STOICHIOMETRIC CONSTRAINTS IN PRIMARY PRODUCERS AFFECT SECONDARY CONSUMERS

Dissertation

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INTRODUCTION

The transfer of nutrients and energy through the aquatic food web shapes trophic interactions and influences food web dynamics. Ecological stoichiometry, the study of the ratios between elements such as carbon, nitrogen and phosphorus in organisms, uses this balance to explain those transfer processes. Elements are the basic building blocks for some of the most important biological and biochemical structures. While carbon is the basic building block of organic matter itself (Hessen and Anderson 2008), phosphorus is essential for nucleic acids and nitrogen is needed for proteins (Sterner and Elser 2002).

The elemental stoichiometry at the base of the food web is determined when autotrophs take up carbon and nutrients to form biomass (Sterner et Elser 2002). As the fixation of CO_2 and the assimilation of the nutrients occur separately they are not strictly coupled, thus making the ratios of the main nutrients in the tissue variable. The main factors influencing this changing nutrient content (C:nutrient ratio) are the interplay between light availability and the nutrient composition of the surrounding medium (Sterner et al. 1997; Urabe et al. 2002; Vitousek 1982). Autotrophs do not maintain a constant elemental composition, meaning they are not homeostatic, but rather reflect the changing nutrient composition of the seawater surrounding them (Dickman et al. 2008; Elser and Hassett 1994; Hecky et al. 1993; Sterner and Elser 2002). An increase in the light intensity can further reduce the nutrient content of the algae by increasing the rate of carbon fixation (Urabe et al. 2002). This will lead to a higher C:nutrient ratio.

Due to the large variations in the nutrient content of the autotrophs, herbivorous consumers are regularly faced with food of changing quality. Unlike their autotrophic food source, however, most herbivorous consumers are constrained with regard to their body C:N:P ratio (Andersen and Hessen 1991; Sterner and Elser 2002) and attempt to maintain homeostasis (Sterner and Elser 2002). The majority of grazers have developed mechanisms to cope with fluctuations in their food quality (Hessen and Anderson 2008). At the primary producer-herbivore interface the C supply of plants usually exceeds the consumers' demand, while N and P are limiting (White 1993). Low availability of N and P has negative effects on the herbivorous grazers, resulting in lower fitness (Brett 1993; DeMott et al. 2004; Elser et al. 2001; Sterner and Hessen 1994; Van Nieuwerburgh et al. 2004). Thus, food with high

C:nutrient ratios is usually of low quality, and the excess C relative to the nutrients has to be stored or excreted. Storage sometimes occurs in the form of lipids in arctic copepods (Jónasdóttir 1999), but most grazers release the excess C. Herbivores have developed a range of mechanisms to dispose of too much C, such as increased activity (Plath and Boersma 2001), futile cycles (Hessen and Anderson 2008; Newsholme et al. 1972; Russell and Cook 1995), increased respiration (Darchambeau et al. 2003; Malzahn et al. 2010; Trier and Mattson 2003), alterations in the digestion of carbon (DeMott and Tessier 2002) and excretion (DeMott et al. 1998).

Apart from direct effects of high C:nutrient ratios on the suitability of primary consumers as food for herbivores other factors play a role. For example, the morphology as well as the biochemical composition of plants may affect food quality. Herbivorous grazers are unable to synthesize some fatty acids de novo and hence depend on the supply of fatty acids from their food (Müller-Navarra 1995b). These essential fatty acids are mostly polyunsaturated fatty acids (PUFA), which play an important role in the building of cell membranes and egg production (Arendt et al. 2005). This indirect biochemical limitation of nutrient-limited algae has been linked to reduced growth rates and lower reproductive output in herbivores (Müller-Navarra 1995b; Ravet and Brett 2006). The morphology of the cell wall structure of algae or the presence of protective structures may also affect the quality of algae as food for herbivores. Further complicating the matter in aquatic systems is the fact that all of these factors are related. Nutrient limited algae change their cell wall structure (Van Donk et al. 1997), and they alter their fatty acid composition so that the amount of these PUFA is strongly decreased (Müller-Navarra 1995a; Weers and Gulati 1997). Thus the question whether direct mineral limitation or indirect biochemical limitation has a more important effect on the consumers is a subject under discussion (Boersma 2000; Gulati and DeMott 1997).

The fact that herbivores maintain a more balanced nutrient composition relative to their prey has led to the prediction that food quality effects caused by low nutrient content in the primary producers will not be passed on to trophic levels beyond that of the herbivorous consumers, as the nutrient imbalances would be eliminated by the herbivore (reviewed bySterner and Elser 2002). Secondary consumers should consequently not be affected by food quality effects at the primary producer-

herbivore interface. However, keeping homeostasis is not without costs. The attempt to buffer the frequently large nutritional imbalances supplied to them by their food often results in lower growth and reproduction in the herbivores (Augustin and Boersma 2006; Boersma 2000; DeMott et al. 2004; Elser et al. 2001; Van Nieuwerburgh et al. 2004). It can therefore be expected that the low food quality experienced by primary consumers should result in lower production of the herbivores and thus a food quantity limitation for higher trophic levels (Sterner and Elser 2002).

Recently it has become apparent that herbivores are not capable of maintaining the strict homeostasis that has previously been assumed. Thus, herbivorous zooplankters change their tissue nutrient content with changes in nutrient stoichiometry of their food (Boersma et al. 2008; Malzahn et al. 2007a; Malzahn et al. 2010). For example, the C:P ratio of herbivores can vary by as much as a factor of two depending on the food quality the animals were exposed to (DeMott and Pape 2005; Plath and Boersma 2001). This implies that both the quantity and the quality of herbivores as food source for their predators could be affected. Higher trophic levels could be faced with imbalances in food quality caused by nutrient limitations at the base of the food web. The propagation of nutrient limitation effects through the food chain is likely to have consequences for the productivity of higher trophic levels and affect the structure of the marine food web. Thus, the main question that I wanted to answer in this thesis is how vulnerable higher consumers are to changes in the nutrient availability of their prey.

Outline of the thesis

The main objective of this thesis was to increase our understanding on aquatic food web interactions in a stoichiometric context, focussing on the effects of stoichiometric constraints and the propagation of nutrient limitation signals onto higher trophic levels.

In a first approach field samplings were carried out to investigate how consumers are affected when the quality at the base of the food web changes. The field sampling was aimed to provide background information, to investigate how relevant changes in the nutrient availability in the phytoplankton are for higher trophic levels (Manuscript I).

Manuscript II is based on an experiment investigating the effect of phosphorus limitation of primary producers on primary and secondary consumers in a tri-trophic food chain. Phosphorus-limited and nutrient-replete copepods were fed to the ctenophore *Pleurobrachia pileus* in a food quality gradient. The results are discussed against the background of increased abundances of jellyfish in temperate oceans.

