community. During the entire study, SAFA accounted for 27.7 - 82.1% of total FA. They consisted mostly of palmitic acid or 16:0 (12.7 – 39.2% of total FA) and, always in lower proportions, of myristic acid or 14:0 (12.4 – 33.9 % of total FA) and of stearic acid or 18:0 (30.9 – 1.2 % of total FA). Saturated acids with 20, 22 carbon atoms did not exceed 1.4% of total FA those with 24 have aleatory behaviour.

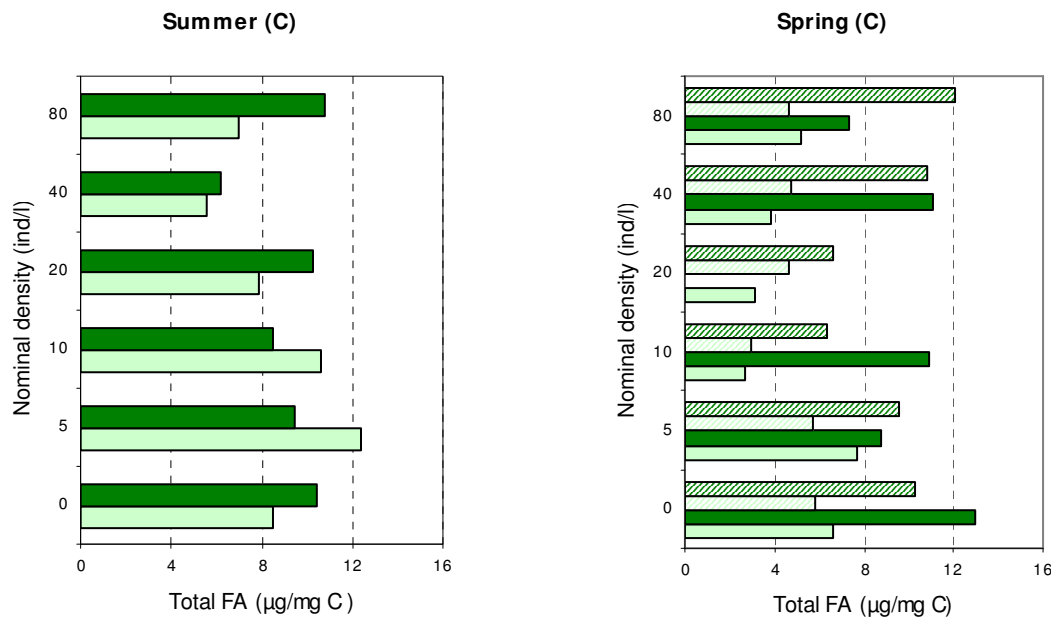
The MUFA accounted for 9.0 -18.0% of total FA, the great majority consisting of palmitoleic acid or 16:1 $\omega$ 7 (5.7 – 9.7% of total FA and 2.9 – 12.3% of total FA), oleic acid or 18:1 $\omega$ 9 (1.3 – 7.7% of total FA) and vaccenic acid or 18:1  $\omega$ 7 (2.8% of total FA). Other MUFA, such as 14:1 $\omega$ 5 and 20:1 $\omega$ 7 were not present. 20:1 $\omega$ 9 appeared in a more sporadic manner and not exceed 1.6%. The PUFA accounted for 7.4 - 57.8% of total FA (spring). They mainly belonged to the linolenate series ( $\omega$ 3) (2.5 – 39.1% of total FA), and also to the linoleates ( $\omega$ 6), but in lower proportions (2.9 – 25.7% of total FA). PUFA with 16 carbon atoms and three or four double bonds were not present. Those with two double bonds (16:2 $\omega$ 4) were relatively constant during the experiment, with lower concentrations (on average 0.4% of total FA).

PUFA with 18 carbon atoms, such as 18:2 $\omega$ 6, linolenic acid or 18:3 $\omega$ 3 and stearidonic acid or 18:4 $\omega$ 3 reached relatively high proportions (**Table 3-2**). PUFA with 20 and 22 carbon atoms were mainly represented by eicosapentaenoic acid (EPA) or 20:5 $\omega$ 3 (1.5 – 16.3% of total FA), and arachidonic acid (AA) or 20:4 $\omega$ 3 (2.1 – 19.5% of total FA). Docosahexaenoic acid (DHA) or 22:6 $\omega$ 3 was only present in the spring seston (1.5 –8.5% of total FA) and its concentrations increased with time.

Since seston contained a considerable amount of 18:2 $\omega$ 6, 18:3 $\omega$ 3 and 18:4 $\omega$ 3, I assume that phytoplankton converted these FA into AA, EPA and DHA. This is supported by decline in 18:2 $\omega$ 6, 18:3 $\omega$ 3 and 18:4 $\omega$ 3 concentrations (**Table 3-2**) that occurred at the same time (final day) when AA, EPA and DHA concentration increased in the experiments.

**Table 3-2:** Fatty acids composition during summer and spring experiments (*copepods*) expressed as a % of total fatty acids. For the purpose of clarity, only major component fatty acids are shown although summary totals include all data (first day - last day; nd = no detected, {1} {2} = sample duplicate)

	summer control	spring control	summer cop5	spring cop5	summer cop10	spring cop10	summer cop20	spring cop20	summer cop40	spring cop40	summer cop80	spring cop80	
SAFA													
C14:0	12.1-7.8	17.3-14.4	10.0-8.8	9.9-16.5	9.6-8.0	33.9-13.4	12.8-5.9	31.3-30.6	14.6-8.5	26.0-11.2	12.4-9.8	20.0-12.4	{1}
		15.9-12.9		17.2-18.3		33.7-15.4		21.3-19.0		19.1-12.8		19.9-7.7	{2}
C16:0	23.8-16.0	22.0-18.9	18.4-18.4	12.8-22.2	19.1-15.6	34.4-18.0	20.1-16.1	34.0-39.2	21.4-18.4	30-15.2	25.3-18.6	19.6-16.7	{1}
		15.4-18.1		16.1-24.5		36.2-22.3		20.5-21.2		18.5-19.8		20.7-20.5	{2}
C18:0	4.0-4.2	15.8-3.6	13.4-3.7	3.8-4.3	3.2-2.9	8.1-2.9	4.5-4.0	7.6-1.21	5.9-4.2	7.5-3.3	4.3-3.0	4.6-4.1	{1}
		3.3-4.2		4.0-5.1		8.2-3.7		4.4-5.1		4.2-12.3		5.2-4.0	{2}
MUFA													
C16:1 $\omega$ 7	8.4-8.1	4.5-6.51	5.7-8.1	8.9-7.3	7.4-9.0	3.5-12.3	8.1-7.3	3.7-16.2	8.5-8.4	3.8-8.6	6.4-8.9	6.9-7.0	{1}
		6.5-5.9		9.7-6.9		4.11-11.5		7.0-5.9		8.1-6.2		7.1-2.9	{2}
C18:1 $\omega$ 9	9.6-9.9	5.5-4.2	15.9-13.2	4.6-3.9	9.8-11.1	2.5-3.7	15.7-20.2	5.7-1.3	12.6-11.5	4.8-4.0	11.0-7.7	5.7-5.7	{1}
		6.2-4.4		6.9-5.4		3.6-3.6		7.3-7.7		6.6-4.5		6.9-2.8	{2}
C18:1 $\omega$ 7	nd-0.5	0.7-1.4	2.8-3.2	1.0-1.6	2.0-3.5	2.0-1.4	2.7-4.1	1.9-0.5	2.3-5.5	2.6-1.7	1.9-3.1	0.8-2.1	{1}
		0.8-2.1		1.3-1.4		2.8-1.2		0.7-1.8		0.8-1.5		1.1-0.9	{2}
C20:1 $\omega$ 9	nd-nd	1.5-nd	nd-0.5	nd-nd	nd-0.5	1.2-nd	nd-1.1	1.6-0.03	nd-1.3	1.5-0.1	nd-0.4	nd-nd	{1}
		0.2-0.2		nd-nd		nd-nd		nd-nd		0.3-1.5		nd-9.0	{2}
C22:1 $\omega$ 9	5.6-3.1	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	{1}
		nd-nd		nd-nd		nd-nd		nd-nd		ndnd		nd-nd	{2}
$\omega$ 6													
C18:2 $\omega$ 6	nd-1.6	4.6-6.1	9.4-3.3	9.7-4.3	5.9-3.1	1.6-4.4	9.1-3.4	1.6-1.8	9.0-3.0	3.4-5.4	5.8-4.1	6.8-5.4	{1}
		5.2-4.0		10.9-5.8		2.0-3.9		6.1-6.1		6.1-4.2		7.0-2.3	{2}
C20:4 $\omega$ 6	6.4-3.1	1.0-1.1	nd-1.0	0.6-1.0	nd-1.2	nd-1.1	nd-1.2	nd-0.2	nd-3.0	0.4-1.2	nd-1.2	0.7-1.9	{1}
		0.5-0.8		nd-1.2		nd-1.0		9.1-nd		0.6-1.6		0.8-0.8	{2}
$\omega$ 3													
C18:3 $\omega$ 3	4.8-3.1	3.7-4.2	10.4-2.9	7.2-2.3	7.4-3.6	1.0-3.1	9.3-2.1	1.0-0.1	9.8-2.5	1.9-4.1	6.7-3.1	5.1-4.5	{1}
		4.0-2.7		8.2-2.7		0.7-3.1		4.4-3.1		4.4-3.1		4.9-2.0	{2}
C18:4 $\omega$ 3	4.0-5.6	3.2-5.5	10.3-3.5	7.2-3.2	6.6-4.0	0.8-5.2	8.3-1.5	0.9-1.9	8.5-2.4	1.4-6.8	5.0-3.7	5.0-8.2	{1}
		4.4-3.1		6.8-0.2		0.3-0.5		4.3-4.4		4.4-4.7		4.4-3.9	{2}
C20:4 $\omega$ 3	8.2-9.4	3.5-8.7	nd-6.7	7.5-5.9	3.5-7.0	1.7-6.4	nd-6.8	2.1-nd	nd-4.9	3.5-8.3	3.9-7.7	6.1-6.8	{1}
		19.5-5.0		5.8-8.1		2.2-4.8		6.5-7.1		7.-6.8		5.4-3.2	{2}
C20:5 $\omega$ 3	8.2-9.4	6.2-13.8	nd-10.0	16.3-11.2	8.1-11.4	2.1-16.6	nd-9.4	2.5-3.2	nd-10-8	4.4-16.3	7.8-14.8	11.2-12.5	{1}
		10.5-8.8		10.6-10.8		1.5-12.3		nd-10.0		11.8-9.8		8.4-5.3	{2}
C22:5 $\omega$ 3	4.0-6.3	nd-0.3	nd-8.5	nd-nd	4.1-7.7	nd-0.4	nd-5.4	nd-0.1	nd-5.8	nd-0.5	3.9-6.7	nd-nd	{1}
		nd-nd		nd-nd		nd-nd		nd-nd		nd-nd		nd-nd	{2}
C22:6 $\omega$ 3	nd-nd	3.2-6-7	nd-nd	7.0-4.3	nd-nd	1.0-5.9	nd-nd	nd1.5	nd-nd	2.3-7.7	nd-nd	5.5-8.5	{1}
		3.9-4.2		nd-4.9		nd-4.8		3.7-6.4		4.2-5.8		2.0-4.2	{2}
tot PUFA ( $\mu$ g/mg C)	3.0-4.7	1.8-6.3	-2.5	0.3-5.0	3.6-3.3	0.4-3.3	5.3-4.4	1.3-0.7	5.4-3.7	0.8-3.2	-2.2	0.3-3.0	{1}
		2.8-3.1		1.9-3.3		2.9-2.9		2.3-3.2		-1.9		0.2-2.3	{2}



**Figure 3.1:** Variation in phytoplanktonic total fatty acid concentrations in the copepod (C) treatment (light green: first day of the experiment; dark green: last day of the experiment; dashed blocks denote the duplicate treatments).

### Summer experiment, *Daphnia* treatments

The total FA concentrations (**Figure 3-2**) in the seston varied from 2.55 to 19.04  $\mu\text{g mg C l}^{-1}$ .

**Table 3-3** summarises the fatty acids composition in the summer phytoplankton community. During the entire study, SAFA accounted for 30.7 - 44.1% of total FA. They mostly consisted of palmitic acid or 16:0 (16.3 – 22.7 % of total FA), myristic acid or 14:0 (10.6 – 32.0% of total FA) and, always in lower proportions, stearic acid or 18:0 (3.3 – 21.8% of total FA). Saturated acids with 20 carbon atoms did not exceed 1,6% , than with 22 or 24 were not present.

The MUFA accounted for 20.1 – 27.0% of total FA, the great majority consisting of oleic acid or 18:1 $\omega$ 9 (8.1 – 15.1% of total FA) and palmitoleic acid or 16:1 $\omega$ 7 (6.1– 9.9% of total FA), and vaccenic acid or 18:1  $\omega$ 7 (1.9 – 4.1% of total FA). 14:1 $\omega$ 5 only accounted for a low proportion of total FA (< 0.5%) and 20:1 $\omega$ 9 appeared in a more sporadic manner never transcending 1.9%. Other MUFA, such as 20:1 $\omega$ 9 and those with 22 and 24 carbon atoms were not present.

The PUFA accounted for 28.5 – 45.2% of total FA. They mainly belonged to the linolenate series ( $\omega$ 3) (18.1 – 32.6% of total FA), and also to the linoleates ( $\omega$ 6), but in lower proportions (8.9 – 17.2% of total FA). PUFA with 16 carbon atoms and three



**Table 3-3:** Fatty acids composition during summer and spring experiments (*Daphnia*) expressed as a % of total fatty acids. For the purpose of clarity, only major component fatty acids are shown although summary totals include all data ( first day - last day; nd = no detected, {1} {2} = sample duplicate)

	summer control	spring contro	summer dap1.25	spring dap1.25	summer dap2.5	spring dap2.5	summer dap5	spring dap5	summer dap10	spring dap10	summer dap20	spring dap20	
SAFA													
C14:0	12.1-7.8	17.3-14.4 15.9-12.9	-9.2	30.5-15.07 20.2-11.2	11.6-8.4	30.4-16.0 16.9-18.1	10.8-7.7	24.3-24.4 18.3-17.3	14.0-8.8	21.3-12.8 -12.3	-8.3	32.0-13.8 37.6-10.6	{1} {2}
C16:0	23.8-16.0	22.0-18.9 15.4-18.1	-17.5	32.9-18.3 19.7-15.9	22.7-17.3	32.4-20.8 14.9-25.6	20.7-16.3	24.0-38.8 15.4-24.2	22.1-18.7	27.9-18.5 -20.7	-16.5	36.6-20.7 36.3-23.8	{1} {2}
C18:0	4.0-4.2	15.8-3.6 3.3-4.2	-3.4	7.9-4.0 4.4-3.7	3.6-3.6	9.8-4.2 4.0-6.1	3.6-3.3	6.6-10.0 4.6-6.2	5.6-3.7	20.8-5.4 -7.2	-3.9	7.8-7.6 8.1-21.8	{1} {2}
MUFA													
C16:1ω7	8.4-8.1	4.5-6.5 6.5-5.9	-9.9	2.3-9.9 7.0-7.4	7.9-7.5	2.3-9.4 9.1-5.2	7.3-7.8	5.9-2.8 8.9-6.0	8.2-8.9	2.8-5.4 nao-2.9	-9	3.3-5.4 3.6-2.6	{1} {2}
C18:1ω9	9.6-9.9	5.5-4.2 6.24.4	-9.3	2.8-4.5 7.3-3.6	9.4-12.2	2.5-4.0 6.9-6.2	10.6-12.3	5.7-4.9 7.0-7.0	15.1-10.6	3.1-4.7 -4.7	-10.6	5.9-7.0 2.1-6.2	{1} {2}
C18:1ω7	1.9-3.0	0.7-1.4 0.8-2.1	-3.6	0.5-1.9 0.8-1.4	1.9-3.1	1.3-1.5 1.2-1.4	2.0-3.2	0.6-2.4 1.2-1.8	3.4-3.3	2.1-1.6 nao-0.8	-3.4	0.6-1.5 1.3-1.2	{1} {2}
C20:1ω9	nd-nd	1.5-nd 0.2-0.2	-0.8	nd-nd 0.7-nd	0.4-0.5	1.5-nd nd-nd	nd-0.5	nd1.1 nd-nd	nd-0.5	1.9-nd -nd	-0.5	nd-nd 1.2-0.6	{1} {2}
C22:1ω9	nd-nd	nd-nd nd-nd	-nd	nd-nd nd-nd	0.4-nd	nd-nd nd-nd	nd-nd	nd-nd nd-nd	nd-nd	nd-nd -nd	-nd	nd-nd nd-nd	{1} {2}
ω6													
C18:2 ω6	5.6-3.1	4.6-6.1 5.2-4.0	-3.3	1.8-6.4 5.7-3.9	5.4-3.0	1.6-4.8 11.5-5.302	6.6-3.6	5.5-1.5 11.6-6.2	8.9-3.3	2.4-4.7 nao-2.6	-0.3	1.7-5.5 1.9-3.3	{1} {2}
C20:4 ω6	nd-10.6	1.0-1.1 0.5-0.8	-nd	nd-0.8 0.7-1.0	nd-1.3	nd-1.1 nd-1.1	nd-1.2	nd-0.5 nd-1.1	nd-1.0	0.5-1.1.0 -0.6	-0.9	nd-0.9 nd-2.0	{1} {2}
ω3													
C18:3ω3	6.4-3.1	3.7-4.2 4.0-2.7	-3.2	1.3-3.7 4.1-1.9	6.3-2.7	1.2-2.8 8.5-2.7	7.5-3.2	4.1-0.7 8.0-3.1	10.0-3.0	1.9-3.5 nao-1.4	-3.3	1.1-3.8 0.6-2.5	{1} {2}
C18:4ω3	4.8-3.1	3.2-5.5 4.4-3.1	-3.8	1.2-6.0 4.2-2.9	5.2-3.4	1.1-0.2 7.6-3.4	6.8-4.1	4.6-1.2 7.5-4.5	8.2-3.5	1.6-5.0 nao-2.7	-4.1	0.9-7.4 0.5-4.0	{1} {2}
C20:4ω3	4.0-5.6	3.5-8.7 19.5-5.0	-5.5	2.1-6.9 7.0-5.3	4.0-7.5	1.8-6.6 5.7-7.4	4.0-7.8	4.6-2.6 4.6-6.8	nd-6.8	2.4-8.0 nao-3.3	-7.3	2.2-7.6 1.7-4.2	{1} {2}
C20:5ω3	8.2-9.4	6.2-13.8 10.5	-10.5	2.9-13.1 11.0-8.8	8.5-10.0	2.8-12.1 10.4-9.4	8.4-10.5	8.4-2.5 8.4-8.5	nd-8.7	3.8-9.6 nao-3.4	-9.7	2.7-9.7 1.4-4.8	{1} {2}
C22:5ω3	4.0-6.3	nd-0.3 nd-nd	-6.8	nd-nd nd-nd	5.0-7.8	nd-nd nd-nd	4.9-9.0	nd-nd nd-nd	nd-7.6	nd-0.3 -nd	-8.3	nd-nd nd-nd	{1} {2}
C22:6ω3	nd-nd	3.2-6.7 3.9-4.2	-nd	nd-nd 4.4-3.6	nd-nd	nd-4.5 nd-nd	nd-nd	4.1-1.2 nd-4.8	nd-nd	1.8-4.4 -1.5	-nd	2.5-4.0 nd-1.9	{1} {2}
tot PUFA (µg/mg C)	3.0-4.7	1.8-6.3 2.8-3.1	3.9-3.8	4.5-2.3 2.5-3.3	4.0-3.7	0.27-4.9 0.21-2.3	2.3-3.7	0.3- 1.7-2.5	1.6-2.2	0.7-5.9 1.9-4.1	2.5-4.8	2.1-3.6 1.6-2.8	{1} {2}

or four double bonds were not present. PUFA with 18 carbon atoms, such as 18:2 $\omega$ 6, 18:3 $\omega$ 3 and 18:4 $\omega$ 3 reached relatively high proportions (see **Table 3-3**).