The nutrient requirements of animals are likely to change throughout their life cycle, meaning that some stages may be more vulnerable to nutrient limitations. In Manuscript III the effects of long- and short-term exposure to food of varying quality during ontogenetic development of a crustacean top-predator was investigated. Larvae of the European lobster in different life stages were exposed to a range of dietary C:N:P ratios simulating the possible food quality variations encountered by the larvae in the field. The observed trend of increasing sea surface temperatures may in future lead to a stoichiometric mismatch between the hatching of the lobster larvae and the availability of high quality food necessary to ensure the larval survival.

Food quality and food quantity have a strong influence on food web productivity. Recent studies have shown that homeostasis in primary consumers is not strict and possibly results in reduced reproduction and variable nutrient composition. In Manuscript IV the combined effects of low food quality and quantity on a top predator are investigated.

In the general discussion I summarize the result of my thesis, and put them in the context of possible future scenarios where limitations may become more severe, given the continuing decrease of nutrient input into coastal seas in combination with increased carbon availability for algae resulting from the increasing CO_2 concentrations in our atmosphere.

List of manuscripts

This thesis consists of four manuscripts which are in preparation for submission (Manuscript I), published (Manuscript II), submitted (Manuscript III) and submitted (Manuscript IV).

MANUSCRIPT I

Schoo, K.L., Aberle, N., Löder, M.G.J., Malzahn, A.M., Boersma, M. (in prep.)

Dietary variability and seasonal changes in trophic relations at the base of the North Sea food web

Conception, sampling, analyses and writing were carried out by K.L. Schoo in co-operation with N. Aberle, A.M. Malzahn, M. Boersma and M.G.J. Löder. Additionally M.G.J. Löder provided abundance data on heterotrophic dinoflagellates and ciliates.

MANUSCRIPT II

Schoo, K.L., Aberle, N., Malzahn, A.M., Boersma, M. (2010).

Does the nutrient stoichiometry of primary producers affect the secondary consumer *Pleurobrachia pileus?* Aquatic Ecology 44: 233–242.

The experiment, analyses and writing were carried out by K. L. Schoo in cooperation with N. Aberle, A.M. Malzahn and M. Boersma.

MANUSCRIPT III

Schoo, K.L., Aberle, N., Malzahn, A.M., Schmalenbach, I., Boersma, M. (submitted)

The reaction of European lobster larvae (*Homarus gammarus*) to different quality food: effects of ontogenetic shifts and pre-feeding history

The experiment was conceptualized and designed by all co-authors. Katherina Schoo conducted the experiment and the analyses. Isabel Schmalenbach provided infrastructure and the lobster larvae; her comments improved the manuscript. N. Aberle, A.M. Malzahn and M. Boersma advised on the interpretation of the data and contributed to the writing.

MANUSCRIPT IV

K.L. Schoo, N. Aberle, A.M. Malzahn, M. Boersma (submitted)

Food quality affects the condition of larval European lobster at low quantities.

The experiment was conducted by K. Schoo. The analyses and writing were done in cooperation with the co-authors.

CHAPTER I

Dietary variability and seasonal changes in trophic relations at the base of the North Sea food web

Katherina L. Schoo, Nicole Aberle, Martin G.J. Löder, Arne M. Malzahn, Maarten Boersma

ABSTRACT

The primary producers at the base of the food web are subject to a great variety of influences. We used a two-dimensional approach including fatty acids and stable isotopes of seston and copepods to examine how this variability affects the trophic interactions between primary producers and copepod consumers. Changes in the fatty acid content, stable isotope signal and composition of the seston samples were observed. Our results indicate that the nutrient content of the seawater, rather than the contributions of autotrophic and heterotrophic fractions in the seston, determined the stable isotope signal of the seston and hence the $\delta^{15}N$ fractionation in the copepods. Using a combination of fatty acid tracers and stable isotopes allowed us to elucidate the feeding preferences of the copepod species studied and their resulting trophic position in the North Sea food web.

INTRODUCTION

The interactions in the marine pelagic food web are very complex and subject to a great variety of influences. Particularly the base of the food web, the interaction between primary producers and consumers, is characterized by a great variability in food quantity (e.g. Sommer 1996; Wiltshire et al. 2008) and quality (e.g.Boersma et al. 2008; Klausmeier et al. 2004; Malzahn et al. 2007a). There is a strong seasonal signal in the availability and quality of microalgae, the main primary producers in temperate oceans, due to the high peak in productivity occurring during blooms. During the spring bloom, for example, phytoplankton biomasses reach a peak which is often followed by a rapid increase in (micro-) zooplankton abundance. The decrease in phytoplankton biomass due to grazing by micro- and mesozooplankton is accompanied by a decrease in phytoplankton quality caused by the depletion of available nutrients in the seawater over the duration of the bloom. This change in quantity and quality at the base of the marine food web has been shown to not only affect the herbivores directly feeding on the algae, but also potentially those secondary consumers that feed on the herbivores (e.g.Malzahn and Boersma 2009).

Despite years of research, the consumer-producer interactions in the pelagic zone are still not completely understood. There are several reasons for this. On the one hand, on the producer side, there are many organisms that are partly heterotrophic, and on the consumer side there is large variation in diet between and within species. Especially copepods, which form an important link between primary producers and higher consumers, need more study, as the trophic position of copepods plays a major role in shaping many aquatic food webs (Hairston and Hairston 1993). Most copepods are omnivores feeding on a wide range of dietary items, such as diatoms, flagellates and ciliates (Kleppel 1993). However, copepods are able to feed selectively (Fileman et al. 2007; Irigoien et al. 2000; Paffenhöfer 1988) and thus they are capable of switching between dietary items of different quality. This switch from feeding lower in the food web as a herbivore to carnivory has consequences for consumers at higher trophic levels, and as such the trophic flexibility of copepods will have consequences for the structure of the whole marine food web. Hence, the objective of the present study is to establish the role of different copepod species in the planktonic food web. A dual tracer approach using stable isotopes and fatty acids was used to investigate seasonal patterns and shifts in trophic positions of major copepod species

As food sources have distinct biochemical compositions that can become incorporated into their consumers, and tracers such as stable isotopes and fatty acids integrate the diet over a longer period of time, tracer approaches are an effective way to investigate trophic interactions (Aberle et al. 2010; El-Sabaawi et al. 2009), as such they have allowed for detailed reconstructions of food sources and trophodynamic interactions (Dalsgaard et al. 2003; Peterson and Fry 1987; Ponsard and Arditi 2000). Stable isotopes are commonly used in ecological studies to deduce trophic position and dietary source (Post 2002; Vander Zanden and Rasmussen 2001). Normally, the $\delta^{15}N$ signal is used to infer the trophic position of an organism as the percentage of ^{15}N relative to ^{14}N in the tissue increases progressively and predictably with increasing trophic position. While the value at which $\delta^{15}N$ fractionates from one trophic level to one level higher averages around 3.4‰ (Minagawa and Wada 1984) the values observed in aquatic animals vary from 2.3‰ to 4.5‰ (McCutchan et al. 2003). Carbon stable isotopes are used to infer the carbon dietary source (Minagawa and Wada 1984). The average trophic fractionation of the carbon stable isotopes is around 1‰ in aquatic animals (McCutchan et al. 2003). Although stable isotopes have been widely used in ecological studies there are still many uncertainties and the assumption of general fractionation patterns of $\delta^{15}N$ and $\delta^{13}C$ enrichment has been criticized, arguing that it disregards the variation in trophic fractionation, especially at lower trophic levels (Vander Zanden and Rasmussen 2001). Moreover, trophic enrichment is not static and it varies both between different consumer species (Post 2002; Vander Zanden and Rasmussen 2001) as well as within species as a result changing food qualities (Vander Zanden and Rasmussen 2001), and differences in specificity of different metabolic processes (Gorokhova and Hansson 1999; Ponsard and Averbuch 1999).

Such large variations are often related to the use of seston as baseline, since the isolation of pure primary producers from the plankton is impossible and filtration results in bulk seston samples containing a mixture of phytoplankton, mixo- and heterotrophic flagellates, ciliates, bacteria and detritus, each with different trophic positions and isotope signals. Since mesozooplankters feed selectively on specific seston groups (e.g. phytoplankton or heterotrophic protists) using seston can introduce errors when assessing trophic positions of mesozooplankton.

Fatty acids (FA) can be used as trophic markers because different potential prey produce specific fatty acids or signatory ratios between fatty acids which may be

retained by the consumers and can thus be used to trace their contribution to the consumers' diet (Dalsgaard et al. 2003). Several fatty acid markers are now in use for marine organisms (Dalsgaard et al. 2003; Graeve et al. 1994; Stevens et al. 2004a). The ratio of 22:6n3 (DHA) to 20:5n3 (EPA) can be used to assess the proportion of dinoflagellates to diatoms in the diet, because dinoflagellates contain high amounts of DHA while diatoms are rich in EPA (Budge and Parrish 1998; Dalsgaard et al. 2003; El-Sabaawi et al. 2010). A high ratio of DHA to EPA could also indicate a carnivorous diet (El-Sabaawi et al. 2009). High amounts of 18:1n9 relative to 18:1n7 have been shown to indicate carnivory in copepods and other crustaceans (Nyssen et al. 2005; Schmidt et al. 2003; Stevens et al. 2004a). Since carnivorous copepods contain larger amounts of polar lipids rich in polyunsaturated fatty acids (PUFA) than herbivorous copepods, the ratio of PUFA to saturated fatty acids (SFA) can be used to identify the degree of carnivory (Stevens et al. 2004b). However, because some of the fatty acids, such as DHA and some polar fatty acids are sometimes preferentially retained by certain copepods, this can obfuscate the dietary signature of primary producers (Dalsgaard et al. 2003; El-Sabaawi et al. 2009). Additionally, some fatty acids can be metabolised and transformed by the consumers (Budge and Parrish 1998). The use of fatty acids on their own to determine the trophic position without precise knowledge of the copepods' metabolism and physiology is therefore difficult.

While both fatty acid and stable isotope analysis have their limitations, the combination of these techniques may provide a more powerful tool to determine trophic interactions in complex food webs (Alfaro et al. 2006; Perga et al. 2006). The advantage of this dual tracer approach is mainly attributed to the fact that FAs are more specific to dietary source than stable carbon isotopes particularly when differences in $\delta^{13}C$ of different carbon sources are small (El-Sabaawi et al. 2009). Combining both techniques thus allows the investigation of seasonal changes in trophic relations and dietary variability in the plankton. Hence, in this study we use these two markers to investigate the between and within species variation in the major copepod species in the Southern North Sea. Given the finding by previous authors (e.g.Kleppel 1993) that different copepod species have different diets, we expect that the within species (seasonal) variation in diet is much smaller than the variation between species.

Table 1: Fatty acid biomarkers and fatty acid trophic markers used in this study

Marker	Diet	Reference
18:1 n7	Bacteria or de novo synthesis	Stevens et al 2004
18:1n9	Carnivory	Graeve et al. 1994
18:1 n9/18:1 n7	Carnivory	Stevens et al 2004 Nyssen et al. 2005
20:5 n3 EPA	Diatoms	Dalsgaard et al. 2003
22:6 n3 DHA	Dinoflagellates	Budge and Parrish 1998
DHA/EPA	Dinoflagellates/diatoms Carnivory	Budge and Parish 1998 Dalsgaard et al. 2003
PUFA/SFA	Carnivory	Stevens et al 2004
D/F	Diatoms/flagellates	Dalsgaard et al 2003 El-Sabaawi et al 2009

Abbreviations used above: **PUFA** = sum of all polyunsaturated fatty acids; **SFA** = sum of all saturated fatty acids; **D** = sum of all diatom markers (16:1 n7 + 20:5 n3 + 16PUFA); **F** = sum of all flagellate markers (22:6 n3 + 18:2 n6 + 18PUFA).

MATERIALS AND METHODS

The rocky island of Helgoland is situated in the southern North Sea, German Bight, about 70 km from the mainland. The long term sampling station Helgoland Roads is located between the main island and the sand dune island (54°11' N, 7°54'E). Due to strong tidal currents and the shallow depth the water column is well mixed (Hickel 1998). Water samples for the analysis of seston composition, stable isotope signature, fatty acid content and nutrient concentrations as well as zooplankton samples were taken by the RV Aade at Helgoland Roads between January 2007 and December 2008.

Sampling was focussed on the base of the food web, represented by the seston (particulate organic matter) and mesozooplankton consumers, represented by copepods. To provide a baseline relevant to the feeding of the primary consumers seston samples were collected at the same time as the zooplankton.

Diatom carbon concentrations were obtained from the Helgoland Roads long-term monitoring program. Samples of surface water for the determination of microzooplankton were preserved with acid Lugol's solution (2% final concentration), and the organisms identified to species level. Many of the dinoflagellates in the plankton of the Helgoland Roads are capable of mixotrophic nutrition via phagotrophy, even if they contain chloroplasts. Hence, for our division of heterotrophic versus autotrophic components in the plankton they were assigned to the microzooplankton. Conversions to carbon content were done with the program 'Count' (Tripos Inc., Germany) and based on calculations by Hillebrand et al. (1999), Menden-Deuer and Lessard (2000) and Putt and Stoecker (1989). Zooplankton samples were obtained by vertical net hauls (mesh size 180 µm and 500 µm. Animals were sorted shortly after collection. Four copepod taxa were sampled: *Calanus helgolandicus*, *Temora longicornis*, *Centropages* spp. and *Acartia* spp. (mainly *A. clausi*) Copepod samples were taken for the analysis of stable isotopes and fatty acids.