PUFA with 20 and 22 carbon atoms were mainly represented by eicosapentaenoic acid (EPA) or 20:5 $\omega$ 3 (7.9 – 10.5%), and arachidonic acid (AA) or 20:4 $\omega$ 3 (4.0 – 7.7% of total FA). Docosahexaenoic acid (EPA) or 22:6 $\omega$ 3 was not present.

### Spring experiment, Daphnia treatment

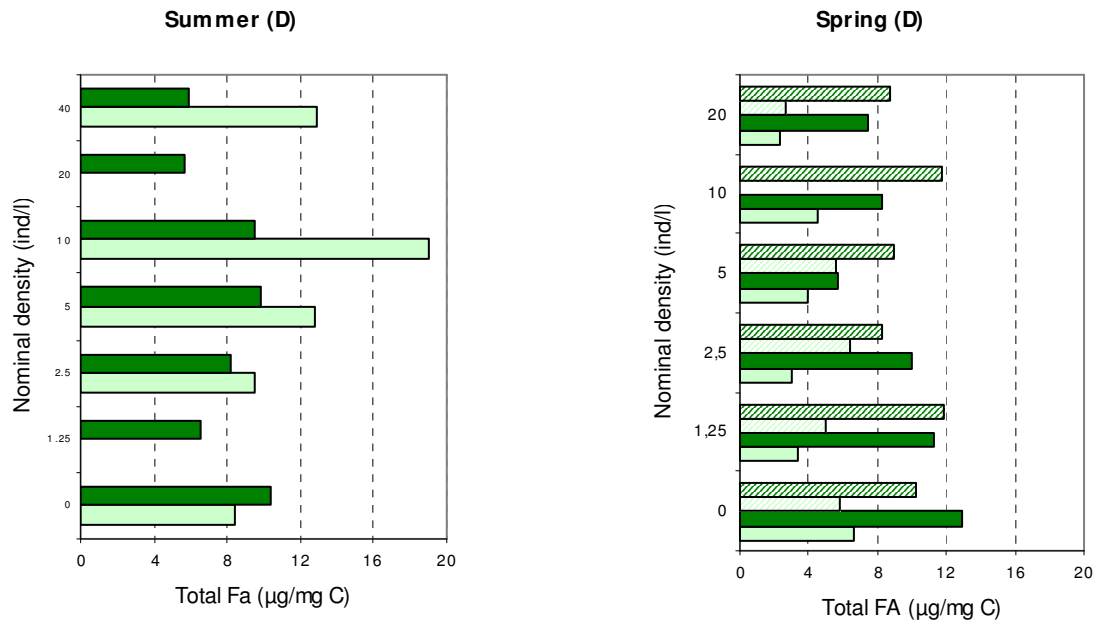
The total FA concentrations (Figure 3-2) in the seston varied from 2.37 to 12.92  $\mu\text{g C l}^{-1}$  (spring).

**Table 3-3** summarises the fatty acid composition in the summer phytoplankton community. During the entire study, SAFA accounted for 37.0 - 85.7% of total FA. They mostly consisted of palmitic acid or 16:0 (14.9 – 38.8% of total FA), myristic acid or 14:0 (7.7 – 14.0% of total FA) and, always in lower proportions, stearic acid or 18:0 (3.2 – 4.2% of total FA). Saturated acids with 20, 22 or 24 carbon atoms accounted for concentrations lesser than 0.9% of total FA.

The MUFA accounted for 5.6 – 17.3% of total FA, the great majority consisting of oleic acid or 18:1 $\omega$ 9 (2.1 – 7.3% of total FA) and palmitoleic acid or 16:1 $\omega$ 7 (2.3 – 9.8% of total FA), and vaccenic acid or 18:1  $\omega$ 7 (0.5 – 2.4% of total FA). 14:1 $\omega$ 5 and 20:1 $\omega$ 9 and those with 22 and 24 carbon atoms were not present.

The PUFA accounted for 6.1 – 49.3% of total FA (spring). They mainly belonged to the linolenate series ( $\omega$ 3) (5.3 – 32.0% of total FA), and also to the linoleates ( $\omega$ 6), but in lower proportions (3.6 – 25.7% of total FA). PUFA with 16 carbon atoms and three or four double bonds were not present. The concentration of PUFA with two double bonds (16:2 $\omega$ 4) was lower in spring (on average 0.41% of total FA) than in summer (on average 1.92% of total FA).

PUFA with 18 carbon atoms, such as 18:2 $\omega$ 6, 18:3 $\omega$ 3 and 18:4 $\omega$ 3 reached relatively high proportions (see **Table 3-3**). PUFA with 20 and 22 carbon atoms were mainly represented by eicosapentaenoic acid (EPA) or 20:5 $\omega$ 3 (1.4 – 13.8% of total FA), and arachidonic acid (AA) or 20:4 $\omega$ 3 (0.5 – 2.0% of total FA). Docosahexaenoic acid (EPA) or 22:6 $\omega$ 3 was only present in the spring seston (1.2 – 6.7% of total FA).



**Figure 3.2:** Variation in phytoplanktonic total fatty acid concentrations in the *Daphnia* (D) treatment (light green: first day of the experiment; dark green: last day of the experiment; dashed blocks denote the duplicate treatments).

### 3.2. Discussion: role of fatty acids in the food web

Zooplankton nutrition is not only determined by food concentration and its elemental composition. Specific essential food compounds like fatty acids (FA) play a significant role in zooplankton productivity. Especially the  $\omega$ 3- and  $\omega$ 6-family are important for metabolic growth and reproduction in zooplankton (Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996, Pond *et al.* 1996, Støttrup *et al.* 1999). Zooplankton must derive these FA from their diet because zooplankton has a limited ability to synthesize PUFA *de novo* (Gurr & Harwood, 1991).

The FA composition of phytoplankton in the experiments exhibited considerable variation. A possible explanation for this behaviour can be found in temperature (Waimann & Smith 1997, Brett & Müller-Navarra 1997), nutritional stress, differential grazing (Kilham *et al.* 1997; Gulati & Demott 1997) or taxonomic differences. Grazing can act on fatty acid composition via two independent mechanisms. First zooplankton can change the physiological state of microalgae through their potential impact on nutrient availability (Gulati & DeMott, 1997). Second zooplankton has the capability to influence the taxonomic composition of phytoplankton by differential grazing.

## Temperature

Temperature has a major effect on the FA composition of cell membranes. Low temperature results in elevated levels of unsaturated FA in polar lipids. This increase in unsaturation lowers melting points and maintains lipids in a liquid state for normal protoplasmic viscosity (Phleger 1991). These induced changes occur especially in PUFA (Nelson *et al.* 2002) which tend to increase their concentrations at lower temperatures, while the total concentration of lipids remains relatively stable (Wainman & Smith 1997). During our spring experiments temperature rose from 12 to 18 °C (**Table 3-1**) while simultaneously the concentrations of PUFAS increased as well as in copepod (**Table 3-2**) and in *Daphnia* (**Table 3-3**) treatments. This is the opposite of the general rule. However this rule was only confirmed to be significant for temperatures under 12 °C (Wainman & Smith 1997). Therefore I suggest that the influence of temperature on fatty acid composition in our experiments was negligible. There was no significant increase in temperature during the summer experiments, nevertheless the PUFA in the copepods treatment decreased with time. In the *Daphnia* treatment not trend was apparent.

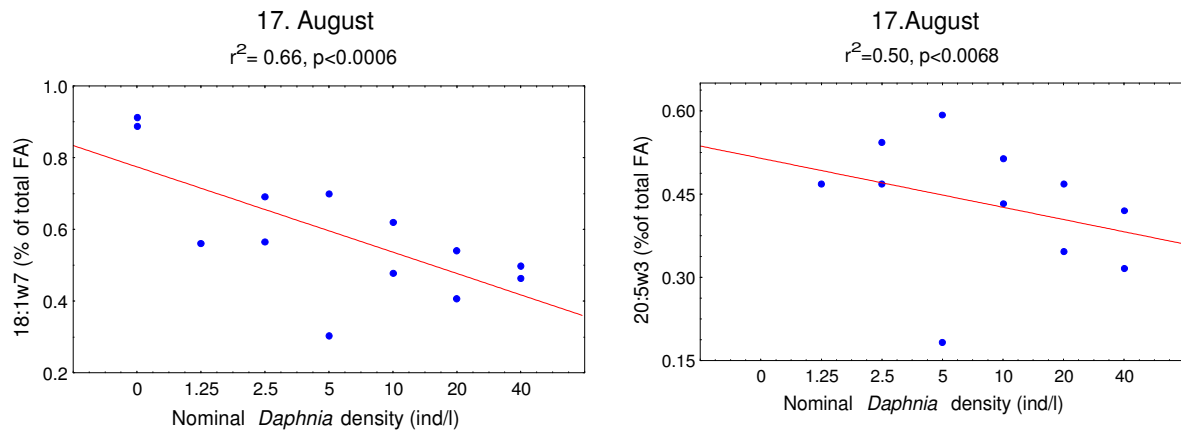
## Nutrients availability

It is generally understood that in freshwater foodwebs, phytoplankton production is limited by the availability of inorganic nutrients, mainly phosphorus, nitrogen and silica or by light (Brett *et al.* 1997; Wainman & Smith 1997). Nutrient limitation tends to depress the rate of cell division more intensively than the carbon accumulation through photosynthesis, and nutrient limitation should therefore result in algal cells that are rich in carbon storage products like lipids (Olsen 1998) or carbohydrates (Kuwata *et al.* 1993). At the start of the spring experiment the phytoplankton already showed signs of P limitation (mean ration of 240:28:1; Feuchtmayr 2004). The C:N:P ratio was independent of time and copepods density, resulting in a prolonged limitation during the experiment. In the *Daphnia* treatments P concentration was negatively influenced by grazer density intensifying the P limitation in this experiment. This could explain the overall increase of total fatty acid per unit carbon (**Figure 3.2**). In contrast seston C:P ratio at the start of the summer experiment were around the Redfield ratio, indicative of P and N sufficiency for phytoplankton growth (Sommer 2003). However, this sufficiency in P disappeared with the beginning of *Daphnia*

growth. Daphnids have high P requirements (Gulati & Demott 1997) and their growth in this experiment caused P limitation in phytoplankton (Sommer 2003). According to Gulati & Demott (1997) P limitation results in a decline in PUFA. This is supported by the PUFA data (**Table 3-2**)

### **Zooplankton grazing effects**

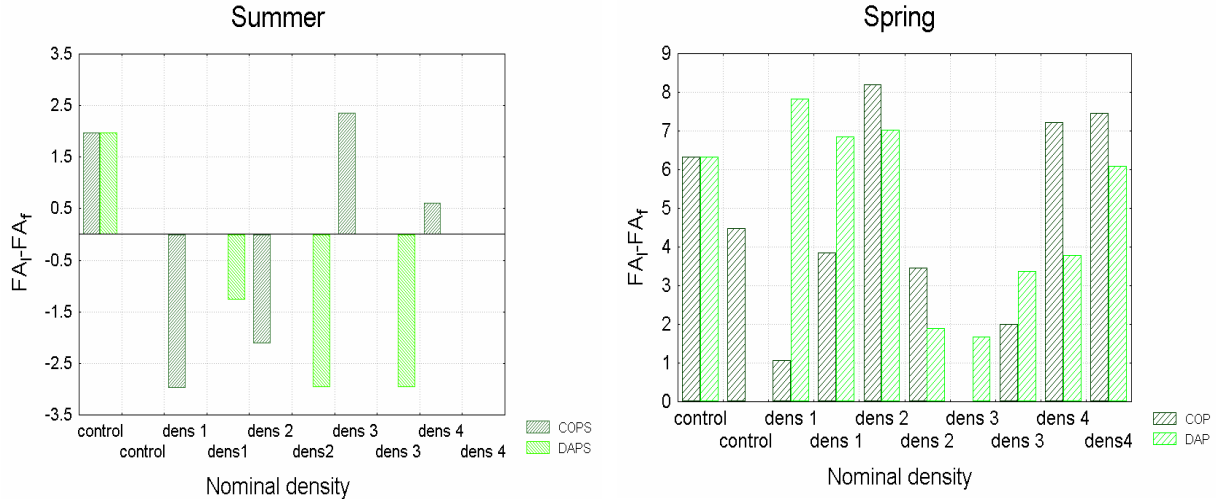
The different grazing behaviour of cladocerans and copepods and its impact on size and species composition of phytoplankton is another possibility to explain differences in concentrations of FA. On the one hand, calanoid copepods (majority in this study) detect their prey remotely and ingest particles selectively on basis of palatability and size (Roman 1977; Poulet & Marsot 1978; Fernandez 1979; DeMott 1988; Olson & Chisholm 1988; Runge & Cembella 1995; Bunay *et al.* 1998). Copepods even have the ability to distinguish between life and dead cells (DeMott 1986 and 1988; Butler *et al.* 1989), but because of their mode of feeding copepods are restricted to relatively large particles. According to Sommer (2003), in our summer experiment, the greatest algal biomass was found in the large size fraction ( $>1000\mu\text{m}^3$ ). Copepods had a positive impact on small phytoplankton, yet negatively affected medium to large sized phytoplankton species ( $>4000\mu\text{m}^3$ ; Sommer 2003). On the other hand cladocerans are able to detect food patches and retain particles by filtering (Gliwicz 1977; Gliwicz & Siedlar 1980; Geller & Müller 1984; Brendelberger 1985) and had in this experiment, a significantly negative impact on most phytoplankton species small in size ( $<4000\mu\text{m}^3$ ; Sommer 2003). These results are supported by the strong negative correlations between *Daphnia* and specific fatty acids (**Figure 3.3**) for small algae (18:1 $\omega$ 7 for cryptophytes *Rhodomonas minuta*, *Cryptomonas spp* and chrysophyte *Rhizochrysis spp* and 20:5 $\omega$ 3 for the diatom *Stephanodiscus parvus*) which decreased with the increase of the *Daphnia* density.



**Figure 3.3:** Relationship between *Daphnia* density and the fatty acids concentration (18:1ω7 and 20:5ω3) during the summer experiment. The dashed line is for the linear regression.

When present in great abundance small algae can cause high concentrations of fatty acids ( $\mu\text{g mgC}^{-1}$ ) due to higher presence of phospholipids per cell in contrast to bigger algae because of their higher surface to volume ratio.

The change of fatty acids concentration ( $\mu\text{g mgC}^{-1}$ ) during the summer experiments showed considerable differences between *Daphnia* and copepods (**Figure 3.4**).

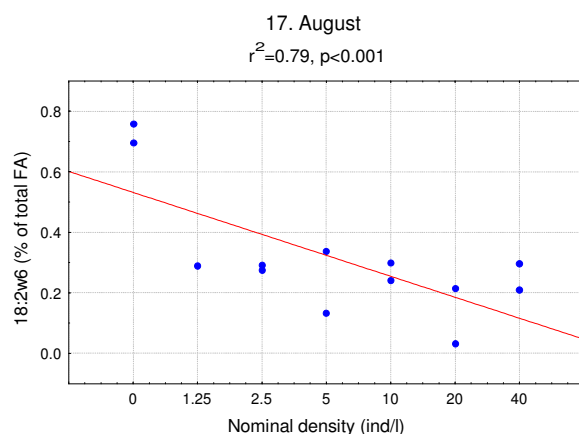


**Figure 3.4:** Difference between final and initial fatty acids concentration ( $\mu\text{g mgC}^{-1}$ ) in the Schöhsee mesocosm

In contrast to the impact by *Daphnia*, in the copepods treatment the fatty acids difference between start and end increased with increasing grazer density. The change in phytoplankton size caused by differential grazing obviously influenced the absolute amount of fatty acids per cell and thereby the food quality.

During the spring experiment the fatty acids concentration ( $\mu\text{g mgC}^{-1}$ ) increased in all treatments and showed no trends and an impact of differential grazing was not perceptible.

It is acknowledged that grazing resistance is an important factor in phytoplankton succession (Sommer *et al.* 1986), where low grazing rates would favor edible species and high grazing rates would favor inedible species (Sterner 1990). Nevertheless it was not possible to verify a good correlations between zooplankton density and fatty acids for specific groups of potentially toxic algae like blue greens in these experiments. In the summer experiment the concentration of 18:2 $\omega$ 6 (biomarker for cryptophyceae) showed a significant negative correlation with *Daphnia* density (**Figure 3.5**). This is in accordance with Sommer (2003) who found that *Daphnia* had a significant negative impact on cryptophytes like *Rodomonas minuta* and *Cryptomonas ssp.*



**Figure 3.5:** Relationship between *Daphnia* density and the 18:2 $\omega$ 6 fatty acid concentration during the summer experiment. The dashed line is for the linear regression.

### Zooplankton growth

Zooplankton growth may depend on the biochemical content of the algal food. There are strong indications that the polyunsaturated fatty acids (PUFA) affect significantly their growth rates in freshwater (Gullati *et al.* 1997, Jónasdóttir *et al.* 1995; Müller-Navarra 1995). Of these PUFA 20:5 $\omega$ 3 (EPA) and 22:6 $\omega$ 3 (DHA) appear to be particularly important (Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996, Pond *et al.* 1996, Støttrup *et al.* 1999). In this study significant correlations between zooplankton growth and EPA and DHA were not registered. It is assumed that the reasons for the failing correlation is the overall low concentration ( $<1.8$  and  $<0.9 \mu\text{g mgC}^{-1}$  respectively) of this PUFA in seston.

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### 3.3. Conclusions

Based on the results of this study we conclude that :

- The quality of the seston is different between summer and spring
- Temperature has a negligible influence on seston quality
- Both experiments were nutrient limited. The intensified P limitation caused by the high P requirement of *Daphnia* influenced seston quality concerning FA negatively in summer
- A grazing impact on seston taxonomic composition was only found in the summer *Daphnia* treatment
- Differential grazing of *Daphnia* and copepods influenced total FA concentration ( $\mu\text{g mgC}^{-1}$ ) in the summer experiments



# CHAPTER 4

## Chapter 4

### Hopavågen Mesocosms: fatty acids in marine water

The summer and spring mesocosm experiments in this fjord lasted for approximately one week. The general initial situation of both mesocosm experiments differed markedly in abiotic (**Table 4-1**) and biotic conditions.

**Table 4-1:** General water parameters and weather conditions during the Hopavågen experimental periods

	Summer	Spring
Period	16 – 22 July 2001	20-27 April 2002
Water temperature (°C)	12.5 – 13.3	8 - 9
Salinity (PSU)	30.8 – 31.1	31.7
Weather conditions	cloudy & rain	dry & sunny
Initial PO <sub>4</sub> <sup>-3</sup> (µg l <sup>-1</sup> )	<2.5	2.5 – 3.6
Initial NO <sub>3</sub> <sup>-</sup> (µg l <sup>-1</sup> )	<1.8	1.8 – 2.5
Seston N (µg l <sup>-1</sup> )	41.0 - 89.1	5.31 – 7.44
Seston P (µg l <sup>-1</sup> )	3.60 – 4.58	0.06 – 0.23
Seston C (µg l <sup>-1</sup> )	279.5 – 362.7	30.84 – 46.40

Average water temperature was 4-5°C higher in summer than in spring. The starting concentrations of dissolved nutrients in summer were much lower than those measured in spring. Summer seston was assumed to be limited in nitrogen (C:N:P ratio of 205:8:1, Sommer 2003), spring seston in phosphorus (C:N:P ratio of 288:48:1, Feuchtmayr 2004). Therefore the Hopavågen food web may be characterised as an nutrient limited system at the time of experimentation.

The summer plankton community was dominated by heterotrophic ciliates and nanoflagellates (Sommer 2003), the spring plankton community by diatoms (Feuchtmayr 2004).