Nutrient content and salinity of the seawater was measured as part of the Helgoland long term data series (Wiltshire et al. 2008). For the determination of the seston stable isotope signature surface water from Helgoland Roads was pre-screened with a 200 µm sieve to remove larger organisms and filtered onto pre-combusted glass fibre filters (GF/C). The filters were examined under a dissecting microscope to remove

any mesozooplankton or large particles and dried at 60°C. In addition to the samples for stable isotope analysis filters were taken for fatty acid analysis of the seston.

Fatty acid analysis

Seston was extracted for the analysis of fatty acids by filtering surface water samples through pre-combusted GF/F filters (Whatman). Three replicate filters were taken on each sampling occasion. The filters were placed in reaction tubes and frozen at -80°C. Copepods for the fatty acid analysis were sorted into reaction tubes and frozen at -80°C until further analysis.

The fatty acids were measured as fatty acid methyl esters (FAMEs). The lipids of the seston were extracted from the filters by dichloromethane:methanol (2:1 vol:vol) at a temperature of -80°C for 48 hours. For the extraction of the fatty acids from the copepods the samples were sonicated instead of being frozen after the addition of the dichloromethane:methanol solution. Water-soluble fractions were removed after centrifugation by washing with 0.88% KCl buffer. The water phase was removed, and the organic remainder was evaporated using nitrogen gas. The esterification was done using methanolic sulphuric acid at 70°C for 30 min. The FAMEs were washed from the methanolic sulphuric acid using n-Hexane. Excess n-Hexane was evaporated using nitrogen gas. All chemicals used were gas chromatography (GC) grade. FAMEs were analyzed by gas chromatography using a Varian CP 8400 gas chromatograph equipped with a DB-225 column (J&W Scientific, 30-m length, 0.25-mm inner diameter [ID], 0.25-mm film). The injector temperature was set to 250°C.

The column oven was set to 80°C, which was heated to 150°C at 30°C min^{-1} after injection , then to 170°C at 6°C min^{-1}, and finally to 220°C at 1.5°C min^{-1}, which was held for 21 min. The complete measurement ran for 60 minutes. The carrier gas was helium at a constant pressure of 12 PSI. The flame ionization detector was set to 300°C. Injection of the 1 µL aliquots of the samples was done in a split-less mode. FAMEs were quantified using calibrations set up for each fatty acid separately and a known amount of C 23:0 was added at the first step of the preparation as an internal standard.

The fatty acid data were calculated to percentage of total fatty acids. The tracer fatty acids and fatty acid trophic markers (FATM) used here are summarized in Table 1. The fatty acid nomenclature employed here is X:Yn-Z, where X is the number of

carbon atoms, Y is the number of double bonds in the fatty acid and Z denotes the position of the double bond from the carboxyl end of the molecule.

Stable isotope analysis

Copepods for stable isotope analysis were rinsed in distilled water and dried in tin capsules. Depending on the size (biomass) of the copepods each tin cup contained between 3 and 30 individuals to meet the analytical requirements for the isotope analysis.

Stable isotope analysis of the samples was performed in two laboratories, at the IfM-GEOMAR in Kiel, Germany, and at the UC Davis stable isotope facility in Davis, California, USA. At the IfM-GEOMAR in Kiel the samples were analysed by using an isotope ratio mass spectrometer (Thermofinnigan EA 1110 CHNS). Samples sent to the UC Davis Stable Isotope Facility were analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The standards used were PeeDee belemnite for C and atmospheric nitrogen for N. During measurements the ratio of the $^{13}C/^{12}C$ and the ratio of the $^{15}N/^{14}N$ stable isotopes were determined. Isotopic abundances are expressed in δ notation in parts per thousand (‰): $\delta = ((R_{sample} / R_{standard})-1) *1000$, where R is the ratio of the heavier isotope to the lighter isotope, i.e. $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Trophic fractionation of stable isotopes is described as the difference of the δ values between food source (A) and consumer (B) using Δ notation, where $\Delta = \delta_B - \delta_A$. A positive Δ value indicates an enrichment of the heavier stable isotope in the consumer B.

The interactions between different biotic and abiotic factors were investigated with linear regression analyses. To test the underlying structure in the fatty acid data a multidimensional scaling (MDS) test was applied to a Bray-Curtis matrix based on untransformed fatty acid data for the copepods.

Apart from detritus and inorganic material that is probably not consumed seston samples consist of autotroph fractions (e.g. diatoms, phytoflagellates) and heterotroph fractions (e.g. ciliates, heterotrophic dinoflagellates). To estimate the $\delta^{15}N$ signal of these different fractions in the seston, we used the following equation

$$\delta^{15}N_{seston} = C_{autotroph} * \delta^{15}N_{autotroph} + C_{heterotroph} * (\delta^{15}N_{autotroph} + 2.2) \quad (1)$$

where $C_{autotroph}$ is the carbon biomass of the autotrophs expressed as fraction of total carbon biomass and $C_{heterotroph}$ is the fraction of the heterotrophic biomass, estimated from the microzooplankton counts, thus $C_{autotroph}$.+ $C_{heterotroph}$ = 1. We assumed a 2.2‰ trophic fractionation between the autotrophic and the heterotrophic fractions of the seston. This level of fractionation between two trophic levels is generally accepted for invertebrates (McCutchan et al. 2003). In this manner the theoretical $\delta^{15}N$ signals of the autotroph and the heterotroph fractions of the seston were calculated. These theoretical values of the different fractions were then used in a standard two source mixing model to assess the diets of the different copepod taxa throughout the season.

RESULTS

Seston

The spring bloom in 2007 was dominated by diatoms (Figure 1). The diatom bloom developed rapidly from mid-April onwards and diatom biomass reached a maximum of 270 µg C l^{-1} in early May. The diatom bloom was instantaneously followed by a bloom of heterotrophic microzooplankton dominated by ciliates. Throughout the rest of the year the microzooplankton was dominated by dinoflagellates reaching a maximum of about 140 µg C l^{-1} in July. Total biomass then decreased to about 100 µg C l^{-1} for the remainder of the summer and declined further following a short secondary bloom in October. During the winter months the biomass remained low at around 20-30 µg C l^{-1}. The spring bloom of 2008 occurred later than in the previous year and the peak diatom biomass (230 µg C l^{-1}) was recorded only in June. The microzooplankton peak biomass of 240 µg C l^{-1} was reached in July.