The maximum seeding density of copepods (80 ind L<sup>-1</sup>) in summer represented about 3 times their maximal abundance in the Hopavågen fjord (N. Tokle, unpublished data) but was only slightly higher than the maximum *in situ* values during the experimental period (~60 ind L<sup>-1</sup>). The zooplankton community in this experiment consisted of a mixed assemblage of smaller calanoid copepods

dominated by *Temora longicornis* (45±5%), *Centropages hamatus* and *Centropages typicus* (together: 24±4%), as well as *Pseudocalanus elongatus* (24±6%) and a few *Acartia longiremes* (5±2%) (Sommer *et al* 2003, Sommer *et al.* 2004).

The mean copepod abundance in the fjord during the spring experiment was 2.3 individuals per litre (top 5 m). According to Feuchmayr (2004) this value underestimated the real abundances due to vertical migration. Saage (2003) found higher copepods abundances and reported 27 copepods per litre in deeper water (10-13 m). The zooplankton community was dominated by copepods (70-80%) consisting of *Calanus finmarchicus* (copepodite stage III-V), *Centropages hamatus* and *C. typicus* and *Oithona sp.* *Semibalanus balanoides* nauplii were abundant too, and these nauplii developed into cyprids during the experiment (Feuchmayr 2004, Saage 2003). Cyprids characteristically do not feed (Todd *et al.* 1996), therefore their influence on the microbial community was regarded as neglectable.

#### 4.1. Results: fatty acids abundance and composition

##### 4.1.1 Fatty acids in the seston

###### **Summer experiment**

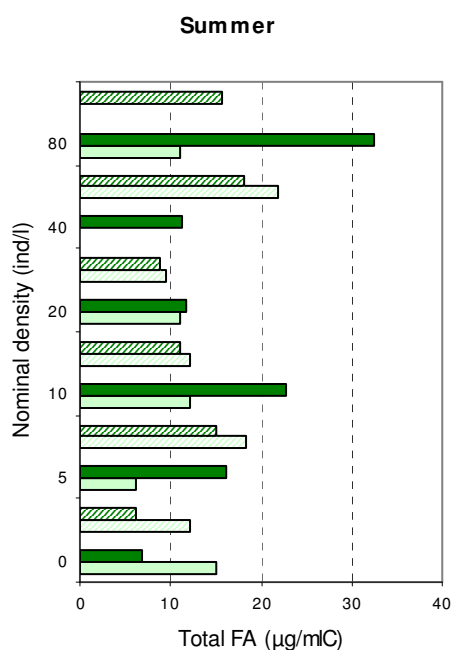
The total FA concentration (**Figure 4.1.**) in the seston varied from 6.19 to 22.66 µg mg C<sup>-1</sup>.

**Table 4-2** summarises the fatty acids composition in the summer phytoplankton community. During the entire study, SAFA accounted for 29.24 – 73.54% of total FA. They consisted mostly of palmitic acid or 16:0 (13.43 – 22.02% of total FA and, always in lower proportions, of myristic acid or 14:0 (5.51 – 12.73% of total FA) and of stearic acid or 18:0 (6.22 – 43.95% of total FA). Saturated acids with 20, 22 or 24 carbon atoms only accounted for a low proportion of total FA (mean <0.5%).

The MUFA accounted for 8.49 – 22.66% of total FA, the great majority consisting of oleic acid or 18:1ω9 (4.47 – 12.36% of total FA), palmitoleic acid or 16:1ω7 (0.23 – 6.1% of total FA), and vaccenic acid or 18:1 ω7 (0.39 – 5.41% of total FA). Other MUFA, such as 14:1ω5, 20:1ω9 and 20:1ω7 appeared in a more sporadic manner in (< 0.5% of total FA).

The PUFA accounted for 17.97 – 48.1% of total FA. They mainly belonged to the linolenate series (ω3) (13.76 – 39.78% of total FA), and also to the linoleates (ω6),

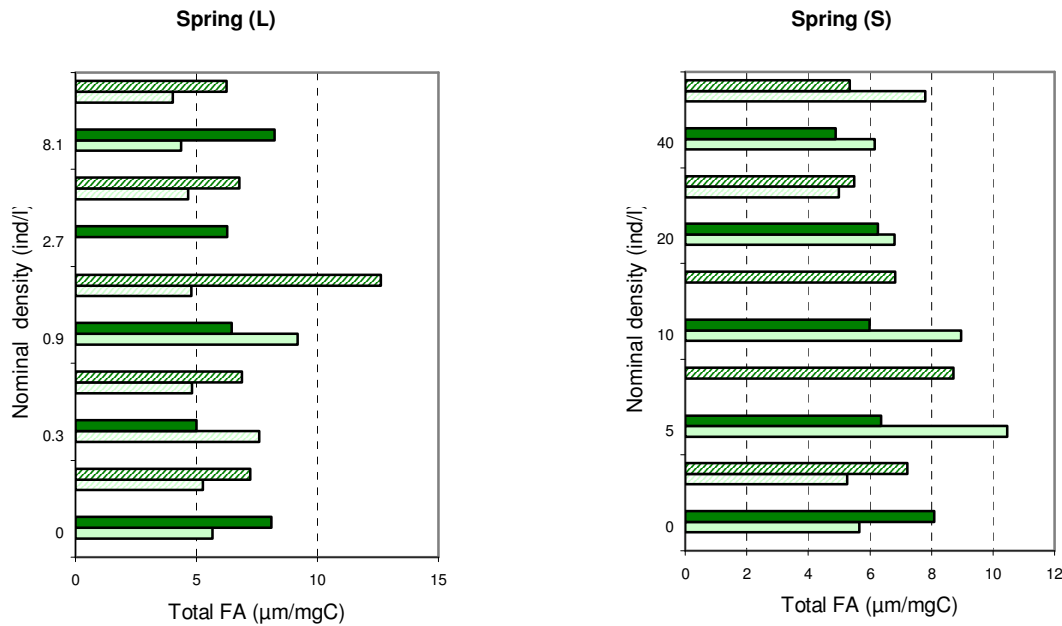
but in lower proportions (4.11 – 9.58% of total FA). PUFA with 16 carbon atoms and three or four double bonds were not detected. Those with two double bonds (16:2 $\omega$ 4) were relatively constant (<0.4% of total FA). PUFA with 18 carbon atoms, such as linoleic acid or 18:2 $\omega$ 6, linolenic acid or 18:3 $\omega$ 3 and stearidonic acid or 18:4 $\omega$ 3 reached relatively high proportions (see **Table 4-2**). PUFA with 20 and 22 carbon atoms were mainly represented by eicosapentaenoic acid (EPA) or 20:5 $\omega$ 3 (3.20 – 10.83% of total FA), docosahexaenoic acid (DHA) or 22:6 $\omega$ 3 (n.d. – 12.05% of total FA) and arachidonic acid (AA) or 20:4 $\omega$ 3 (n.d. – 1.17 % of total FA).



**Figure 4.1:** Variation in phytoplanktonic total fatty acid concentrations in the summer copepod treatment (light green: first day of the experiment; dark green: last day of the experiment; dashed blocks denote the duplicate treatments).

### Spring experiment

The total FA concentration (**Figure 4.1.**) in the seston varied from 4.02 to 9.17  $\mu\text{g mg C}^{-1}$  (large copepods treatment – hereafter referred to as L) and from 4.89 to 10.45  $\mu\text{g mg C}^{-1}$  (small copepods treatment – hereafter referred to as S).



**Figure 4.2:** Variation in phytoplanktonic total fatty acid concentrations in the spring Copepod treatment (light green: first day of the experiment; dark green: last day of the experiment; dashed blocks denote the duplicate treatments).

**Table 4-2** summarises the fatty acid composition in the spring phytoplankton community. During the entire study, SAFA accounted for 53.54 – 77.70% of total FA (L) and for 43.54 to 78.37 of total FA (S). They consisted mostly of palmitic acid or 16:0 (15.13 – 52.52% of total FA and 24.45 – 46.03% of total FA, large and small respectively) and, always in lower proportions, of myristic acid or 14:0 (10.93 – 59.63 % of total FA and 11.54 – 19.73% of total FA) and of stearic acid or 18:0 (2.05 – 19.64% of total FA and 2.36 – 10.10% of total FA). Saturated acids with 20, 22 were only sporadic and this with 24 were not detected.

The MUFA accounted for 6.26 – 28.05% of total FA (L) and 11.80 – 30.32% of total FA (S), the great majority consisting of oleic acid or 18:1 $\omega$ 9 (2.40 – 7.72% of total FA and 3.24 – 7.51% of total FA), palmitoleic acid or 16:1 $\omega$ 7 (1.24 – 19.23% of total FA and 2.86 – 23.85% of total FA), and vaccenic acid or 18:1  $\omega$ 7 (0.96 – 3.19% of total FA and 1.29 – 3.00% of total FA). Other MUFA, such as 14:1 $\omega$ 5, 20:1 $\omega$ 9 and 20:1 $\omega$ 7 appeared in a more sporadic manner (< 0.5%of total FA).

The PUFA accounted for 2.52 – 35.12% of total FA (L) and for 8.87 –37.30% of total FA (S). They mainly belonged to the linolenate series ( $\omega$ 3) (1.53 – 30.17% of total FA and 8.81 – 30.07% of total FA), and also to the linoleates ( $\omega$ 6), but in lower proportions (1.72 – 5.16% of total FA and 1.89- 4.83). PUFA with 16 carbon atoms and three or four double bonds were not detected. Those with two double bonds

**Table 4-2: Fatty acids composition** during summer and spring experiments expressed as % of total fatty acids. For the purpose of clarity only major component fatty acids are shown although summary totals include all data. ( first day - last day; {1} {2} = replicate)

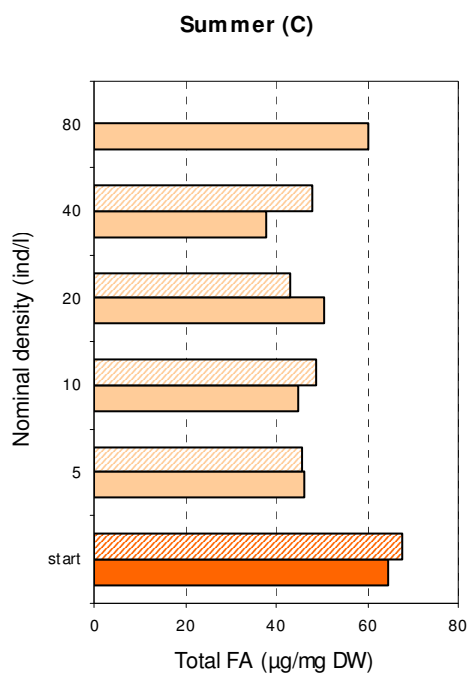
	summer control	spring Lcontrol	Scontrol	summer cop5	spring l1.3	S5	summer cop10	spring l2.5	S10	summer cop20	spring l5	S20	summer cop40	spring l10
SAFA														
14:00	7.78-9.10	12.60-16.38	12.6-16.38	10.18-5.51	10.93-15.77	12.07-18.5	8.89-6.25	12.54-17.72	12.15-1.75	9.48-10.18	-19.8	14.96-19.73	10.21-12.73	18.57-13.11
	10.57-8.25	25.61-15.32	25.6-15.32	10.76-5.73	24.57-16.25	-18.00	8.37-7.92	14.53-59.66	-1.7	9.40-10.57	27.96-20.87	15.56-18.98	7.95-8.55	25.95-16.88
16:00	13.43-18.04	34.77-32.25	34.77-32.25	19.22-15.17	29.14-19.60	27.23-34.2	19.82-20.74	34.81-34.06	24.45-33.68	18.91-20.47	-35.6	46.03-35.15	18.66-19.56	52.52-40.37
	20.30-18.07	46.02-31.84	46.02-31.84	22.02-18.25	46.74-36.39	-29.94	17.22-17.81	43.61-15.13	-28.8	18.55-19.22	52.30-34.96	44.82-37.38	14.40-17.87	48.56-41.89
18:00	7.59-10.96	4.69-3.30	4.69-3.30	6.77-30.47	4.06-19.64	3.22-6.10	8.57-29.89	5.28-4.63	2.36-4.49	6.81-9.95	-5.5	10.10-6.02	6.22-6.98	8.15-4.58
	9.03-12.62	4.10-5.24	4.10-5.24	7.49-27.39	4.88-4.99	-3.37	9.71-12.89	6.63-2.05	-4.1	6.66-8.90	4.13-4.82	-4.08	20.45-16.05	7.52-4.46
MUFA														
16:1ω7	4.42-6.14	9.07-15.02	9.07-15.92	5.34-3.55	10.12-7.99	10.56-17.40	4.21-2.27	8.18-11.56	13.21-17.66	4.92-3.68	-5.0	4.78-10.63	5.77-3.99	3.48-1.24
	4.53-6.06	4.81-19.23	4.81-19.23	0.23-3.21	3.70-14.07	-20.46	3.91-4.01	6.55-5.67	-23.9	5.46-4.50	3.24-1.37	2.87-10.88	4.73-2.85	2.50-5.10
18:1ω9	13.98-9.92	5.83-3.97	5.83-3.97	9.79-8.88	5.14-4.61	4.72-4.54	12.36-8.11	5.94-4.80	4.11-4.05	10.43-9.65	-4.7	6.48-5.18	9.57-9.81	7.72-6.25
	10.37-9.03	5.61-5.72	5.61-5.72	11.64-8.43	4.00-5.15	-4.70	10.83-9.85	5.85-2.41	-3.24	9.05-10.41	2.72-4.72	7.51-4.45	9.60-9.64	3.05-5.61
18:1ω7	4.26-5.41	1.89-2.97	1.89-2.97	3.31-4.27	2.23-3.10	2.30-2.29	3.34-0.39	1.97-2.87	1.84-3.00	3.43-2.54	-2.5	1.30-1.92	3.65-2.81	1.18-3.19
	0.76-4.22	1.38-2.76	1.38-2.76	3.42-2.56	1.26-2.38	-2.01	3.39-2.40	1.58-1.68	-2.88	3.37-2.99	0.96-2.20	1.82-1.88	3.89-2.29	0.70-2.51
20:1ω9	nd-nd	nd-nd	nd-0.07	nd-nd	nd-nd	nd-0.06	nd-0.41	nd-nd	nd-nd	nd-nd	nd-nd	nd-0.12	nd-nd	nd-nd
	nd-nd	nd-nd	nd-0.07	nd-nd	nd-nd	nd-nd	nd-0.23	nd-0.04	nd-nd	0.27-nd	nd-nd	nd-nd	nd-nd	nd-nd
22:1ω9	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	-nd	nd-nd	nd-nd	nd-nd
	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd
ω6														
18:2ω6	6.29-6.20	3.88-4.02	3.88-4.02	5.01-4.53	4.50-4.88	4.37-3.47	5.88-7.82	4.04-3.89	3.85-3.68	5.53-7.49	-3.84	2.00-3.16	5.70-8.53	0.99-4.36
	5.49-4.91	1.58-1.24	1.57-1.24	5.08-6.07	1.22-3.99	-3.71	5.74-6.56	3.13-2.17	-3.17	5.16-7.45	0.79-4.42	2.69-2.55	5.34-8.57	0.55-2.60
20:4ω6	0.74-nd	nd-0.16	nd-0.13	0.91-0.32	0.46-0.17	nd-nd	0.87-0.30	nd-0.17	nd-0.18	0.83-0.32	-3.7	nd-nd	0.90-0.40	nd-0.11
	0.44-nd	nd-0.09	0.31-0.07	1.04-0.30	nd-0.13	nd-0.1	0.62-0.71	nd-0.07	-0.15	0.94-0.36	nd-nd	nd-nd	0.67-nd	nd-nd
ω3														
18:3ω3	7.06-1.97	4.09-3.31	4.09-3.31	4.94-3.51	5.48-1.21	5.67-2.66	6.08-2.83	4.41-4.02	4.88-3.13	6.05-4.83	-5.25	nd-2.43	5.75-5.69	nd-9.32
	6.78-2.50	4.72-3.96	4.72-3.96	5.08-2.04	8.45-4.13	-3.11	6.17-3.68	3.07-2.33	-2.04	5.69-4.76	3.62-7.58	2.23-2.75	4.64-4.20	4.68-5.81
18:4ω3	8.14-3.85	8.75-5.69	8.75-5.69	5.61-4.74	11.05-4.79	11.17-4.05	5.74-3.19	8.65-7.34	11.62-5.08	6.57-5.08	-10.9	4.46-5.81	6.54-6.27	1.53-11.49
	7.34-4.58	3.01-6.88	3.01-6.88	5.51-2.98	2.12-5.79	-5.50	7.27-4.98	6.07-3.95	-2.04	6.54-5.15	1.56-12.52	4.60-5.53	5.50-5.08	1.20-10.45
20:4ω3	1.18-nd	nd-0.13	nd-0.13	1.06-0.62	nd-0.27	nd-nd	0.79-0.46	nd-0.15	nd-0.18	0.97-nd	-nd	nd-nd	0.93-nd	nd-nd
	0.66-nd	0.31-0.07	0.31-0.07	0.99-0.61	0.31-nd	-0.01	0.91-0.87	nd-0.05	-0.2	1.17-nd	0.28-0.00	nd-nd	0.73-nd	nd-nd
20:5ω3 (EPA)	10.83-9.25	4.00-4.30	4.00-4.30	9.19-6.78	5.78-6.05	6.26-nd	7.84-4.89	4.24-3.61	8.25-3.10	9.54-6.53	-nd	1.81-2.04	9.29-5.56	nd-2.34
	6.57-9.82	0.54-2.46	0.54-2.46	8.25-5.76	0.48-2.67	-3.79	8.54-9.28	3.03-1.70	-4.1	10.73-7.59	0.38-3.04	2.87-3.18	7.72-7.44	0.33-2.41
22:5ω3	nd-nd	nd-0.08	nd-0.07	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	0.30-nd	-nd	nd-nd	0.34-nd	nd-nd
	nd-nd	nd-0.04	nd-0.04	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	nd-nd	0.37-nd	nd-nd	nd-nd	nd-nd	nd-nd
22:6ω3 (DHA)	11.96-7.68	5.35-1.34	5.35-1.34	9.68-nd	7.40-2.21	7.85-0.65	8.63-6.92	5.01-1.00	8.32-0.77	10.49-9.38	-1.5	nd-0.66	9.50-7.37	nd-0.58
	7.90-nd	0.24-0.78	0.24-0.78	9.03-6.91	0.17-0.94	-0.94	11.02-11.74	nd-0.52	-0.85	12.05-10.38	0.15-1.33	4.1-0.85	7.08-11.30	nd-0.75
tot SAFA	29.24-49.06	56.71-55.57	56.71-55.57	42.47-58.67	47.39-63.15	46.62-60.49	42.41-60.93	57.21-58.54	43.53-57.20	39.42-48.77	-61.37	78.37-65.27	39.57-47.58	85.10-60.51
	46.94-47.95	77.50-53.54	77.50-53.54	47.28-60.17	77.83-60.0	-52.73	39.72-42.89	70.73-77.71	-54.36	36.46-45.03	85.93-62.26	71.30-66.62	48.45-48.52	86.53-64.34
tot MUFA	22.67-21.7	16.79-23.14	16.79-23.14	18.44-17.20	17.49-15.89	17.58-24.51	19.92-11.18	16.08-19.76	19.16-24.65	18.78-15.87	-12.55	12.76-18.42	19.33-16.62	12.38-10.68
	15.89-19.31	11.80-28.05	11.80-28.05	15.29-14.40	8.95-21.75	-27.39	18.14-16.49	13.97-10.87	-30.32	18.16-17.90	6.92-8.29	12.19-17.32	18.21-14.91	6.25-13.22
tot PUFA	48.09-29.47	26.50-21.30	26.49-21.29	39.09-24.12	35.12-20.96	35.79-15.00	37.68-27.89	26.69-21.65	37.30-18.15	41.81-35.35	-26.08	8.87-16.30	41.10-35.81	2.5-28.80
	37.17-32.74	10.70-18.41	10.70-18.41	37.43-25.43	13.22-19.25	-19.87	42.14-40.62	15.29-11.43	-15.31	45.38-37.08	7.14-29.45	16.50-16.06	33.34-35.58	7.22-22.44

(16:2 $\omega$ 4) were present at end of experiment and not exceed 2.34% of total FA. PUFA with 18 carbon atoms, such as linoleic acid or 18:2 $\omega$ 6, linolenic acid or 18:3 $\omega$ 3 and stearidonic acid or 18:4 $\omega$ 3 reached relatively high proportions (see **Table 4-2**). PUFA with 20 and 22 carbon atoms were mainly represented by eicosapentaenoic acid (EPA) or 20:5 $\omega$ 3 (not detected – 6.05% of total FA and no detected – 8.24% of total FA), docosahexaenoic acid (DHA) or 22:6 $\omega$ 3 (no detected – 7.40% of total FA and no detected – 7.85% of total FA) and arachidonic acid (AA) or 20:4 $\omega$ 3 (no detected – 3.65% of total FA and no detected – 1.99% of total FA).

#### 4.1.2. Fatty acids in copepods

##### *Summer experiment*

The total FA concentrations in copepods (**Figure 4.3**) varied from 37.68 to 67.79  $\mu\text{g mg}^{-1}$  DW. **Table 4-3** summarises the fatty acid composition in the zooplankton community. Predominant fatty acids were 22:6 $\omega$ 3 (DHA), 20:5 $\omega$ 3 (EPA), 16:0 and 18:4  $\omega$ 3. In this experiment the biomarker fatty acid for flagellates (18:1 $\omega$ 9) was present in significant amounts (13.1 - 19.4% of total FA) and the  $\omega$ 3-family fatty acids were clearly the dominant fatty acids (45.1 – 59.4% of total FA).



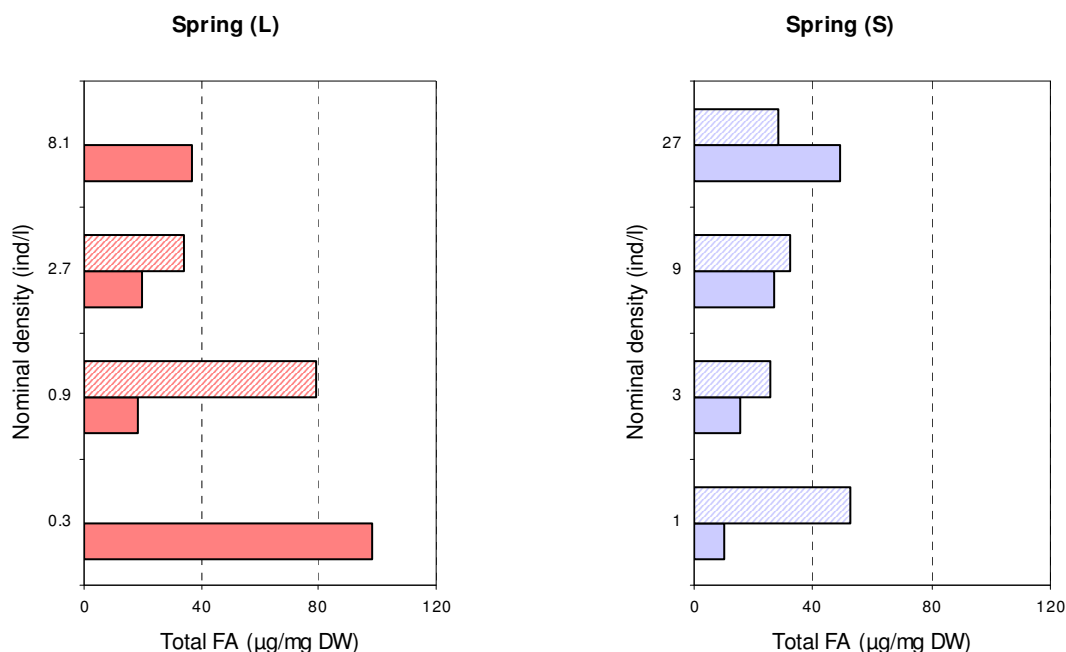
**Figure 4.3:** Variation in copepod total fatty acid concentrations in summer (light orange: first day of the experiment; dark orange: start values; dashed blocks denote the duplicate treatments)

**Spring experiment****Large copepod treatment (L)**

The total FA concentrations (**Figure 4.4**) varied from 18.35 to 98.05  $\mu\text{g mg}^{-1}$  DW. **Table 4-3** summarises the fatty acids composition in the zooplankton community. Predominant fatty acids were 20:5 $\omega$  (EPA), 16:0, 22:0 and 22:6 $\omega$  (DHA). In this treatment the biomarker fatty acid for diatoms 16:1 $\omega$ 7 was present in significant amounts (6.7 – 11.4% of total FA) and SAFA were the dominant fatty acids (53.6 – 62.9% of total FA)

**Small copepod treatment (S)**

The total FA concentrations (**Figure 4.4**) varied from 10.31 to 52.53  $\mu\text{g mg}^{-1}$  DW. **Table 4-3** summarises the fatty acids composition in the zooplankton community. Predominant fatty acids were 16:0, 18:0, 22:6 $\omega$ 3 (DHA) and 20:5 $\omega$ 3 (EPA). In these treatments the biomarker fatty acid for diatoms 16:1 $\omega$ 7 was present in significant amounts (10.3 – 15.4% of total FA) and the SAFA were the dominant fatty acids (52.1 – 59.3% of total FA)



**Figure 4.4:** Variation in copepod total fatty acid concentrations in the spring large copepod treatment (L) and spring small copepod treatment (S)(dashed blocks denote the duplicate treatments)



**Table 4-3:** Animal fatty acid composition during summer and spring experiments expressed as % of total fatty acids. For the purpose of clarity only major component fatty acids are shown although summary totals include all data. ({1} {2} = replicate)

	summer cop5	spring l1.3	S5	summer cop10	spring l2.5	S10	summer cop20	spring l5	S20	summer cop40	spring l10	S40	summer cop80	
<b>SAFA</b>														
14:0	3.07	39.53	34.91	3.42	24.25	82.51	2.19	42.47	34.08	2.85		30.34	1.88	
	3.26	38.04	36.05	3.50	38.49	8.69	2.73	37.87	31.55	2.74	40.12	31.02	1.95	
16:0	11.82	10.29	16.65	12.96	6.34	2.78	9.45	10.18	14.91	12.99		21.10	9.21	{1}
	13.26	11.04	15.06	14.55	7.40	3.10	10.95	11.72	12.26	11.83	13.40	20.90	8.83	{2}
18:0	3.33	0.66	1.80	3.65	0.76	0.41	2.85	0.44	1.50	3.25		2.07	2.42	{1}
	10.27	1.21	1.65	4.15	1.02	0.38	2.54	1.06	1.40	2.96	0.96	2.07	2.21	{2}
<b>MUFA</b>														
16:1ω7	2.11	10.43	12.68	2.13	6.58	2.23	2.22	10.66	12.02	1.88		10.30	2.06	{1}
	1.52	11.39	15.38	1.88	8.11	3.82	2.54	10.65	12.18	1.94	10.10	12.51	1.96	{2}
18:1ω9	13.43	2.06	3.30	3.87	1.47	0.21	15.99	1.40	3.32	19.36		2.83	15.02	{1}
	15.97	1.06	1.25	13.28	3.28	0.79	13.09	3.46	4.14	16.37	3.16	4.09	14.54	{2}
18:1ω7	1.96	0.59	1.39	13.90	0.20	0.14	1.95	0.44	nd	1.78		1.44	1.63	{1}
	1.77	0.04	0.41	2.01	0.95	0.38	1.63	0.92	1.19	1.81	0.91	1.61	1.61	{2}
20:1ω9	nd	0.07	nd	nd	0.08	nd	nd	0.03	0.10	nd		nd	nd	{1}
	nd	0.04	0.07	nd	0.06	0.24	nd	0.07	0.05	nd	0.03	0.93	nd	{2}
22:1ω9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	{1}
	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	{2}
<b>ω6</b>														
18:2ω6	2.81	1.22	1.12	2.65	0.79	0.27	3.44	0.77	1.23	3.02		1.40	3.22	{1}
	2.66	1.49	1.56	2.65	1.79	0.38	2.53	1.70	1.61	2.81	1.49	1.69	3.38	{2}
20:4ω6	0.97	nd	0.11	1.20	0.42	nd	1.10	0.16	0.14	0.99		0.13	1.03	{1}
	0.69	0.15	nd	1.14	0.19	2.60	1.40	0.14	0.16	1.12	nd	0.15	1.06	{2}
<b>ω3</b>														
18:3ω3	2.14	0.08	0.41	2.16	4.36	0.37	0.40	0.05	0.29	1.38		2.04	0.41	{1}
	4.71	1.90	1.96	2.96	0.08	0.02	1.08	1.75	0.19	1.60	0.32	2.54	0.35	{2}
18:4ω3	4.81	6.27	7.95	4.79	1.03	1.58	7.06	5.79	8.97	5.00		7.13	6.39	{1}
	4.63	9.35	8.65	0.36	10.64	2.50	4.79	10.50	11.44	4.92	8.63	0.84	6.59	{2}
20:4ω3	1.68	0.67	0.58	1.97	1.54	nd	1.82	0.75	0.61	1.92		0.50	1.98	{1}
	1.61	0.64	nd	2.01	0.72	1.89	2.19	0.52	0.64	1.82	0.67	0.59	1.98	{2}
20:5ω3 (EPA)	16.57	10.13	7.46	15.29	20.96	1.79	16.53	10.75	8.23	13.51		7.74	17.40	{1}
	12.53	9.84	9.56	16.09	11.21	38.61	18.92	7.05	10.62	15.74	7.25	9.62	17.36	{2}
22:5ω3	1.66	0.30	0.22	1.96	0.63	0.05	0.83	0.30	0.26	1.46		0.24	1.03	{1}
	0.95	3.20	0.23	1.58	0.30	1.08	1.38	0.19	0.32	1.44	0.24	0.23	1.24	{2}
22:6ω3 (DHA)	27.13	3.05	3.50	24.40	6.10	0.77	28.19	3.09	3.37	24.51		5.06	30.56	{1}
	20.63	0.05	3.41	26.92	3.17	13.80	28.57	1.77	3.57	27.18	2.31	6.23	31.54	{2}
tot SAFA	20.47	60.62	59.33	21.24	53.63	89.14	16.02	62.86	55.58	20.12		57.92	14.52	{1}
	28.47	58.87	54.40	24.48	56.85	31.68	17.34	58.37	52.06	18.48	61.63	56.92	13.61	{2}
total ω3	54.27	20.57	20.10	50.86	34.81	4.64	55.23	20.79	21.77	48.15		22.72	58.12	{1}
	45.07	25.02	24.34	50.23	26.19	57.90	57.33	21.82	26.85	53.04	19.46	20.20	59.43	{2}

## 4.2. Discussion: role of fatty acids in the food web

### 4.2.1. Fatty acids in seston

The total amount of fatty acids ranging from  $37.15 \pm 9.87 \mu\text{g l}^{-1}$  to  $46.9 \pm 16.4 \mu\text{g l}^{-1}$  during the summer experiment and from  $21.31 \pm 6.7$  to  $24.03 \pm 6.44 \mu\text{g l}^{-1}$  for the large copepods treatment (L) and from  $18.95 \pm 6.7$  to  $32.88 \pm 9.9 \mu\text{g l}^{-1}$  for the small copepods treatment (S) during spring didn't match completely with early reports. Reuss & Poulsen (2002) reported, that the amount of total fatty acids ranged from 55 to  $132 \mu\text{g l}^{-1}$  during spring bloom and from 1 to  $5 \mu\text{g l}^{-1}$  during the post bloom.

In agreement with early reports (Reitan *et al.* 1994, Hazzard & Kleppel 2003) the 14:0, 16:0 and 16:1 fatty acids were the most abundant fatty acids in spring. Other studies (Støttrup & Jensen 1990, Jónasdóttir *et al.* 1995, Pond *et al.* 1996) included 20:5 $\omega$ 3 and 22:6 $\omega$ 3 among the most abundant fatty acids as in our summer experiment. However, the absolute concentrations of 20:5 $\omega$ 3 and 22:6 $\omega$ 3 were low in summer seston ( $\sim 0.03 \text{ mg l}^{-1}$ ) and scarce in spring ( $< 0.003 \text{ mg l}^{-1}$ ). Previous studies verified a reduction in the synthesis of  $\omega$ 3-PUFAS (Reitan *et al.* 1994, Gulati & Demott 1997, Schmidt & Jónasdóttir 1997) in microalgal growth under nutrient limitation resulting in the reduced relative contents of 20:5 $\omega$ 3 (EPA) and 22:6  $\omega$ 3 (DHA).

Although only the spring phytoplankton community was dominated by diatoms, the initial percent profiles of 16:0, 20:5 $\omega$ 3, 22:6 $\omega$ 3, total  $\omega$ 3 and total  $\omega$ 6 fatty acids of seston (**Table: 4-2**) in both seasons were in agreement with other studies on diatom growth under nutrient limitation (Reitan *et al.* 1994, Volkman *et al.* 1989). However, the fatty acid 16:1 $\omega$ 7 another biomarker for diatoms (Desvilettes *et al.* 1997) had significantly higher values in spring than in summer.

In contrast, the concentration of the fatty acid 22:6 $\omega$ 3 was twice as high in summer than in spring. According to Pond *et al.* (1996) high 22:6 $\omega$ 3 concentrations can be associated with ciliates presence. Ciliates derive this fatty acid from their microplanktonic prey, particularly small flagellates and dinoflagellates (Sargent *et al.* 1995a). The high values of the fatty acid 22:6 $\omega$ 3 in the summer experiment

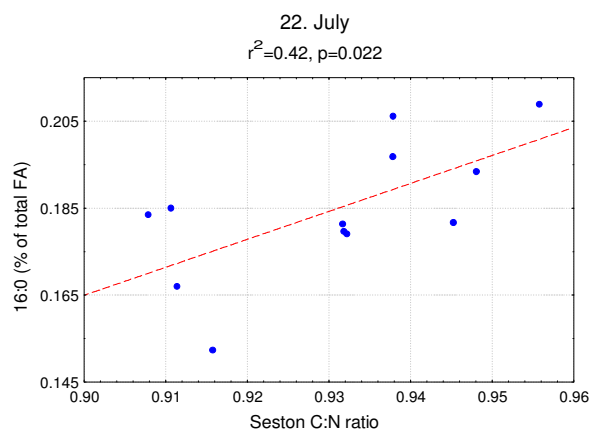
corresponded with the dominance of heterotrophic ciliates and nanoflagellates in the summer plankton community (Sommer 2003). The potential transfer of this fatty acid from flagellates to ciliates is supported by Zöllner (2004) who found a cascading predation impact in this mesocosm experiment.

### **Nutrient influence on fatty acids**

Both experiments showed a decrease over time in the amount of PUFA compensated by the increasing content of SAFA and MUFA all treatments. Such a change in fatty acid composition is indicative of nutrient limitation (Reitan *et al.* 1994, Müller-Navarra 1995, Gulati & Demott 1997).

### **Summer experiment**

In summer an influence of N limitation could only be demonstrated for the fatty acid 16:0 (**Figure 4.5**). This significantly positive correlation between the C:N ratio and the content of 16:0 in seston explained only 42% of the variation of this fatty acid in the seston, because grazing and the resulting phytoplankton succession had an important impact too.

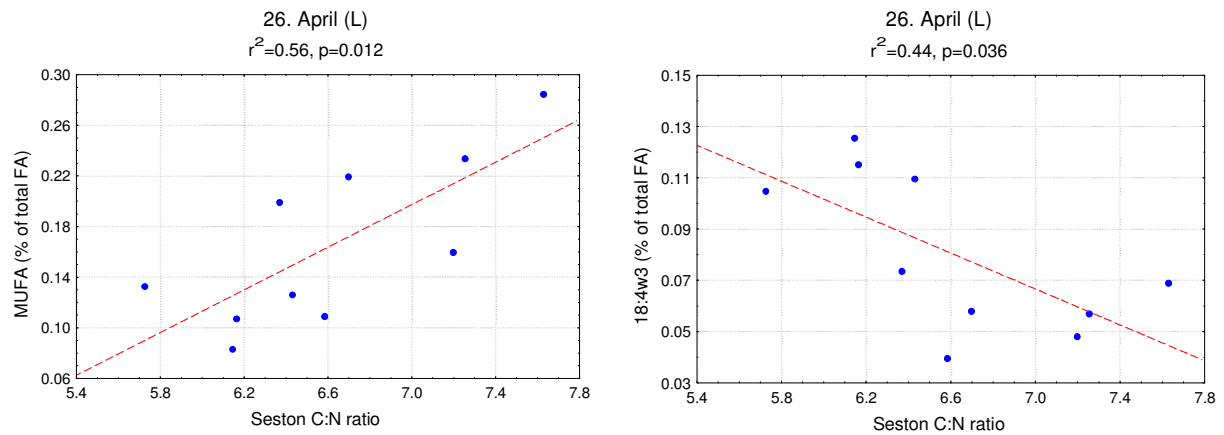


**Figure 4.5:** Relationship between fatty acid concentration in seston and seston C:N ratio in summer. The dashed line is for the linear regression. All data were normalized.

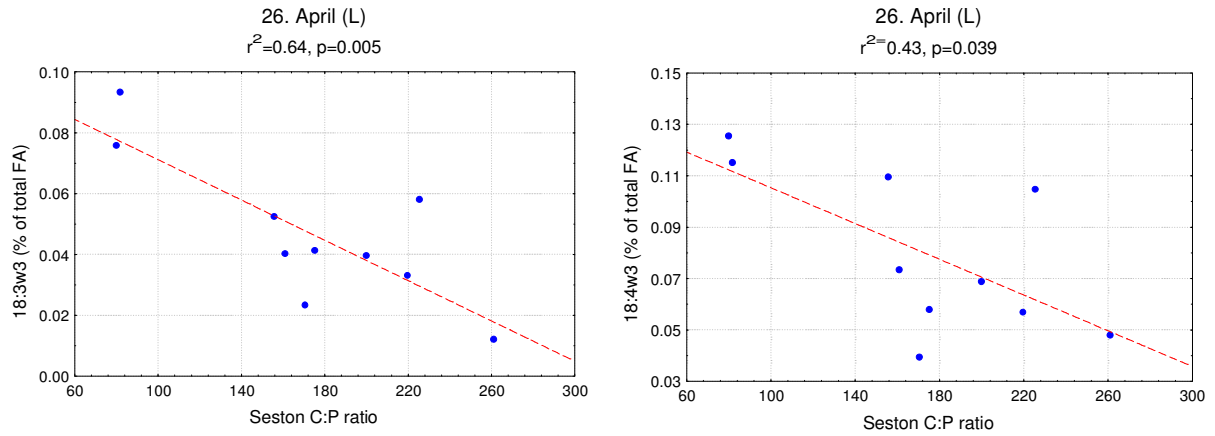
### **Spring experiment, large copepod treatments**

In the P limited spring experiment there was a significantly positive correlation between C:N ration and MUFA in the large copepod treatments (**Figure 4.6** and

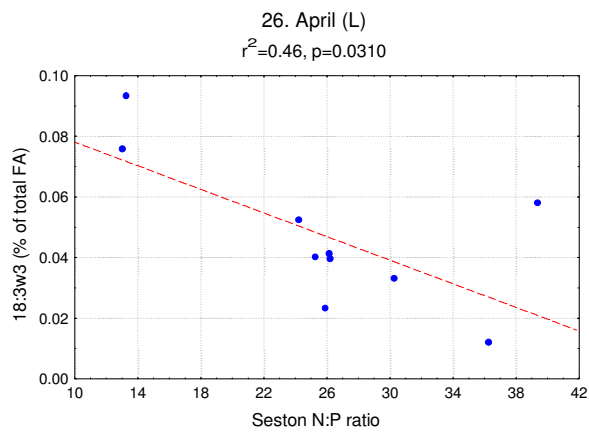
**Figure 4.8).** The increase of MUFA in algae under limited growth conditions was previously confirmed (e.g. by Reitan *et al.* 1994, Müller-Navarra 1995). C:N and C:P had significantly negative correlations with the fatty acid 18:4 $\omega$ 3 (**Figure 4.6** and **4.7**). This could be an indication that, under nutrient limitation, algae are not able to transform 18:3 $\omega$ 3 to 18:4 $\omega$ 3. The C:P ratio was negatively correlated with the polyunsaturated fatty acids (PUFA) 18:3 $\omega$ 3 and 18:4 $\omega$ 3 (**Figure 4.7** and **4.8**). The decrease of PUFA in relation to the SAFA and MUFA during nutritional limitation was already demonstrated (Reitan *et al.* 1994, Müller-Navarra 1995, Gulati & Demott 1997, Shin *et al.* 2000). The N:P ratio was negatively correlated with 18:3 $\omega$ 3 (**Figure 4.8**) too. This results provided additional evidence for the important influence of nutrients on fatty acid composition.