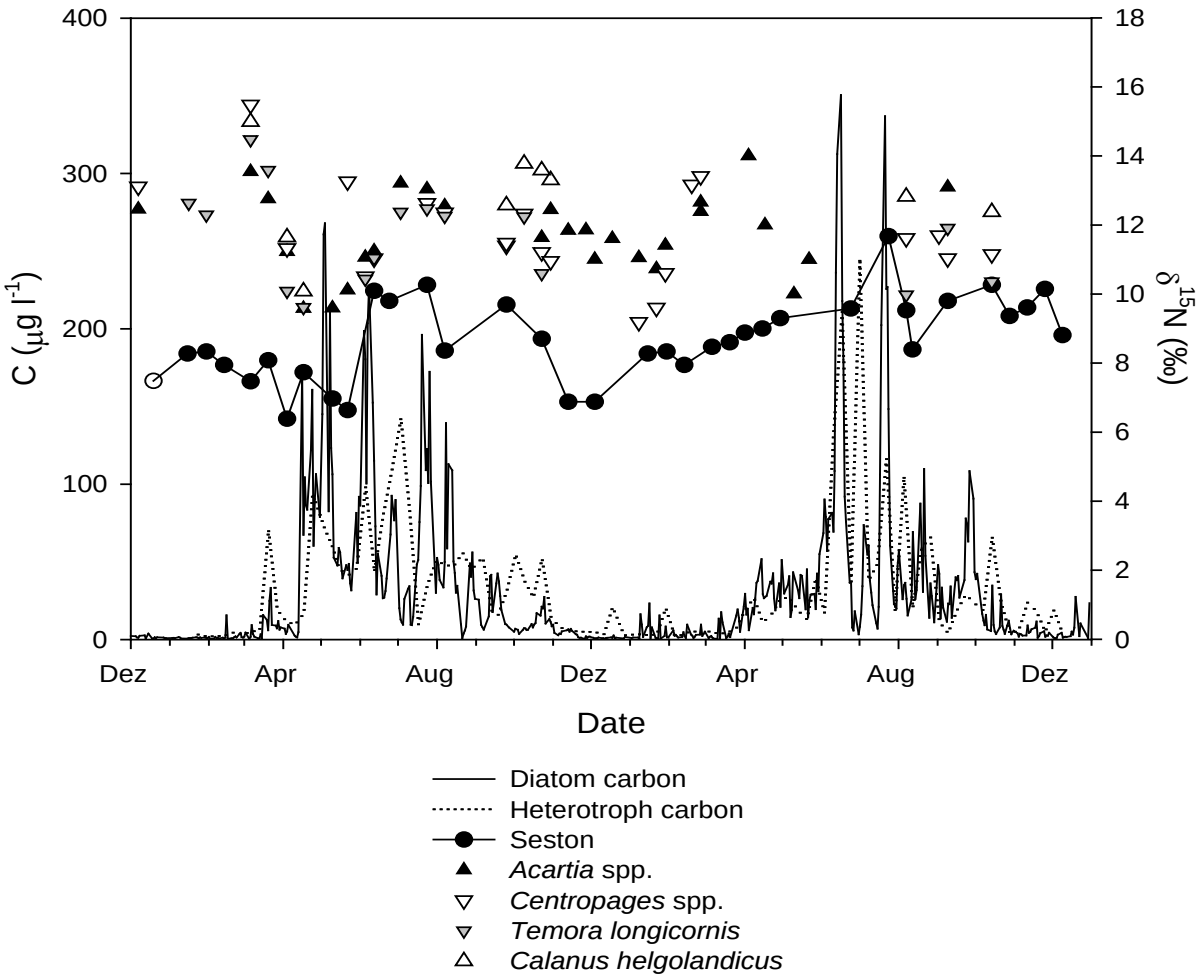

Figure 1: $\delta^{15}N$ (‰) of the seston and four copepod species as well as carbon biomass (µg l^{-1}) of diatoms and heterotrophic microzooplankton at Helgoland Roads from January 2007 to December 2008.

The $\delta^{15}N$ stable isotope signal of the seston ranged between 6.3‰ in spring 2007 and 11.7‰ in summer 2008 (Figure 1). The $\delta^{15}N$ of the seston decreased from 8 ‰ in winter to 6‰ at the start of the spring bloom. Following the diatom peak the $\delta^{15}N$ increased again, reaching values of 10‰ in July. This corresponded to the period of the highest heterotrophic biomass. The $\delta^{15}N$ decreased during the winter months, with decreasing seston biomass. The $\delta^{15}N$ signal of the seston increased again rapidly in February of 2008 and continued to increase until the summer. A drop in the $\delta^{15}N$ stable isotope values was observed in August 2008, followed by an increase during an autumn bloom of diatoms and heterotrophic dinoflagellates in October (Figure 1).

A significant positive correlation was found between the $\delta^{15}N$ signature of the seston and the diatom biomass (r^2= 0.18, $p<0.05$; Figure 2A). The correlation between the $\delta^{15}N$ of the seston and the biomass of the heterotrophic organisms was also significant (linear regression analysis, r^2 = 0.20, $p<0.01$; Figure 2B), indicating a strong influence of both the diatoms and the heterotrophic fraction on the seston $\delta^{15}N$ stable isotope signal.

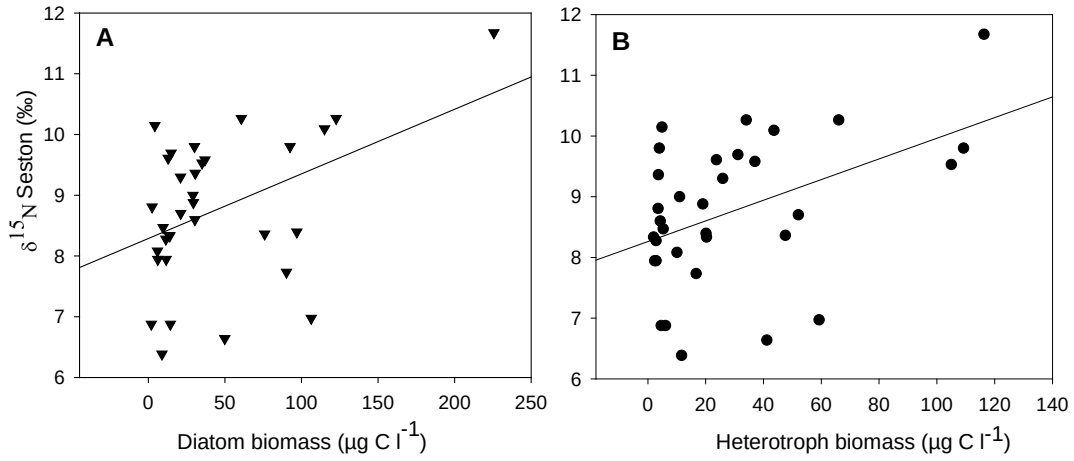

Figure 2: Correlation for diatom biomass (A) and heterotroph biomass (B) with $\delta^{15}N$ of the seston