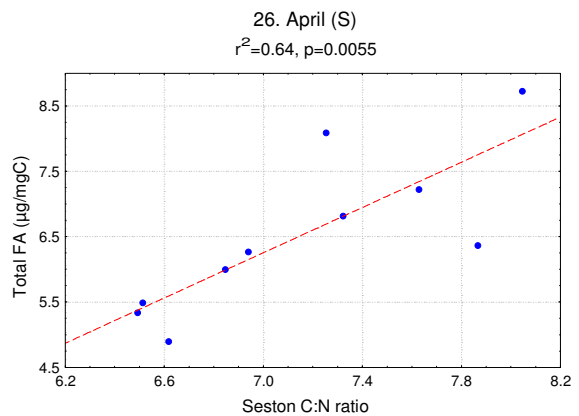
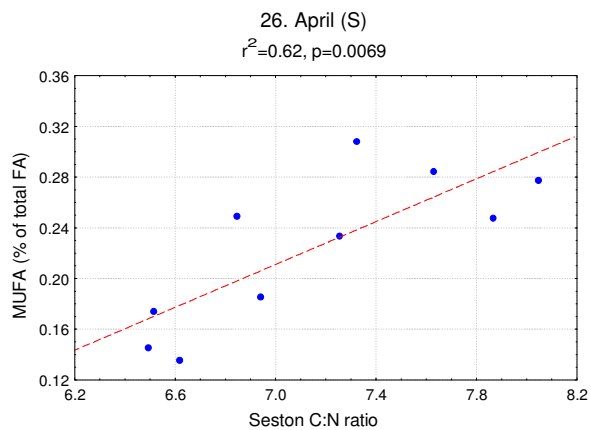
**Figure 4.6:** Relationship between fatty acid concentration in seston and seston C:N ratio in spring, large copepod treatment. The dashed line is for the linear regression. All data were normalized.

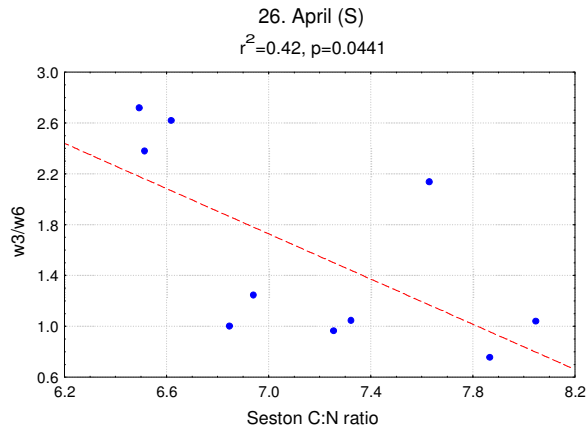


**Figure 4.7:** Relationship between fatty acid concentration in seston and seston C:P ratio in spring, large copepod treatment. The dashed line is for the linear regression. All data were normalized.

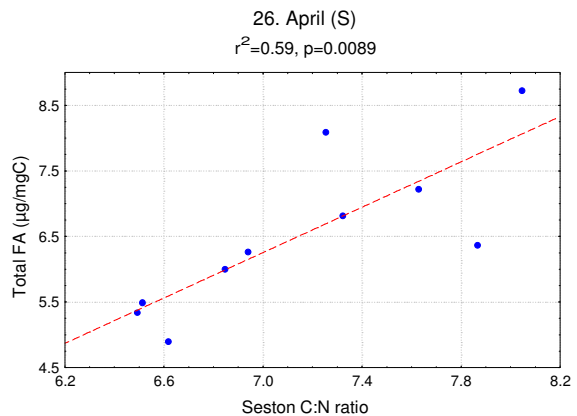
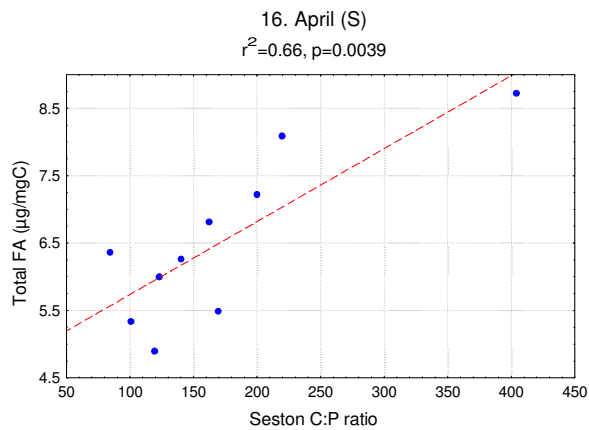


**Figure 4.8:** Relationship between fatty acid concentration in seston and seston N:P ratio in spring, large copepod treatment. The dashed line is for the linear regression. All data were normalized.





**Figure 4.9a:** Relationship between fatty acid concentration in seston and seston C:N ratio in spring, small copepod treatment. The dashed line is for the linear regression. All data were normalized.



**Figure 4.9b:** Relationship between fatty acid concentration in seston and seston C:P and N:P ratio in spring, small copepod treatment. The dashed line is for the linear regression. All data were normalized.

### Spring experiment, small copepod treatments

There was a significantly positive correlation of total fatty acids with the C:N, C:P and N:P ratio in the small copepod treatments (**Figure 4.9a** and **b**). Lipid accumulation is an usual response of algae to nutrient limitation. This is partially a result of steady lipid synthesis combined with reduced cell division rate and protein synthesis due to reduced availability of nutrients (Reitan *et al.* 1994).

The simultaneous change of fatty acid composition to more saturated ones (SAFA and MUFA) is supported in the significantly positive correlation between MUFA and

### C:N ratio (**Figure 4.9a**)

The quality of the seston (here shown by the  $\omega_3/\omega_6$  ratio – **Figure 4.9a**) is also explained to 42% by nutrient limitation, the remaining variation probably caused by zooplankton grazing and phytoplankton succession.

## **Zooplankton grazing effects**

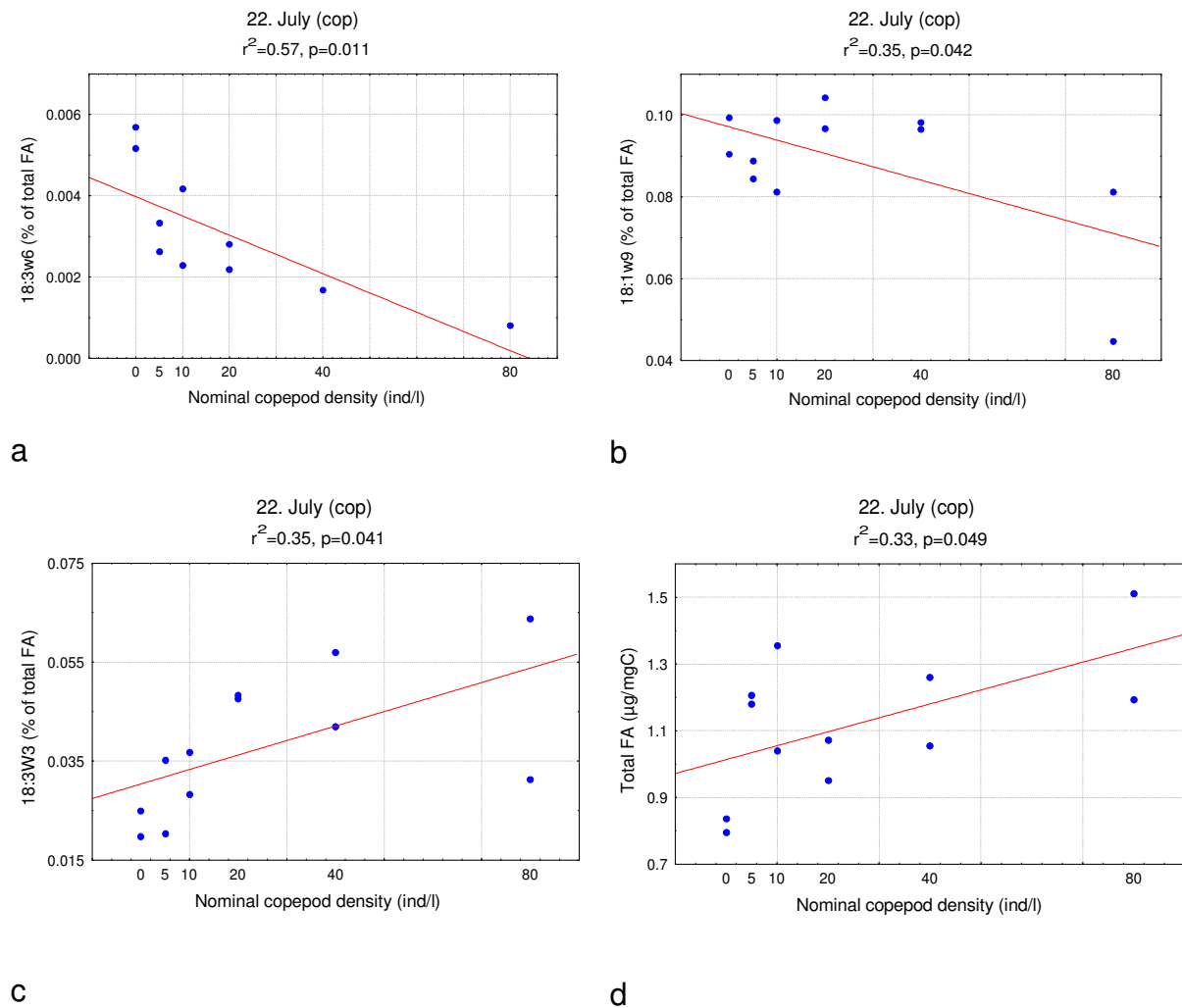
### **Summer experiment**

In the summer experiment the seston fatty acids 18:3 $\omega_6$  (precursor of arachidonic acid - Vance & Vance 1985) and 18:1 $\omega_9$  associated with dinoflagellates and Haptophyceae (Desvillate *et al.* 1997, Reuss & Poulsen 2002, Hamm & Roussau 2003) showed a good relationships with copepod grazing. The declining concentration was explained to 57% and 35%, respectively, by grazing control (**Figure 4.10a** and **b**). The decreasing concentration of the fatty acid 18:1 $\omega_9$  along the copepod density gradient corresponded with the negative impact of copepods on the dinoflagellate *Gymnodinium sp* found by Sommer 2003.

The positive correlation between copepod density and total fatty acids (**Figure 4.10 d**) could be explained by nutrient limitation or phytoplankton succession. The total fatty acid content of seston doesn't only depend on the physiological status of microalgae but also on the species composition (Viso & Marty 1993). In general small algae have higher concentrations of fatty acids ( $\mu\text{g mgC}^{-1}$ ) in contrast to bigger algae. Their higher surface to volume ratio generates higher concentrations of phospholipids. The negative correlation of 18:1 $\omega_9$  with copepod density (**Figure 4.10b**) indicated a decrease of larger cells (*Gymnodinium sp*,  $1770\mu\text{m}^3$ ). The positive correlation with 18:3 $\omega_3$  (**Figure 4.10c**) supported a species shift in the phytoplankton community. The fatty acid 18:3 $\omega_3$  plays a central role in the syntheses of essential fatty acids. The increase of this important fatty acid could be explained by higher biomass of smaller species with relative higher content of fatty acids.

The dominance of the effect of phytoplankton species shift on fatty acids composition over the effects of nutrient limitation was supported by results found by Sommer (2003), who found that N and P were only rarely correlated to copepod abundance.

The copepods in our experiment had a strong negative impact on ciliates and relative large algae (biovolume 500 to 18000  $\mu\text{m}^3$ ). Small flagellates (biovolume 30 to 200  $\mu\text{m}^3$ ) were positively affected. This displacement of larger species in favour of smaller ones was caused by two different mechanisms. First copepods choose larger particles in preference to smaller ones (Kleppel 1993, Adrian & Schneider-Olt 1999) and second the copepods diminished ciliate grazing pressure on nanoflagellates by preferably grazing on ciliates (Sommer 2003). This trophic cascade copepods-ciliates-nanoflagellates benefited the growth of small algae in dependence with copepod density.



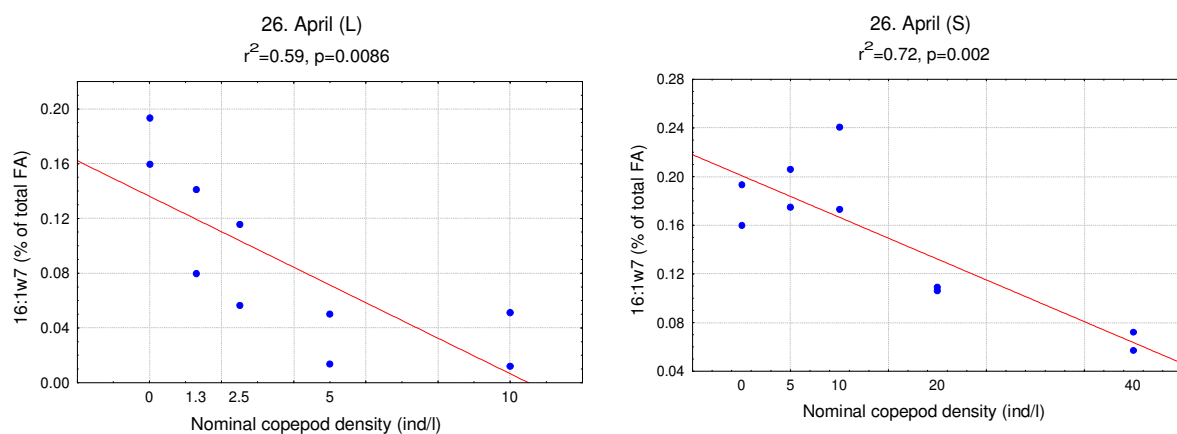
**Figure 4.10:** Relationship between copepod density and the fatty acids concentration in seston



during the summer experiment. The dashed line is for the linear regression. All data were normalized.

### Spring experiment

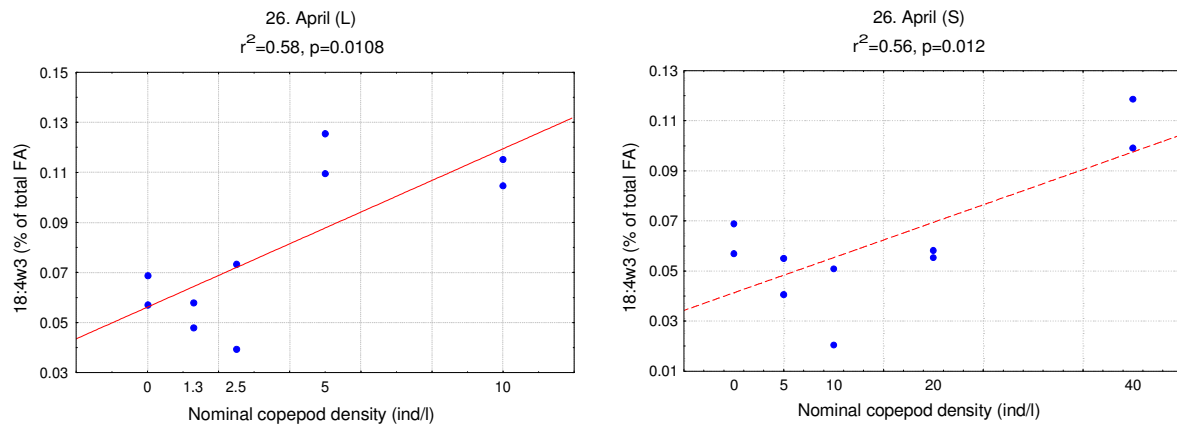
One of the principal fatty acids of diatoms is 16:1 $\omega$ 7 (Sargent & Falk-Peterson 1988, Desvillette *et al.* 1997). The decline of 16:1 $\omega$ 7 in seston with increasing copepod density suggested grazing control of diatoms for the small and large copepods in the spring experiment (**Figure 4.11**) being in agreement with the results found by Feuchtmayr (2004). Additionally this fatty acid was the quantitatively most important MUFA in the copepods fatty acids profile (**Table 4-3**). This demonstrated that diatoms were part of the diet in both treatments. However the fact that copepods fed on diatoms does not necessarily imply that this algae was an adequate food source for copepod growth success. Some studies provide evidence for the bad nutritional quality of diatoms for copepods (Ban *et al.* 1992, Peterson & Kimmerer 1994, Koski *et al.* 1998). The presence of aldehydes in this algae group caused a “birth control effect” (Miralto *et al.* 1999). This substance inhibits the envelopment of copepod eggs and thus diminishes hatching success.



**Figure 4.11:** Relationship between copepod density and the fatty acid concentration in seston during the spring experiment. The dashed line is for the linear regression ((L)= large copepod treatment, (S)= small copepod treatment). All data were normalized.

The change in phytoplankton composition was supported by the significant positive correlation of 18:4 $\omega$ 3 and copepod density in both treatments (**Figure 4.12**). 18:4 $\omega$ 3 is one of the major fatty acids of Chrysophyceae and Cryptophyceae (Desvillette *et al.* 1997, Hygum *et al.* 2000) and in accordance with this the cryptophyte *Teleaulax*

*acuta* benefited most strongly from copepod grazing (Feuchtmayr 2004).



**Figure 4.12:** Relationship between copepods density and the fatty acid concentration in seston during the spring experiment. The dashed line is for the linear regression((L)= large copepod treatment (S)= small copepod treatment). All data were normalized.

The variation of special fatty acids (16:1ω7 and 18:4ω3) confirmed a species shift in the phytoplankton community from diatom-dominated to greater importance of small flagellates in both treatment.

### **Seston quality**

One of the most common biomarkers used for diatom dominance is a high 16:1ω7/16:0 ratio (Hygum et al. 2000, Reuss & Poulsen 2002). In general, values ranging from 0.5 to 2.0 are applied as indication of diatom dominance, while values well below 0.5 stand for flagellate dominance (Viso & Marty 1993, Budge *et al.* 2001, Reuss & Poulsen 2002). Values of the 16:1ω7/16:0 ratio smaller than 0.34 indicated a predominance of flagellates in our summer phytoplankton community. This was in agreement with the ciliates/flagellate dominance found by Sommer (2003) and with the cascading predation impact found by Zöllner (2004). In the spring experiment the 16:1ω7/16:0 ratio was generally lower than 0.5, although the phytoplankton community was dominated by diatoms (Feuchtmayr 2004). The large variation in the 16:1ω7/16:0 ratio observed in different studies may be caused by varying, dominating diatom species and by environmental conditions as well as the

physiological condition of the plankton. It is essential for planktonic algae to maintain at least some metabolic processes to survive under conditions unfavourable for vegetative growth, such as nutrient deficiency. The algae reduce metabolic activity and accumulate storage products like lipids until environmental conditions improve. Shin *et al.* (2000) suggested that the Polyunsaturation Index<sup>1</sup> of C<sub>16</sub> fatty acids could be a useful indicator for the ecophysiological state of marine diatom populations.