The nutrient concentrations at Helgoland Roads showed that phosphate (PO_4) concentrations increased from 0.6 µmol l^{-1} at the beginning of the year 2007 to 1.6 µmol l^{-1} in early spring. Following the onset of the phytoplankton spring bloom the phosphate concentrations dropped to below detection limit and increased again in late summer. Nitrate (NO_3) and ammonium (NH_4) concentrations followed a similar pattern, with nitrate concentrations rapidly dropping from 43 µmol l^{-1} in early spring to below detection limit in summer and increasing in autumn. Ammonium concentrations were around 1 µmol l^{-1} in early spring, below detection limit throughout May and June and increased to around 3 µmol l^{-1} in winter 2007. Silicate concentrations ranged from 22 µmol l^{-1} in March to 0.2 µmol l^{-1} through May and

June. The nutrient concentration patterns were similar for 2007 and 2008. A strong correlation of the nutrient concentrations with the $\delta^{15}N$ seston signal was observed. The $\delta^{15}N$ showed negative correlations to the concentrations of SiO_2 (linear regression analysis, r^2= 0.16, $p<0.05$) and NH_4 (r^2=0.01, $p<0.05$) (Figure 3A), and NO_3 (r^2= 0.24, $p<0.01$) and PO_4 (r^2= 0.19, $p<0.01$) (Figure 3B). Combining all of the potential parameters correlating with the sestonic $\delta^{15}N$ signal in a multiple regression, yielded a total explained variance of 40%, and two significant explanatory variables: total dissolved nitrogen and the diatom biomass. Interestingly, the percentage of autrotroph biomass relative to the heterotroph biomass did not enter the equation, and using equation 1 to compute the $\delta^{15}N$ signals for the two different fractions showed a strong correlation between the total signal and the computed $\delta^{15}N$ signal of the two fractions (Figure 4). Thus, the primary driver of the $\delta^{15}N$ signal of the total seston is the nitrogen availability to the primary producers combined with the total available living biomass.

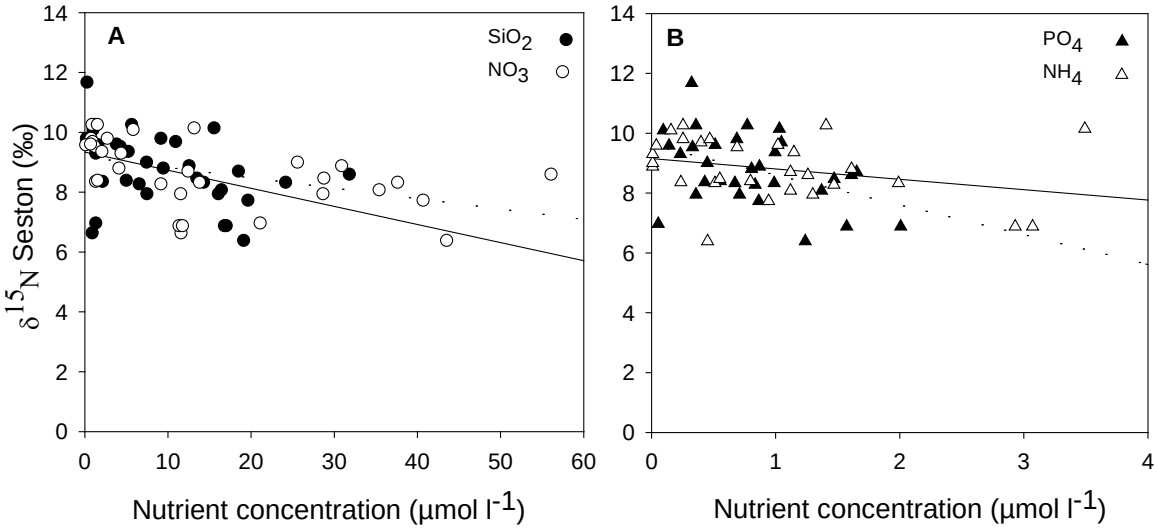

Figure 3: Correlations between seston $\delta^{15}N$ and nutrient concentrations. (A) SiO_2 and NO_3 (dashed line is linear regression for NO_3). (B) PO_4 and NH_4 (dashed line is linear regression for NH_4).

The $\delta^{13}C$ signal of the seston showed a range from -17 to -24‰. A steep change in the signal from -17 to -23‰ was observed in early spring 2007. The seston signal showed strong variations during the summer before a strong increase in November 2007. The $\delta^{13}C$ was not significantly correlated to the biomass of the diatoms or the

heterotrophs, nor did we find any correlations between $\delta^{13}C$ signal of the seston and the nutrient concentrations.