In the large copepod treatment the Polyunsaturation Index increased from 1.5 to 2.0 (mean values) during the spring experimental period, in the small copepod treatment from 2.8 to 3.1 (mean values). Kuwata *et al.* (1993) found values of 0.38 for diatom vegetative cells, 1.17 for resting cells and 2.39 for resting spores. A possible development of resting cells is supported by results of Feuchtmayr (2004), who found a significant decrease of dissolved silicate with increasing number of large and small copepods in our experiments. When diatoms enter the process of forming resting cells they can absorb large amounts of silica to produce new thickened cell walls (Kuwata & Takahashi 1990, Kuwata *et al.* 1993). Microscopic evidence for resting spores could not be supplied (Feuchtmayr 2004), resting cells however, are similar in appearance to vegetative cells and therefore difficult to distinguish. Additionally changes in physiological state under nutrient limitation reportedly caused an increase in the ratio of saturated and monounsaturated fatty acids to PUFA (Reitan *et al.* 1994, Müller-Navarra 1995, Gulati & Demott 1997, Shin *et al.* 2000) as shown in our experiment.

Based on the information mentioned above I assume that, already at the beginning of the spring experiment, the natural algae community was in a bad physiological condition, which grew worse during the experiments.

### **Zooplankton growth**

In general, the growth rates of zooplankton are likely to be limited by the availability and quality of food (Jónasdóttir 1994, Jónasdóttir *et al.* 1995, Pond *et al.* 1996). Food availability may fall below the critical concentration induced by the seasonality of seston quantity (e. g. after the spring phytoplankton bloom). Quality of food mainly depends on plankton community composition (e.g. heterotrophic ciliate and nanoflagellates -summer experiment; diatoms - spring experiment) and their

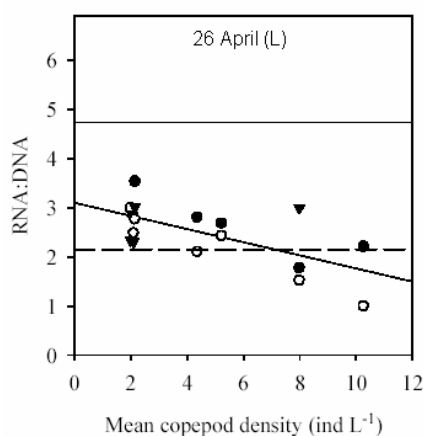
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<sup>1</sup> Polyunsaturation Index of fatty acid (C<sub>16</sub>) =  $\frac{C_{16}(\text{two or more double bonds})}{\text{Total } C_{16}}$

biochemical compounds.

For some copepods, the 20:5 $\omega$ 3 (EPA) and 22:6  $\omega$ 3 (DHA) fatty acids appear to be particularly important and have been correlated with copepod growth and development (Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996, Pond *et al.* 1996, Støttrup *et al.* 1999). In this study the lack of correlation between these fatty acids and growth was partially caused by the already low concentration of these fatty acids in the seston at the beginning of the experiments (see page 24) and also because of overall copepod mortality. In general, the different treatments contained at the end of the experiments fewer individuals than in the beginning.

Copepod growth or nutritional status can be determined by measurements of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The ratio of the two (RNA:DNA) has confirmed to be a useful tool in the determination of nutritional status of various zooplankton organisms (Vrede *et al.* 2002, Wagner *et al.* 1998). An increased RNA:DNA ratio indicates a higher growth potential. At the beginning of the spring experiment the RNA:DNA ratios were relatively high ( $\sim 4.7$ ), at the end these ratios decreased with the increasing copepod density (**Figure 4.13**). Even in the treatment with the lowest copepod density the RNA:DNA ratio was significantly lower compared to the initial values. The relative good initial nutritional status of the copepods in our experiment declined with time and copepod density in general correspondence with seston quality and quantity.



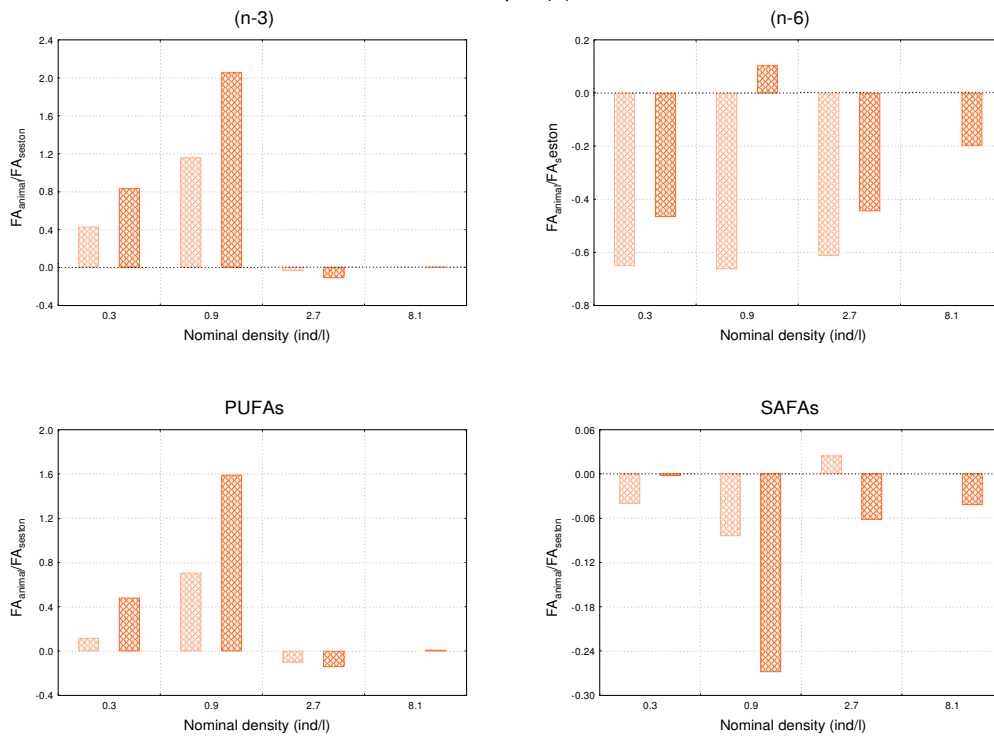
**Figure 4.13:** RNA:DNA ratios of *Calanus finmarchichus*. The line through the data points represents the significant linear regressions for all C3-C5 copepodites grouped. The horizontal line indicates the initial value, the dashed horizontal line denotes the RNA:DNA ratio of C4 after 5 days of starvation ( $\circ$ C4  $\bullet$ C3  $\blacktriangledown$ C5,  $r^2=0.44$ ,  $p=0.03$ )

This was supported by an additional indicator of food conditions of copepods, the sex

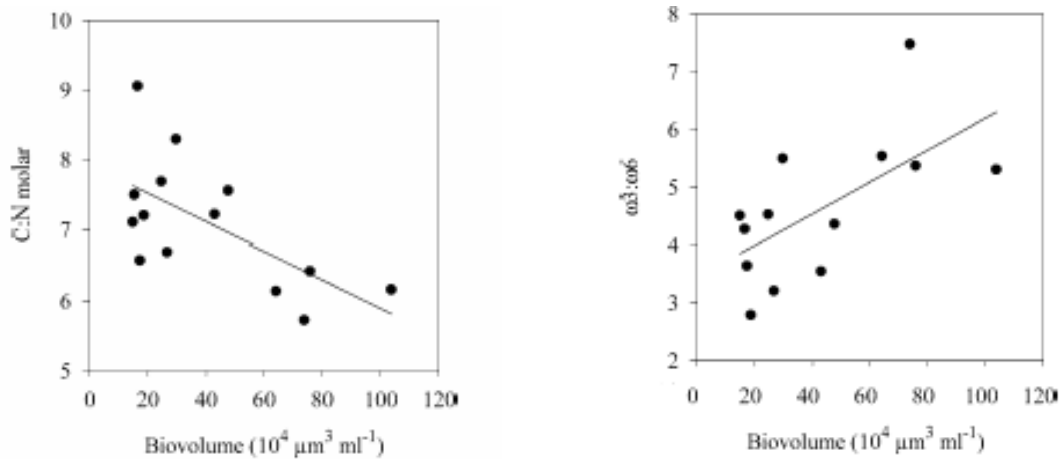
ratio. Hygum *et al.* (2000) found that *C. finmarchichus* growing under good food conditions produce significantly more males than females. The numbers of female and male copepods were more or less equal during low resources (1:1). The same observation was made in our spring experiment (1:1.3, Saage 2003).

Additional information can be obtained from an enrichment index ( $FA_{\text{animal}}/FA_{\text{seston}} = [\% \text{ of total animal FA} / \% \text{ of total seston FA}] - 1$ ). In general, depletion of fatty acid content of the copepods in contrast to the seston fatty acids occurred in  $\omega 6$  and SAFA while total PUFA and  $\omega 3$  were enriched (**Figure 4.14**, **Figure 4.15** and **Figure 4.16**). The fatty acid composition of copepods showed no correlation with copepod density in all experiments. Therefore, changes in the enrichment index must to be attributed to changes in seston fatty acid composition. The mainly essential PUFA and  $\omega 3$  fatty acid amounts in seston was closer to copepod needs at high copepod densities in both spring experiments. This improvement in nutritional quality of the seston probably resulted from the species shift to small flagellates caused by selective copepod grazing. The change from larger cells to smaller ones improved the food quality concerning fatty acids and C:N ratio (**Figure 4.15**). Nevertheless this improvement in seston quality could not be utilized by the copepods because of the unfavourably sized food particles.

26. April (L)

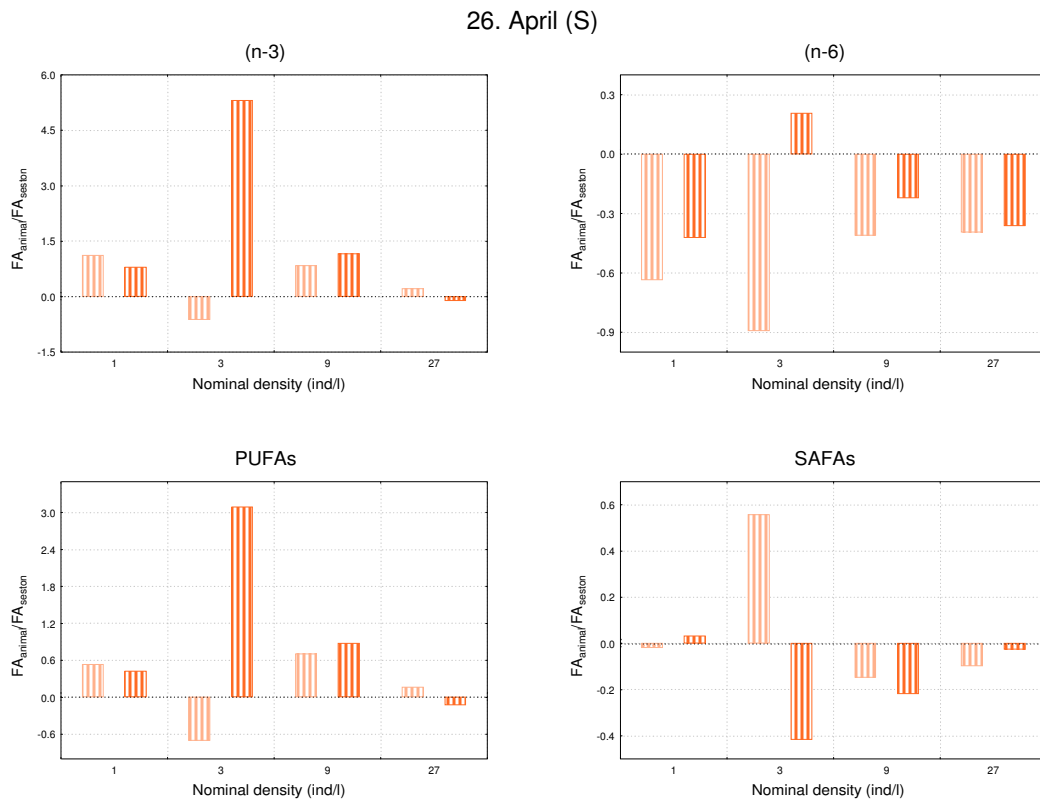


**Figure 4.14:** Enrichment index of copepods based on % fatty acids values in the large copepods treatment (light orange: bag 1; dark orange: bag 2)

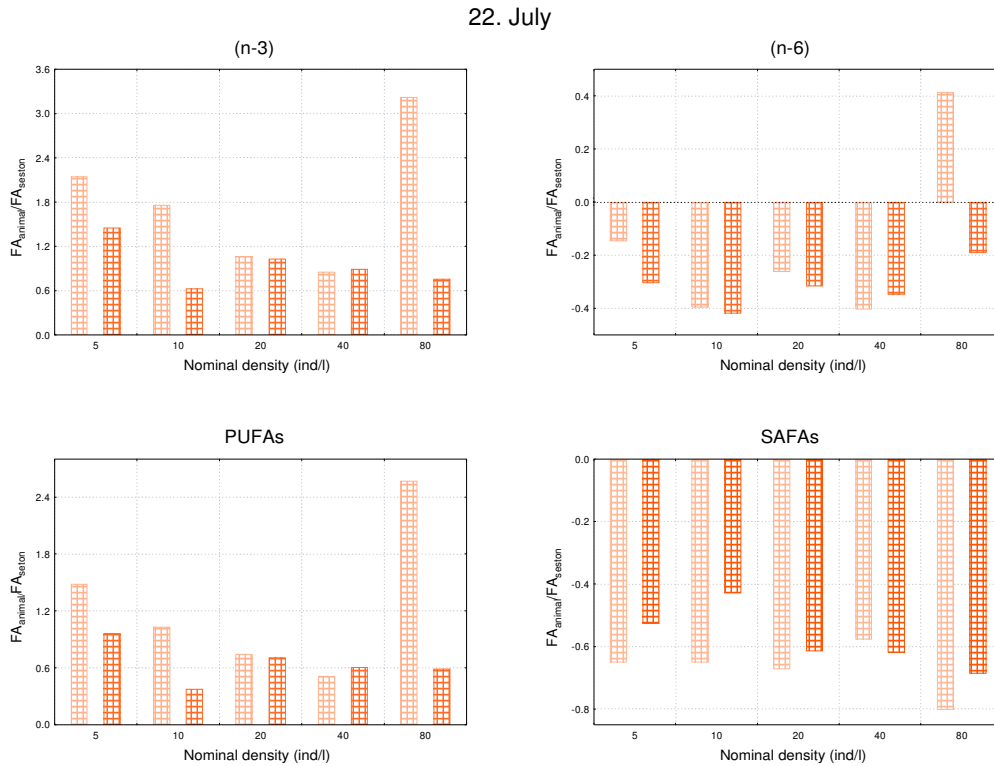


**Figure 4.15:** The relation between the C:N molar ratio and the biovolumes of phytoplankton smaller than  $1000\mu\text{m}^3$  ( $r^2 = 0.41$ ,  $p = 0.014$ )

SAFA are already known as storage lipids. The altogether relatively low ratio for SAFA (Figure 4.15 Figure 4.16 and Figure 4.17) supported further the general view, that the seston was a poor diet for copepods. In the summer experiment this effect was more pronounced than in the spring experiments.



**Figure 4.16:** Enrichment index of copepods based on % fatty acid values in the small copepods treatment (light orange: bag 1; dark orange: bag 2)



**Figure 4.17:** Enrichment index of copepods based on % fatty acid values in the copepod treatment (light orange: bag 1; dark orange: bag 2)

### 4.3. Conclusions

Based on the results of this study I concluded that :

- The fatty acids composition in copepods was different in the summer and spring experiments in relation to the plankton species composition
- The low proportions of PUFA indicated a low nutritional value of food particles. The lack of N and P was a result of nutrient depletion after the spring bloom
- Results supported the idea that ratios between SAFA, MUFA and PUFA change with variable nutrient limitation and may be used as an indicator for the physiological status of the algae community
- Most of the variance associated with fatty acid changes in seston was related to the physiological state of the populations and the species successions
- Apparently, nutrient limitation of the phytoplankton can alter trophic interactions, reducing transfer of energy to herbivorous zooplankton
- In addition to the low food quantity the low growth rates in this study might be



due to poor seston quality possibly linked to the nutritional limitation of the algae

- Dietary components like PUFA (e. g. C20 and C22) can explain low copepod growth rates because they are not able to synthesize these essential PUFA.
- All copepods exhibited bad nutritional condition, reflected by high mortality and low RNA/DNA, despite being reared at different food quantities and, to some extent, also food qualities.
- Certainly on the basis of fatty acid composition and RNA/DNA ratio, the nutritional quality of seston was a dominant factor regulating the growth of zooplankton.

# CHAPTER 5

## Chapter 5

### Kiel Fjord Mesocosms: fatty acids in brackish water

In this fjord, summer mesocosm experiments lasted for nine days and the spring experiments for approximately two weeks. The general initial situation of both mesocosm experiments differed markedly in their abiotic (**Table 5-1**) and biotic conditions.

**Table 5-1:** General water parameters and weather conditions during the Kiel Fjord experimental periods

Study site	Period	Water temp (°C)	Salinity (PSU)	Weather conditions
Kiel Fjord Summer	4 -13 September 2002	19.4 – 20.0	11.8 – 14.3	dry & sunny
Kiel Fjord Spring	3 – 16 April 2003	4 – 6	14.4	dry & sunny

Water temperature was ~ 15°C higher in summer than in spring so that intense and rapid development of zooplankton was expected. Summer initial seston C:N:P ratios scattered widely (C:N=6-12, C:P=~130-200, N:P=13-22). Compared to the Redfield Ratio this seston showed relative deficiencies of N and P with respect to C (C:N generally >8; C:P generally >140). In the spring seston (Feuchtmayr 2004) nitrogen was available in excess (C:N:P of 106:24:1).

The summer plankton community had a high diversity and was dominated by dinoflagellates (Sommer 2003). In contrast spring plankton community was dominated by dinoflagellates and cryptophytes (Feuchtmayr 2004).

The maximum seeding density of copepods (80 ind L<sup>-1</sup>) in summer represented about 8 times the long-term abundance of calanoid copepods in the Kiel Bight. But abundances of calanoids may sometimes exceed 60 ind L<sup>-1</sup> at this time of the year. The zooplankton community in this experiment was almost entirely composed of the calanoid *Acartia clausi* (>90%) (Sommer 2003). For the spring experiment the copepod abundance was comparable to mean interim values for April-June (12,4 to 73.6 ind/l - Albjerg *et al.* 1996) and the copepod community consisted mainly of *Centropages hamatus* and *Acartia clausi*.

## 5.1. Results: fatty acids abundance and composition

### 5.1.1. Fatty acids in the seston

#### *Summer experiment*

The total FA concentrations (**Figure 5.1**) in the seston varied from 6.44 to 276.41  $\mu\text{g mg C}^{-1}$ .

**Table 5-2** summarises the fatty acids composition in the summer phytoplankton community. During the entire study, SAFA accounted for 62.65 – 91.99% of total FA. Generally, they consisted mostly of palmitic acid or 16:0 (11.28 – 50.38% of total FA) and, always in lower proportions, of myristic acid or 14:0 (6.57 – 42.21% of total FA) and by stearic acid or 18:0 (13.49 – 2.31% of total FA). At the beginning of the experiment tridecanoic acid or 13:0 was the most abundant fatty acid (>25% summer) and disappeared in the course of time. Saturated acid with 15, 20, 22 or 24 carbon atoms FA increase did not exceed 2.8% of the total fatty acids.

The MUFA accounted for 3.21 – 27.15% of total FA, great majority consisting of vaccenic acid or 18:1  $\omega$ 7 (0.59 – 14.75% of total FA), palmitoleic acid or 16:1 $\omega$ 7 (1.06 – 9.42 % of total FA) and oleic acid or 18:1 $\omega$ 9 (0.73 – 5.30% of total FA). Other MUFA, such as 14:1 $\omega$ 5, 20:1 $\omega$ 9 and 20:1 $\omega$ 7 only accounted for a low proportion of total FA (<0.5%).

The PUFA accounted for 2.60 – 11.07% of total FA. They mainly belonged to the linolenate series ( $\omega$ 3) (0.44 – 11.13% of total FA), and also to the linoleates ( $\omega$ 6), but in lower proportions (0.91– 2.79% of total FA). PUFA with 16 carbon atoms and three or four double bonds were not represented. Those with two double bonds (16:2 $\omega$ 4 ) tend to increase with the time but they did not exceed 1.1%. Concentration of 18:2 $\omega$ 6 and 18:3 $\omega$ 3 18:4 $\omega$ 3 decreased with time.

PUFA with 20 and 22 carbon atoms were mainly represented by eicosapentaenoic acid (EPA) or 20:5 $\omega$ 3 (nd – 2.72% of total FA) and arachidonic acid (AA) or 20:4 $\omega$ 6 (0.04 – 1.39 % of total FA). While the docosahexaenoic acid (DHA) or 22:6 $\omega$ 3 and EPA concentrations decreased, the concentration of AA increased (nd – 1.80% of total FA) with time.

### ***Spring experiment***

The total FA concentrations (**Figure 5.1**) in the seston varied from 2.52 to 21.26  $\mu\text{g mg C}^{-1}$  (spring).

**Table 5-2** summarises the fatty acids composition in the spring phytoplankton community. During the entire study, SAFA accounted for 45.78 – 92.00% of total FA. Generally, they consisted mostly of palmitic acid or 16:0 (10.74 – 54.02% of total FA) and, always in lower proportions, by myristic acid or 14:0 (4.24 – 19.33 % of total FA) and of stearic acid or 18:0 (1.51 – 14.99 % of total FA). At the beginning of the experiment tridecanoic acid or 13:0 was the most abundant fatty acid (>15 spring) and disappeared the course of time. Saturated acid with 15, 20, 22 or 24 carbon atoms FA increase by and by but did not exceed 1.4% of total fatty acids.

The MUFA accounted for 3.45 – 18.92 % of total FA, the great majority consisting of vaccenic acid or 18:1  $\omega$ 7 (0.04 – 14.50% of total FA), palmitoleic acid or 16:1 $\omega$ 7 (0.49 – 3.74% of total FA) and oleic acid or 18:1 $\omega$ 9 (nd – 3.52% of total FA). In comparison with the summer experiment the initial concentrations of 18:1 $\omega$ 9 in the spring are lower and disappeared with time. Other MUFA, such as 14:1 $\omega$ 5, 20:1 $\omega$ 9 and 20:1 $\omega$ 7 only accounted for a low proportion of total FA (<0.6%).

The PUFA accounted for 4.54 – 34.27 % of total FA. The mainly belonged to the linolenate series ( $\omega$ 3) (2.80 – 26.63% of total FA), and also to the linoleates ( $\omega$ 6), but in lower proportions (1.14 – 6.10% of total FA). PUFA with 16 carbon atoms and three or four double bonds were not represented. Those with two double bonds (16:2 $\omega$ 4 ) tend to increase with time but they did not exceed 1.4%. PUFA with 18 carbon atoms, such as 18:2 $\omega$ 6 or linoleic acid, 18:3 $\omega$ 3 or linolenic acid and 18:4 $\omega$ 3 or stearidonic acid had higher proportions in spring than in the summer. While concentration of 18:2 $\omega$ 6 and 18:3 $\omega$ 3 increased, concentration of 18:4 $\omega$ 3 had aleatory behaviour.

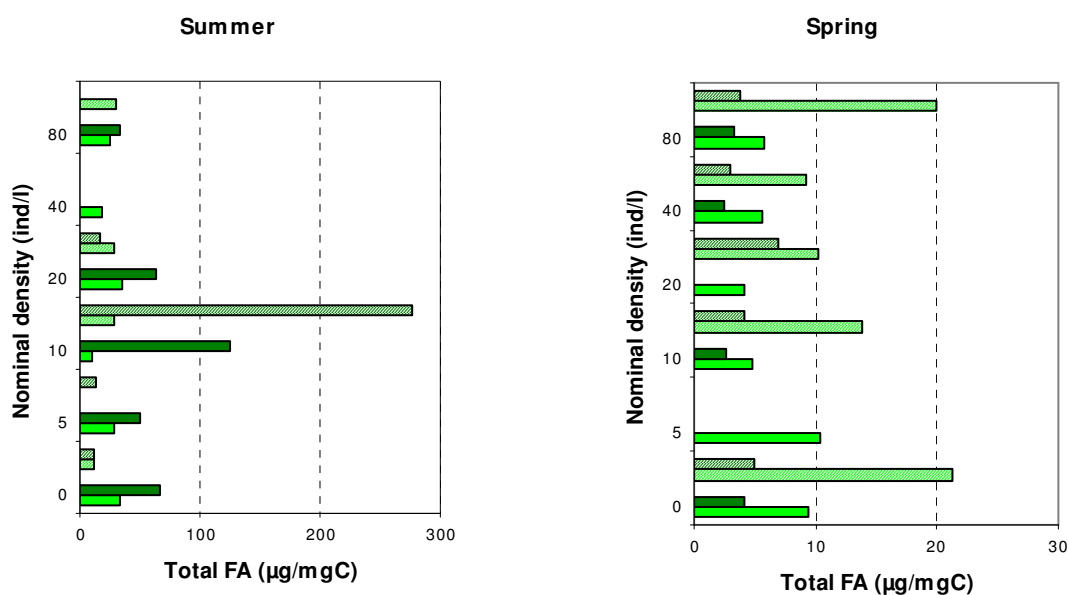
PUFA with 20 and 22 carbon atoms were mainly represented by eicosapentaenoic acid (EPA) or 20:5 $\omega$ 3 (nd – 4.56% of total FA), and arachidonic acid (AA) or 20:4 $\omega$ 6 (0.02 – 0.48% of total FA). Docosahexaenoic acid (DHA) or 22:6 $\omega$ 3 concentrations increase with the time(0.52 – 6.68% of total FA).

**Table 5-2:** Fatty acids composition during summer and spring experiments expressed as % of total fatty acids. For the purpose of clarity only major component fatty acids are shown although summary totals include all data. ( first day - last day; {1} {2} = replicate)

	summer control	spring control	summer cop5	spring cop5	summer cop10	spring cop10	summer cop20	spring cop20	summer cop40	spring cop40	summer cop80	spring cop80	
<b>SAFA</b>													
13:00	55.26-nd 0.60-nd	41.10-nd 52.06-nd	37.19-nd -nd	15.46- 41.55-nd	1.59-0.17 32.15-0.06	43.88-nd 3.67-	50.15-nd 50.66-nd	47.08-nd 30.15-nd	25.09- -	52.89-nd 24.74-nd	39.29-0.14 43.94-	65.86-nd -nd	
14:00	10.49-22.77 20.30-29.20	10.50-12.74 7.13-8.44	16.51-25.76 -23.9	11.90- 11.22-11.18	18.52-33.03 6.65-39.55	8.74-7.89 19.33-	12.38-17.95 9.75-37.54	7.99-7.48 10.92-14.16	11.34- -	7.53-16.32 6.72-8.55	17.36-42.21 6.57-	4.24-6.40 -8.12	{1} {2}
16:00	11.29-47.10 32.98-47.26	23.22-38.52 15.30-27.59	16.03-47.47 -46.04	31.80- 29.20-44.30	35.55-45.10 17.19-29.65	19.05-29.60 54.02-	13.43-50.38 16.50-40.31	17.42-25.64 34.53-49.01	23.91- -	18.45-50.05 30.15-37.99	16.07-33.54 12.70-	10.74-27.73 -37.03	{1} {2}
18:00	1.75-8.27 4.56-9.06	2.49-11.09 1.18-12.23	2.31-6.97 -9.4	4.48- 4.04-14.99	4.67-7.72 13.49-3.81	1.60-12.57 10.01-	2.04-11.16 1.91-8.07	2.23-9.83 3.57-13.47	11.99- -	2.58-11.42 6.90-10.83	1.96-7.35 9.9-	1.51-11.22 -16.82	{1} {2}
<b>MUFA</b>													
16:1ω7	7.33-3.32 8.25-2.15	0.95-1.24 1.10-2.21	9.42-2.81 -2.8	1.39- 0.72-1.59	7.12-1.57 2.89-6.63	1.21-3.74 1.11-	7.09-2.41 4.68-2.07	1.07-2.74 0.97-0.96	3.84- -	0.49-1.05 1.73-1.05	9.32-1.06 3.74-	1.19-2.76 -2.23	{1} {2}
18:1ω9	2.60-2.83 14.76-0.89	5.08-13.69 3.23-10.34	7.97-2.30 -2.4	7.88- 2.84-4.37	7.01-0.59 3.72-4.57	4.40-12.14 1.32-	4.82-2.02 3.70-0.72	3.78-9.84 6.16-2.72	2.04- -	4.30-3.20 8.70-14.50	5.26-4.23 9.90-	0.04-10.59 -12.94	{1} {2}
18:1ω7	0.83-2.50 1.85-1.09	0.74-nd 0.79-nd	0.86-1.61 -1.7	0.97- 0.76-nd	1.57-0.74 1.00-0.73	0.95-nd 0.81-	0.98-2.56 1.01-0.79	0.74-nd 1.02-nd	5.30- -	0.56-nd 1.43-nd	0.96-0.93 1.33-	3.52-nd -nd	{1} {2}
20:1ω9	0.02-0.13 0.29-nd	0.07-0.43 0.07-0.60	0.13-0.22 -0.4	0.12- 0.14-0.20	0.38-nd 0.42-0.06	0.08-0.61 0.21-	0.03-0.23 0.10-0.21	0.14-0.35 0.12-1.18	0.28- -	0.14-0.20 0.30-0.35	0.17-0.23 0.38-	0.05-0.32 -0.16	{1} {2}
22:1ω9	nd-nd nd-nd	0.06-0.16 0.07-0.35	nd-nd -nd	nd- 0.06-0.24	nd-nd nd-nd	0.05-0.26 nd- nd-nd	0.08-0.28 nd-nd 0.19-0.37	nd- nd- nd-	nd- nd- nd-	0.06-nd 0.22-nd nd-	nd-nd nd- nd-	0.05-0.08 -0.56	{1} {2}
<b>ω6</b>													
18:2ω6	0.80-0.18 1.53-0.22	2.57-3.02 3.05-5.25	0.72-1.73 -1.6	4.25- 1.09-2.02	1.63-0.10 1.62-0.60	3.13-5.11 0.67-	0.73-0.25 1.01-0.14	2.22-5.63 1.29-1.59	1.66- -	1.34-1.47 1.68-3.04	0.71-0.24 1.56-	2.13-5.64 -3.90	{1} {2}
20:4ω6	0.04-0.46 0.05-0.40	0.07-0.18 0.11-0.35	0.11-0.49 -0.3	0.07- 0.16-0.30	0.42-0.49 0.59-0.62	0.12-0.10 0.10-	0.11-0.61 0.22-1.39	0.11-0.9 0.48-0.32	0.25- -	0.13-0.32 0.21-0.31	0.07-0.50 0.25-	0.02-0.13 -0.27	{1} {2}
<b>ω3</b>													
18:3ω3	0.93-0.37 1.99-0.58	0.14-0.23 0.13-0.55	0.59-0.37 -0.7	0.37- 0.08-0.53	2.68-0.64 2.91-0.44	0.12-0.31 nd-	0.94-0.63 0.21-1.14	0.08-0.26 0.23-0.50	2.91- -	0.1-0.15 0.16-0.31	0.65-0.44 2.36-	0.31-0.27 -0.91	{1} {2}
18:4ω3	0.85-0.09 0.29-nd	3.50-2.20 4.77-6.40	0.55-0.12 -0.2	4.73- 1.18-1.52	1.77-nd 1.34-0.97	4.73-0.26 0.90-	0.71-0.07 1.66-nd	3.39-7.96 0.14-0.69	0.48- -	2.23-1.34 2.43-3.29	0.56-nd 1.12-	2.99-7.53 -3.75	{1} {2}
20:4ω3	0.04-0.46 0.05-0.39	0.17-0.27 nd-0.40	0.11-0.49 -0.3	0.24- 0.08-0.09	0.42-0.49 0.59-0.62	1.76-0.33 0.37-	0.11-0.61 0.22-1.39	nd-0.38 0.11-nd	0.25- -	0.14-0.11 0.26-0.30	0.07-0.50 0.26-	0.18-0.65 -0.32	{1} {2}
20:5ω3 (EPA)	1.66-0.22 1.74-nd	0.85-1.07 nd-3.15	0.79-0.35 -15.0	1.37- 0.35-0.75	2.72-nd 1.67-2.74	nd-4.11 0.11-	1.06-0.66 1.70-nd	nd-4.56 0.47-0.27	1.30- -	0.60-0.46 0.97-1.35	1.16-nd 1.20-	0.82-4.08 -1.60	{1} {2}
22:5ω3	0.22-nd 0.41-nd	0.32-0.65 0.15-0.52	0.15-nd -nd	0.59- 0.27-nd	0.54-0.27 0.33-0.49	0.36-0.44 nd-	0.06-nd 0.37-nd	0.35-0.41 nd-nd	0.31- -	0.34-0.49 nd-0.52	0.20-nd 0.22-	0.22-0.50 -nd	{1} {2}
22:6ω3 (DHA)	1.69-0.1 1.80-nd	2.34-2.09 3.18-5.44	0.51-0.15 -0.14	3.52- 0.88-0.99	2.47-nd 1.36-1.29	3.37-4.64 0.59-	0.77-nd 1.65-nd	2.72-6.68 1.16-0.52	1.18- -	1.72-1.16 1.46-1.67	0.64-nd 1.03-	2.19-5.04 -2.01	{1} {2}
tot SAFA	80.21-83.72 62.65-90.96	79.56-66.69 77.13-52.12	74.67-85.51 -74.65	67.19- 19.59-	63.41-91.99 73.71-75.96	89.08-76.27 75.11-54.35	79.78-85.42 80.36-90.21	92.00- 77.81-45.78	76.40- 11.07-	82.80-82.44 83.96-82.62	76.88-88.13 71.86-	72.52-62.08 83.65-48.74	
tot PUFA	7.47-4.35 9.63-2.89	12.92-14.39 16.93-30.46	4.70-4.96 -4.62	19.59- -	14.57-2.62 14.30-9.41	5.92-10.50 17.86-21.74	5.71-4.72 7.94-3.87	4.54- 13.24-34.27	11.07- -	6.61-7.29 9.66-10.55	5.43-2.60 9.8-	11.58-16.46 10.97-33.13	

**Table 5-3:** Animal fatty acids composition during summer and spring experiments expressed as % of total fatty acids.  
 For the purpose of clarity only major component fatty acids were shown although summary totals include all data.  
 (first day - last day; {1} {2} = replicate)

	summer cop5	spring cop5	summer cop10	spring cop10	summer cop20	spring cop20	summer cop40	spring cop40	summer cop80	spring cop80	
<b>SAFA</b>											
13:00	44.10		1.11		2.90		1.89		20.03		
	28.09		0.48		0.00		35.15		0.52		
14:00	11.01	3.40	12.00	3.12	11.72	3.00	9.68		1.97	1.77	{1}
	4.39		13.60	2.24	12.30	2.95	6.22	2.43	8.44	1.44	{2}
16:00	17.35	22.97	34.60	18.67	29.76	20.71	39.37		4.57	13.49	{1}
	18.41		38.64	18.26	37.28	16.87	17.33	15.22	45.36	12.05	{2}
18:00	6.11	8.33	16.13	5.52	15.24	4.78	19.52		21.02	3.60	{1}
	13.60		14.62	5.90	16.21	5.84	8.76	4.25	23.11	3.42	{2}
<b>MUFA</b>											
16:1 $\omega$ 7	4.93	1.77	5.13	2.26	6.97	2.25	2.17		0.91	3.01	{1}
	1.49		5.50	2.44	2.16	2.05	1.67	2.02	0.70	2.58	{2}
18:1 $\omega$ 9	2.08	2.97	5.52	4.09	5.11	3.86	5.24		4.63	3.83	{1}
	2.59		4.74	4.64	4.89	4.25	0.44	4.11	0.79	4.27	{2}
18:1 $\omega$ 7	1.21		1.05		1.15		0.97		nd		{1}
	0.46		0.83		1.09		1.45		0.42		{2}
20:1 $\omega$ 9	0.12	0.57	0.57	0.50	0.35	0.46	nd		0.39	0.37	{1}
	0.25		0.00	0.43	0.31	0.55	0.24	0.43	0.29	0.36	{2}
22:1 $\omega$ 9	nd	6.44	nd	2.46	nd	2.02	nd		nd	0.57	{1}
	nd		nd	1.15	nd	1.09	nd	1.28	nd	0.77	{2}
<b><math>\omega</math>6</b>											
18:2 $\omega$ 6	0.53	2.97	0.81	4.09	0.86	3.86	0.59		1.21	3.83	{1}
	0.78		0.75	4.64	0.53	4.25	0.77	4.11	0.32	4.27	{2}
20:4 $\omega$ 6	0.89	0.18	0.91	0.16	0.96	0.10	0.68		0.32	0.08	{1}
	1.71		1.20	0.10	1.32	0.19	0.96	0.08	3.25	0.07	{2}
<b><math>\omega</math>3</b>											
18:3 $\omega$ 3	0.23	2.85	0.42	3.78	0.29	3.29	0.31		0.56	4.68	{1}
	0.58		0.54	3.57	0.60	4.57	0.10	4.80	0.46	6.23	{2}
18:4 $\omega$ 3	0.19	3.01	0.08	6.45	0.30	6.44	0.36		0.49	9.69	{1}
	0.31		0.65	6.50	0.29	6.86	0.59	8.54	0.13	10.48	{2}
20:4 $\omega$ 3	0.89	1.06	0.91	1.58	0.96	1.57	0.68		0.32	2.13	{1}
	1.71		1.20	1.74	1.32	1.56	0.96	1.81	3.25	2.48	{2}
20:5 $\omega$ 3 (EPA)	2.86	4.44	4.36	5.47	6.02	5.90	2.55		0.91	7.57	{1}
	6.63		4.10	7.18	2.38	7.62	3.72	5.98	1.38	8.13	{2}
22:5 $\omega$ 3	2.86	0.88	4.36	1.35	6.02	1.22	2.55		0.91	0.53	{1}
	6.63		4.10	1.05	2.38	1.00	3.72	0.96	1.38	0.49	{2}
22:6 $\omega$ 3 (DHA)	2.59	6.79	5.01	8.07	6.00	8.63	5.22		8.43	11.18	{1}
	9.74		4.05	11.17	nd	12.18	0.24	8.63	2.49	10.98	{2}



**Figure 5.1:** Variation in phytoplanktonic total fatty acid concentrations in the copepod treatment (light green: first day of the experiment; dark green: last day of the experiment; dashed blocks denote the duplicate treatments. Look out! Graphics with different scales.

### 5.1.2. Fatty acids in the copepods

**Table 5-3** summarises the fatty acids composition in the summer and spring zooplankton community.

#### **Summer experiment**

The total FA concentrations (**Figure 5.2**) varied from 7.61 to 53.12  $\mu\text{g mg}^{-1}$  DW. **Table 5-3** summarises the fatty acids composition in the zooplankton community. Predominantly fatty acids were the 16:0, 18:0, 22:6 $\omega$ 3 (DHA) and 20:5 $\omega$ 3 (EPA). In this experiment biomarker fatty acids from bacteria (13:0 and 15:0), diatoms (16:1 $\omega$ 7) and flagellates (18:1 $\omega$ 9) were clearly present.