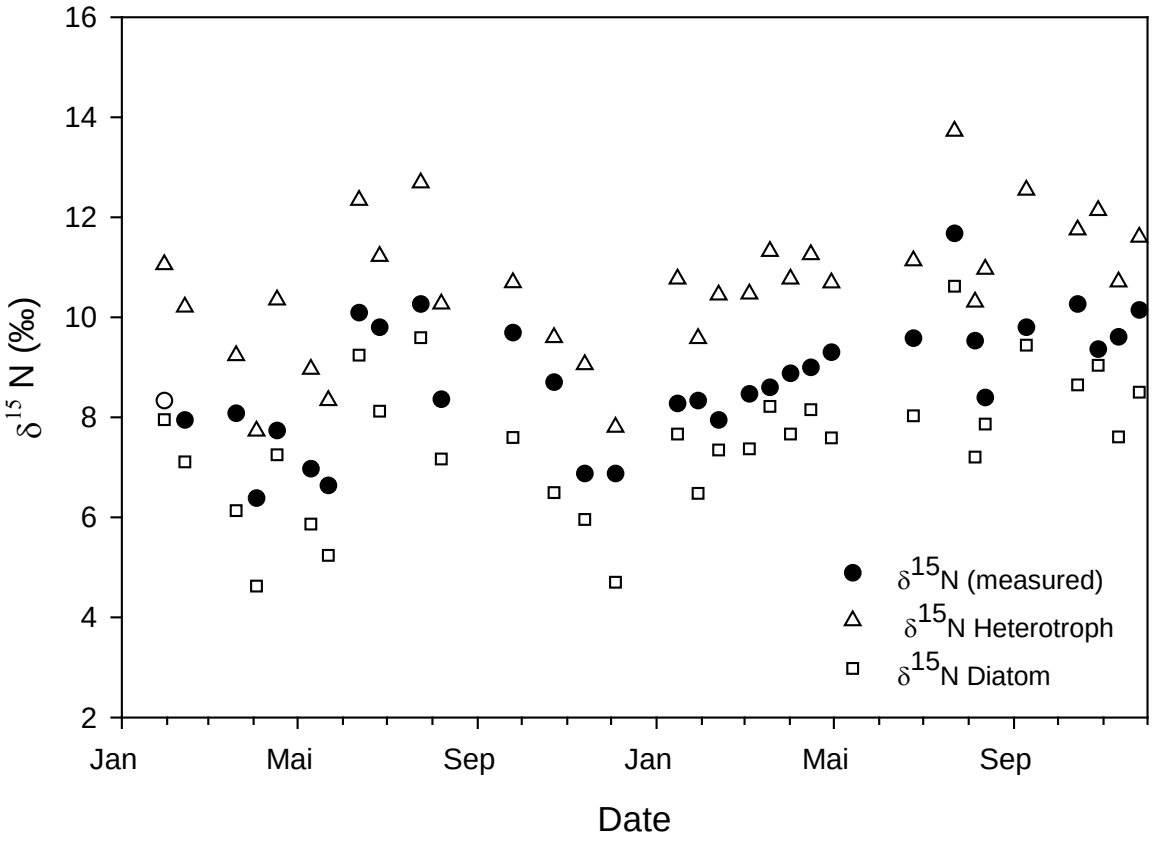

Figure 4: Seston $\delta^{15}N$ (‰) and calculated $\delta^{15}N$ for diatom and heterotroph fractions.

The fatty acid content of the seston changed according to the seston composition (Figure 5). There was a strong seasonal change in the relative amounts of certain fatty acids. During the diatom bloom in May 2007 high amounts of eicosapentanoic acid (20:5 n3, EPA) were recorded (Figure 5). Concurrently to the increase in heterotrophic biomass in June 2007 increased amounts of the dinoflagellate tracer fatty acids 18:1 n9 and 22:6 n3 (DHA) were measured. Throughout summer and autumn the concentration of 18:1 n9 remained high in the seston, while DHA displayed a second peak in late summer. The dominant fatty acids during the winter months were again those associated with heterotrophic organisms, in particular 18:1 n9.

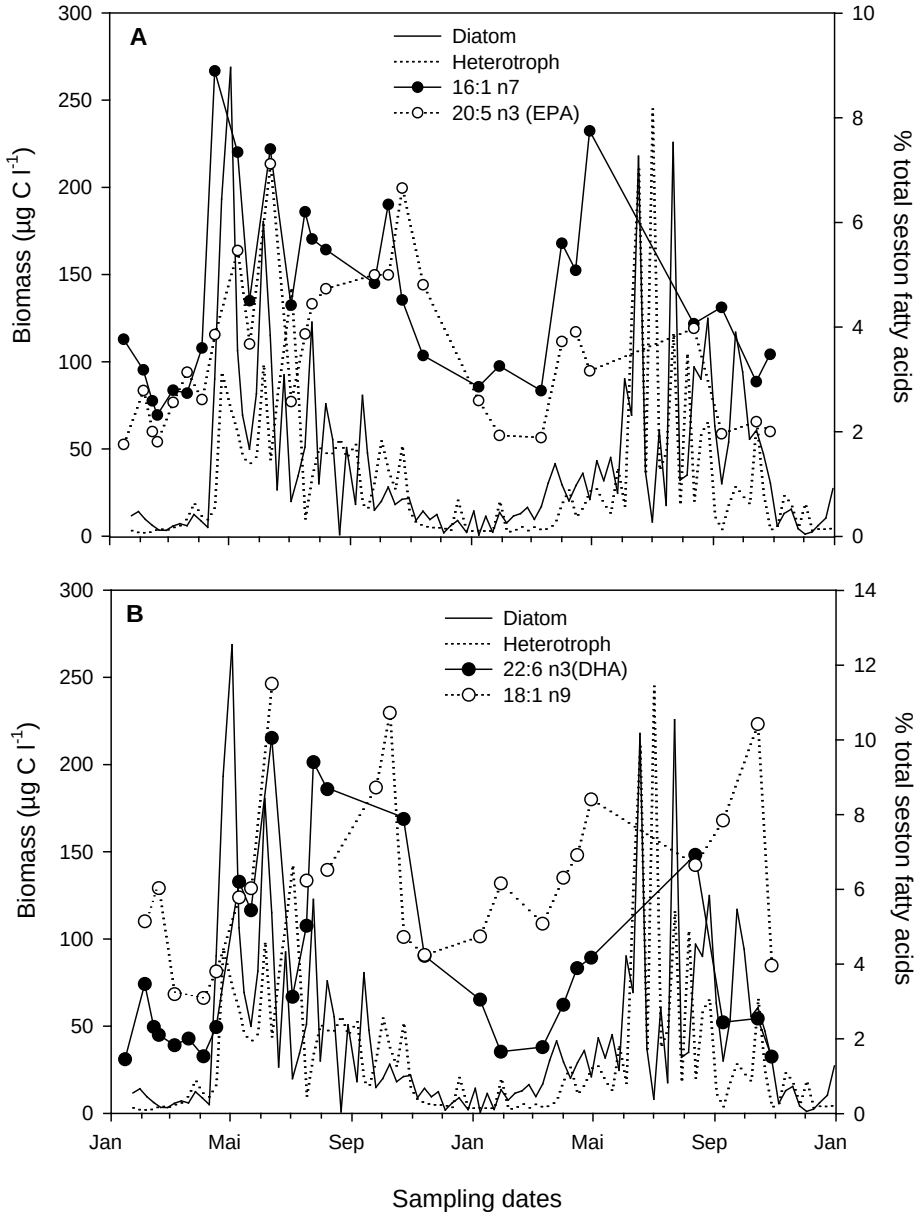

Figure 5: Seasonal variability of diatom fatty acid markers (A) and dinoflagellate fatty acid markers (B) overlaid on diatom and microzooplankton biomass.

The $\delta^{15}N$ signal of the seston correlated with 18:1 n7 (linear regression analysis: r^2= 0.19, $p<0.05$), 18:1 n9 (r^2= 0.48, $p<0.001$) and the diatom-specific fatty acid 18:4 n3 (r^2= 0.27, $p<0.01$). A strong correlation between the $\delta^{15}N$ of the seston and the ratio between 18:1 n9 and 18:1 n7 (r^2= 0.18, $p<0.05$) was also detected. No significant correlations between the fatty acids from the seston and the $\delta^{13}C$ signal were found.