#### **Spring experiment**

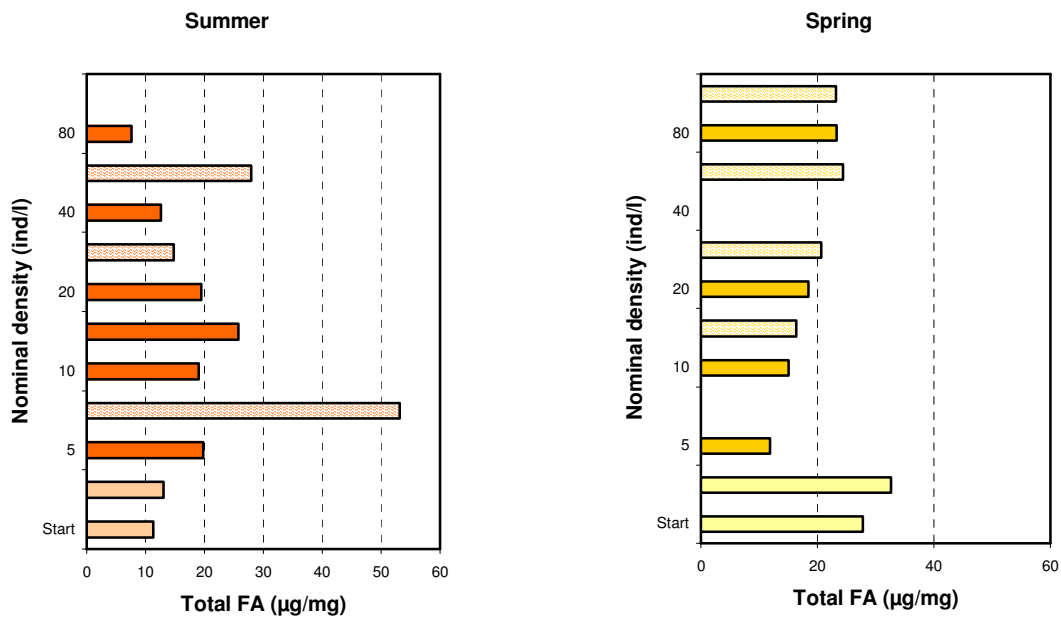
The total FA concentrations (**Figure 5.2**) varied from 15.00 to 24.38  $\mu\text{g mg}^{-1}$  DW.

**Table 5-3** summarises the fatty acid composition in the zooplankton community.

Predominantly fatty acids were 18:1 $\omega$ 9, 16:0, 22:6 $\omega$  (DHA) and 20:5 $\omega$  (EPA). Bacterial biomarkers were slightly (15:0) or not present (13:0). By comparison  $\omega$ 3



fatty acids were higher in the spring experiment than in summer.



**Figure 5.2:** Variation in copepods total fatty acid concentrations (light colour: start values, dashed blocks denote the duplicate treatments)

## 5.2. Discussion: role of fatty acids in the food web

### 5.2.1 Fatty acids in seston

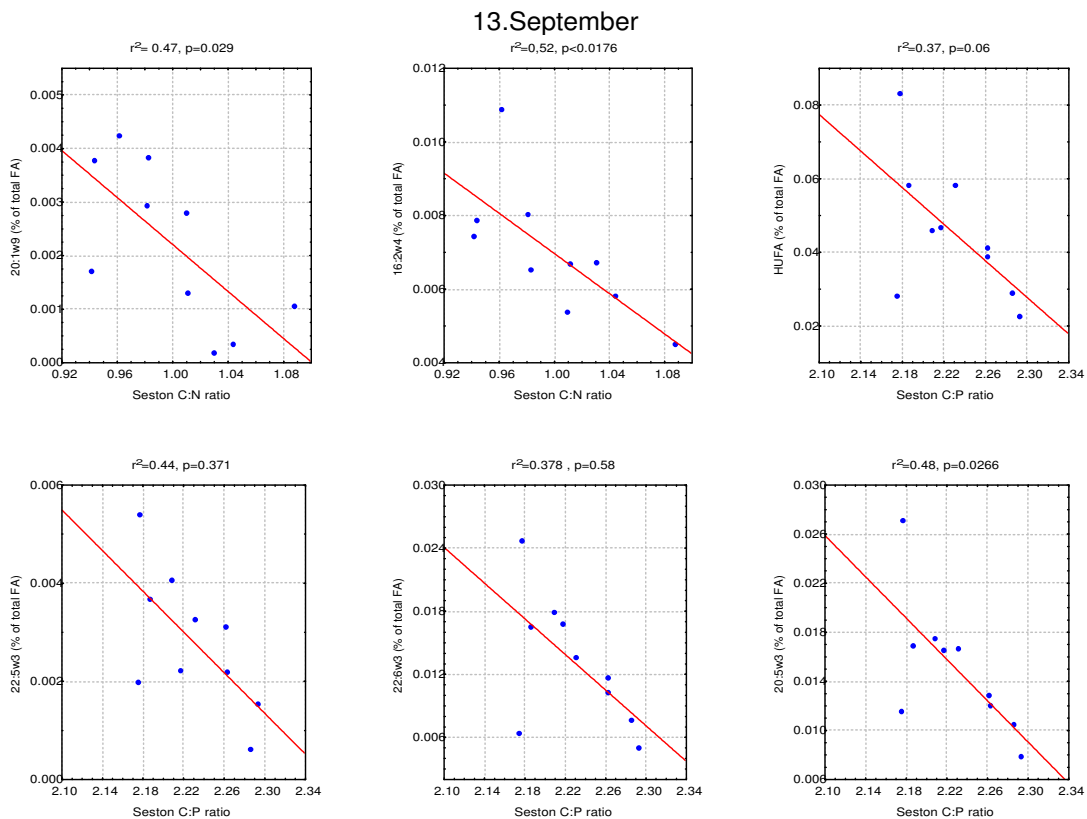
#### Nutrient influence on fatty acids

##### Summer experiment

At the start of this experiment the seston quantity was high, yet the seston was relatively deficient in N and P with respect to C. This nutrient limitation should cause variations in the seston fatty acid composition (Reitan et al. 1994, Müller-Navarra 1995, Gulati & DeMott 1997). In this experiment the decrease in the amount of PUFA over time was compensated by the increasing content of SAFA (**Table 5-2**). A simultaneous increase of MUFA was not found. An influence of N was only confirmed for the fatty acids 20:1 $\omega$ 9 and 16:2 $\omega$ 4 (PUFA) (**Figure 5.3**), an influence of P for the HUFA, 22:5 $\omega$ 3, 22:6 $\omega$ 3 and 20:5 $\omega$ 3 respectively (**Figure 5.3**). This decrease of HUFA and PUFA with diminished P and N content supported the dependence of fatty acid concentration on nutrient availability (Reitan et al. 1994,

Brett & Müller-Navarra 1997, Wainman 1997).

Since quantities of detritus may be assumed to be high (Sommer 2003), the contribution of P and N to seston C and to seston fatty acids may have been distorted. However, the significance of N and P for the production of essential PUFA in phytoplankton, and therefore for seston food quality was furthermore supported.



**Figure 5.3:** Relationship between fatty acid concentration in seston and seston C:N:P ratio in summer. The dashed line is for the linear regression. All data was normalized.

### Spring experiment

As expected for an experiment without nutrient limitation correlations between seston fatty acid concentration and seston C:N:P ratio were generally not found.

## Zooplankton grazing effects

### Summer experiment

Sommer (2003) has confirmed a significant negative grazing impact on seston for *Pseudo-nitzschia sp*, *Coschinodiscus sp* (diatoms) and *Prorocentrum micans*

(dinoflagellates). There was no significant correlation between fatty acids and copepod density. Probably the high amount of detritus in this experiment obscured the grazing effect on fatty acid composition.

### **Spring experiment**

Feuchmayr (2004) has found a significant negative grazing impact on seston for large phytoplankton species ( $> 1000\mu\text{m}^3$ ) and a positive impact on picophytoplankton and bacterial growth with increasing copepod density. In concern to fatty acids it was not possible to verify any significant correlation.

### **Seston quality and zooplankton growth**

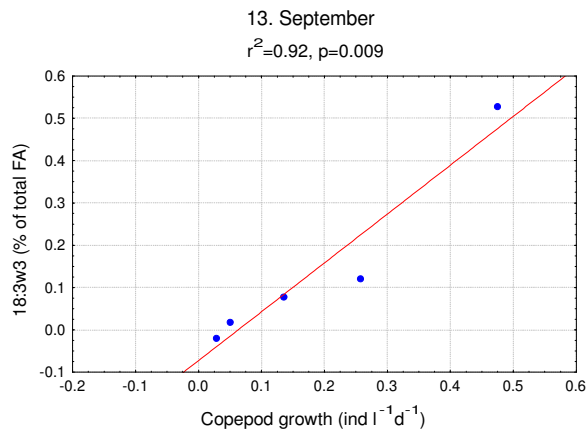
The  $\omega 3:\omega 6$  ratio has been suggested as an important indicator in metabolic growth and reproduction process in crustaceans (Ahlgren *et al.* 1990, Jónasdóttir *et al.* 1995). Jónasdóttir (1994) reported seston  $\omega 3:\omega 6$  ratios of 25 for highest egg-production by *A. tonsa* and *A. hudsonica* in laboratory experiment, while rates at a ratio of 3 were very low. The present study recorded  $\omega 3:\omega 6$  ratios of 0.9 (summer) and 3.1 (spring), indicating low food quality in seston.

The most common copepod in this experiments, *Acartia spp* is an opportunistic copepod. This species does not build up energy, but rather invests its entire metabolic production in egg production as soon as food concentration (9.5 to 24 h after ingestion) become favourable. The egg production rates decrease rapidly when food conditions deteriorate (Kiørboe *et al.* 1985, Tester & Turner 1990). According to Jónasdóttir (1994) decline of phytoplankton blooms and senescent algal cells cause a decrease in eggs production.

### **Summer experiment**

The SAFA were already present in very high concentrations (mean 73.9% of total FA) at the beginning of the experiment and increased (86.3% of total FA) with time in all copepod treatments and correspondingly the PUFA decreased. This fatty acid profile indicated an insufficient nutritional quality of seston (Parrish *et al.* 2005) as well as the low  $\omega 3:\omega 6$  ratio mentioned above. The presence of huge amounts of detritus and the low quality of seston suggested, that this experiment was conducted

during a phytoplankton senescent phase. However the low quality appeared to be partly compensated by the high quantity of organic matter, since absolute copepod growth rates were positive for copepod densities < 30 ind/l (Sommer 2003). Nevertheless a significantly positive correlation between growth and the fatty acid 18:3 $\omega$ 3 was found, indicating that relative food quality had an influence on copepod growths (**Figure 5.4**). The fatty acid 18:3 $\omega$ 3 is the precursor of the essential  $\omega$ 3 fatty acids and therefore of great importance in fatty acid synthesis.



**Figure 5.4:** Relationship between fatty acid concentration in seston and copepod growth in summer. The dashed line is for the linear regression.

### *Spring experiment*

The dominance of dinoflagellates and ciliates found in the spring experiment (Feuchtmayr 2004) indicated a post-bloom situation (Jónastóttir *et al.* 1995). Although in the spring experiment the quality of seston was superior to the summer seston quality, significant correlations with copepod growth could not be found. Probably the insufficient quantity of available food asserted a stronger influence on copepod growth than the quality of the seston.

### **5.2.2. Fatty acids in zooplankton**

The fatty acid composition of copepods differed concerning two aspects between the spring and the summer experiment. First, the bacterial biomarker fatty acid 13:0 was present in very high amounts in the copepods of the summer experiment (max. 44.1%) and was absent in the spring experiment. This high concentration in summer suggested that the copepods fed on detritus particles, which occurred in high amounts (Sommer 2003). Second, the essential  $\omega$ 3 fatty acids were significantly higher in spring than in the summer experiment being in accordance with seston quality.

### **5.3. Conclusions**

- The influence of nutrient availability on PUFA was furthermore confirmed.
- Seston quality was generally insufficient for copepod growths.
- In summer the lacking quality was partially compensated by seston quantity and enabled copepod growth.
- Detrital matter seemed to be part of the copepod diet in summer.

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## Summary

Most studies on the fatty acid composition of phytoplankton are based on laboratory experiments, and numerous studies using fatty acids as food web traces were conducted with monoalgal cultures. However, fatty acid studies with natural phytoplankton communities (food quality) and the passage of these to the next food web levels (biomarkers) are not abundant. It is important to consider the differences between analyses of fatty acids from natural plankton and laboratory studies. Seston does not only contain phytoplankton, but also some portion of bacteria, protozoa and non-living particles (detritus), especially in regions with high nutrient contents. Because it is almost impossible to quantitatively separate algae from the other particles, the fatty acid composition of phytoplankton can be disguised in natural waters.

The aim of this studies was to describe and compare the composition of fatty acids in natural phytoplankton and their transfer to the mesozooplankton community under different nutrient availability and grazing pressure. Furthermore the viability of using phytoplankton fatty acid biomarkers found in monoalgal culture studies was tested. The fatty acid composition of planktonic particulate matter (seston and zooplankton) was examined during summer and spring experiments carried out in Lake Schöhsee (Germany), the Hopavågen lagoon (Norway) and the Kiel Fjord (Germany). At all study sites, mesocosm experiments were carried out in polyethylene bags (volume ~1.5 or 3.4 m<sup>3</sup>) suspended in several floats. Each treatment consisted of a logarithmically scaled gradient of copepod or *Daphnia* densities. The copepods originated from natural assemblages, the *Daphnia* were laboratory-reared.

In all experiments the nutrient availability influenced the fatty acid contents in seston. These results supported the idea that the ratio between SAFA, MUFA and PUFA change with variable nutrient limitation and may be used as an indicator of the physiological status of the algae. Increasing nutrient limitation lead to reduced food quality of phytoplankton because of the decrease in essential PUFA. Therefore nutrient limitation of phytoplankton can alter trophic interactions. The low food quality can inhibit zooplankton growth, because most zooplankton species are not able to synthesize *de novo* these fatty acids essential for growth and reproduction. However the results of the Kiel fjord experiment suggested that the lacking quality of seston can partially be compensated by seston quantity.

The relative bad nutritional status of copepods in the Hopavågen experiment caused by food deficiency in quantity and quality was proven by the low RNA/DNA ratio. It was demonstrated, that this ratio already established in fish larvae can be a useful tool in the analysis of copepod growth dynamics.

Generally, the results from my study has confirmed that analysis of the fatty acid composition can provide general biomarkers for natural plankton communities (18:4 $\omega$ 3 and 18:2 $\omega$ 6 for chryptophyceae, 16:1 $\omega$ 7 and 20:5 $\omega$ 3 for diatom, 18:1 $\omega$ 9 for dinoflagellates, 22:6 $\omega$ 3 here indirect for ciliates, 13:0 for bacteria) to be used in food web studies. However their use can be disturbed by high concentration of detritus. In the Kiel fjord experiments with relative high detritus amounts was not possible to confirm any correlation between copepod density and biomarkers although algae counts proved a grazing pressure on phytoplankton. Furthermore fatty acid biomarkers make it possible to establish qualitative changes in seston, when quantitative changes were not found.



**Zusammenfassend**

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## Zusammenfassung

Die meisten Studien über die Fettsäurezusammensetzung im Phytoplankton beruhen auf Laborexperimenten, und zahlreiche Studien zur Nutzung von Fettsäuren als Biomarker für Nahrungsnetzanalysen wurden mit Algenmonokulturen durchgeführt. Fettsäurestudien über natürliche Phytoplanktongemeinschaften (Futterqualität) und deren Transfer in die nächste Stufe der Nahrungskette (Biomarker) sind jedoch nicht sehr zahlreich. Es ist wichtig, die Unterschiede zwischen Fettsäureanalysen von natürlichem Plankton und Laborstudien zu beachten. Seston enthält nicht nur Phytoplankton, sondern auch Bakterien, Protozoa und tote Partikel (Detritus). Weil es fast unmöglich ist, die Algen quantitativ von den anderen Partikeln zu trennen, kann die Fettsäurezusammensetzung des Phytoplankton in natürlichen Gewässern maskiert sein.

Das Ziel dieser Studie war es, die Zusammensetzung von Fettsäuren im natürlichen Phytoplankton und ihren Transfer in die Mesozooplanktongemeinschaft unter verschiedenen Nahrungstoffbedingungen und unterschiedlichem Fraßdruck zu beschreiben und zu vergleichen. Desweiteren wurde die mögliche Verwendung von Phytoplankton-Fettsäurebiomarkern getestet, die in Studien mit Algenmonokulturen gefunden wurden. Die Fettsäurezusammensetzung des Planktons (Seston und Zooplankton) wurde im Sommer und im Frühling an drei Standorten, dem Schöhsee (Deutschland), dem Hopavågen Fjord (Norwegen) und in der Kieler Förde (Deutschland) untersucht. An allen drei Orten wurden Mesokosmosexperimente in Polyethylensäcken (Volumen 1.5 bzw. 3.4 m<sup>3</sup>) durchgeführt. In jedem Treatment wurde ein logarithmisch skaliertes Gradient der Copepoden- bzw. der Daphnia-Dichte eingesetzt. Die Copepoden stammten aus Fängen in der natürlichen Umgebung, die Daphnien wurden im Labor gezüchtet.

In allen Experimenten beeinflusste die Nährstoffverfügbarkeit den Fettsäuregehalt im Seston. Diese Ergebnisse unterstützen die Idee, daß sich das Verhältnis zwischen SAFA, MUFA und PUFA mit unterschiedlicher Nährstofflimitation verändert und als ein Indicator für den physiologischen Status der Algen benutzt werden kann. Zunehmende Nährstofflimitation führt zur einer Verschlechterung der Nahrungsqualität des Phytoplanktons, weil die essentiellen PUFA abnehmen. Deshalb kann die Nährstofflimitierung des Phytoplanktons zu einer Veränderung der trophischen Interaktionen führen. Die schlechte Nahrungsqualität kann das

Zooplanktonwachstum limitieren, weil die meisten Zooplanktonarten die für Wachstum und Reproduktion essentiellen Fettsäuren nicht selbst synthetisieren können. Die Ergebnisse aus den Experimenten in der Kieler Förde jedoch zeigen, daß die mangelnde Qualität des Sestons teilweise durch die Quantität ausgeglichen werden kann.

Der relativ schlechte Ernährungszustand der Copepoden im Hopavågen-Experiment, verursacht durch ein Mangel an Futterqualität und Futtermenge, wurde durch das niedrige RNA/DNA-Verhältnis bestätigt. Es könnte gezeigt werden, daß dieses Verhältnis, das für Fischlarven schon etabliert ist, ein nützliches Instrument zur Bestimmung der Wachstumdynamik von Copepoden ist.

In Rahmen dieser Mesokosmosstudien könnte ich bestätigen, daß die Analyse der Fettsäurezusammensetzung auch in natürlichen Planktongemeinschaften zeigt, daß artspezifische Fettsäurebiomarker (18:4 $\omega$ 3 und 18:2 $\omega$ 6 für Chryptophyceae, 16:1 $\omega$ 7 und 20:5 $\omega$ 3 für Diatomen, 18:1 $\omega$ 9 für Dinoflagellates, 22:6 $\omega$ 3 hier indirekt für Ciliaten, 13:0 für Bacteria) in Nahrungsnetzstudien eingesetzt werden können.

Die Nutzung von Fettsäurebiomarkern kann von Detritusgehalt des Sestons gestört werden. Im Kieler Förde-Experiment mit relativ hohem Detritusanteil im Seston war es nicht möglich, irgendeine Korrelation zwischen Copepodendichte und Biomarkern festzustellen, obwohl Phytoplanktonzählung einen Fraßdruck auf die Algen gezeigt haben. Desweiteren ermöglichen es Fettsäurebiomarker qualitative Veränderung im Seston festzustellen, selbst wenn quantitative Veränderungen nicht feststellbar sind.

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## Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation, abgesehen von der Beratung durch meinen akademischen Lehrer, selbstständig von mir angefertigt wurde und dass sie nach Form und Inhalt meine eigene Arbeit ist. Des Weiteren versichere ich, dass die vorliegende Dissertation weder ganz noch zum Teil bei einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegen hat.

Kiel, den 16.3.2005

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**Teile dieser Arbeit oder einzelne Datensätze würden bereits wie folgt vorab veröffentlicht:**

- Becker C, Feuchtmayr H, **Brepohl D**, Santer B & Boersma M (2004) Differential impacts of copepods and cladocerans on lake seston, and resulting effects on zooplankton growth. *Hydrobiologia* 526:197-207
- Becker C, **Brepohl D**, Feuchtmayr H, Zöllner E, Sommer F, Clemmesen C, Sommer U & Boersma M (2005) Impacts of copepods on marine seston and resulting effects on *Calanus finmarchicus* RNA:DNA ratios. *Mar Biol* 146:431-541