Copepods

The $\delta^{15}N$ signature of the copepods showed strong seasonal fluctuations (Figure 1). The $\delta^{15}N$ signals ranged from 9‰ to 15‰. Overall, the highest average $\delta^{15}N$ throughout the sampling period was recorded in *Calanus helgolandicus*, followed by *Centropages* spp. and *Acartia* spp., while the lowest $\delta^{15}N$ was observed in *Temora longicornis.*

The trophic fractionation of the copepods relative to the seston was calculated and expressed as $\Delta\delta^{15}N$ of the copepods. This value also showed a wide range, from as low as 1‰ to 8‰, with strong differences between species and seasons. The $\Delta\delta^{15}N$ of the copepods was highest in winter, declined with the onset of the spring bloom and reached its lowest level in early summer. This pattern displays the opposite trajectory to the diatom biomass and could indicate an increased feeding on autotrophic organisms during the spring bloom. The $\Delta\delta^{15}N$ of most copepods increased again in July and remained elevated through the autumn. The highest difference in trophic enrichment between species was observed in autumn, where the $\Delta\delta^{15}N$ values ranged from 1.8‰ to 6.4‰. In *Acartia* spp. the lowest enrichment coincided with the spring bloom, indicating that this copepod species fed on a herbivorous diet during that particular time. Enrichment was higher in late autumn and winter, when the diatom biomass was lowest. A similar pattern was observed in *C. helgolandicus. T. longicornis* showed a high level of enrichment in spring and late summer, while the highest level of enrichment for *Centropages spp.* was recorded in July and August. *Centropages* spp. displayed the highest increase in $\Delta\delta^{15}N$ in the winter with values rising from 0.9‰ in January to 5.5‰ in late February. Using the computed values of the heterotrophic and autotrophic fraction in the seston for the mixing model to determine copepod feeding habits yielded no further information, as in most cases the computed fractions were not within the 0-1 range where they should be. This, combined with the fact that the seston $\delta^{15}N$ signal was independent of the ratio of autotrophs to heterotrophs, shows that we certainly did not identify all of the potential food source in the seston.

The $\delta^{13}C$ of copepods showed strong fluctuations. The highest $\delta^{13}C$ signals were recorded in May 2007 around the time of the diatom spring bloom before decreasing again. The $\delta^{13}C$ signal of *Acartia* spp. varied from -23 to -18‰. The highest $\delta^{13}C$ signals for this copepod were observed in May 2007 and September 2008. The lowest values (-23‰) were found in early March 2007, with another strong decrease

in the spring of 2008. A very similar pattern was observed for the $\delta^{13}C$ of *T. longicornis* and *Centropages* spp.. The $\delta^{13}C$ for *C. helgolandicus* was slightly lower, i.e. less enriched, than that of the other copepods throughout the sampling period.

Table 2: Correlations between seston fatty acids and copepod fatty acids. * denotes p<0.05, ** denotes p<0.01. n.s. identifies no significant correlation.

Fatty acid	*Acartia* spp.	*T. longicornis*	*Centropages* spp.	*C. helgolandicus*
18:1 n7	**	n.s.	**	n.s.
18:1 n9/18:1 n7	*	**	n.s.	n.s.
18:4 n3	n.s.	*	*	*
20:5 n3 (EPA)	**	*	n.s.	**
22:6 n3 (DHA)	*	*	**	*
DHA/EPA	n.s.	n.s.	n.s.	n.s.
PUFA/SFA	**	**	n.s.	n.s.
D/F	*	**	**	**

The fatty acid content of the four copepod species sampled was correlated with some specific fatty acid markers in the seston. *Acartia* spp. showed correlations to the diatom fatty acids 16:1 n7, 20:5 n3 (EPA), as well as to 18:1 n7 and the dinoflagellate fatty acid 22:6 n3 (DHA) (Table 2). Additionally the fatty acid content of *Acartia* spp. was correlated to the FATM 18:1 n9/18:1 n7, PUFA/SFA and D/F. The fatty acid signature of *T. longicornis* was strongly correlated to the diatom fatty acids 16:1 n7 and 20:5 n3 (EPA), as well as to the dinoflagellate markers 18:4 n3 and 22:6 n3 (DHA) and to 18:1 n9. Significant correlations to the FATM 18:1 n9/18:1 n7, PUFA/SFA and D/F were also recorded. Fatty acids in *Centropages* spp. were significantly correlated to the fatty acids 18:1 n7, 18:4 n3 and DHA in the seston. A correlation with the FATM D/F was found. *C. helgolandicus* showed significant correlations with the diatom fatty acids 16:1 n7 and EPA and to the dinoflagellate

fatty acids 18:4 3 and DHA. The fatty acid content of C. *helgolandicus* was also correlated with the FATM D/F.

Some strong correlations between the $\delta^{15}N$ of the copepods and their fatty acid markers, i.e. the fatty acids incorporated by the copepods were observed. The $\delta^{15}N$ of *Acartia* spp. correlated significantly with two fatty acid marker for diatoms (16:1n7 and D/F). There was also a strong correlation to the carnivory marker DHA/EPA in *Acartia* spp. *Centropages* spp. displayed the strongest correlations between $\delta^{15}N$ and fatty acid markers for carnivory, such as DHA/EPA and PUFA/SFA. No correlations were found between the $\delta^{15}N$ of *T. longicornis* or *C. helgolandicus* and the fatty acid markers. Significant correlations between the $\delta^{13}C$ signal and FATMs were only observed for *T. longicornis*.

Stable isotopes and fatty acids

In order to check whether the combination of stable isotope data and fatty acid markers is useful in determining the trophic position of consumers the $\delta^{15}N$ values were plotted versus fatty acid trophic markers (Figure 6). The relative positions of the copepods on the plot give an indication of the dietary preference and the resulting trophic position. *C. helgolandicus* has the highest $\delta^{15}N$ values, almost one trophic level above that of the other copepods, and also the highest concentration of the carnivory markers 18:1 n9/18:1 n7 and PUFA/SFA. The low ratio of DHA/EPA in this copepod is indicative of a high amount of dinoflagellates relative to diatoms in its diet, which could further explain the higher trophic position. The other three copepods examined in this study show similar $\delta^{15}N$ values, but have slightly different fatty acid profiles. The fatty acid composition of *T. longicornis* reveals a preference for dinoflagellates, indicated by the high D/F ratio. Confounding this is the low DHA/EPA ratio observed, which indicates a high proportion of diatoms (EPA) relative to dinoflagellates (DHA) in the diet of this copepod. *Centropages spp.* on the other hand contained a high ratio of DHA/EPA and a low amount of D/F. Both the fatty acid spectrum and the $\delta^{15}N$ values of *Acartia spp.* indicate the omnivorous feeding of this copepod, not exhibiting any clear feeding preference.

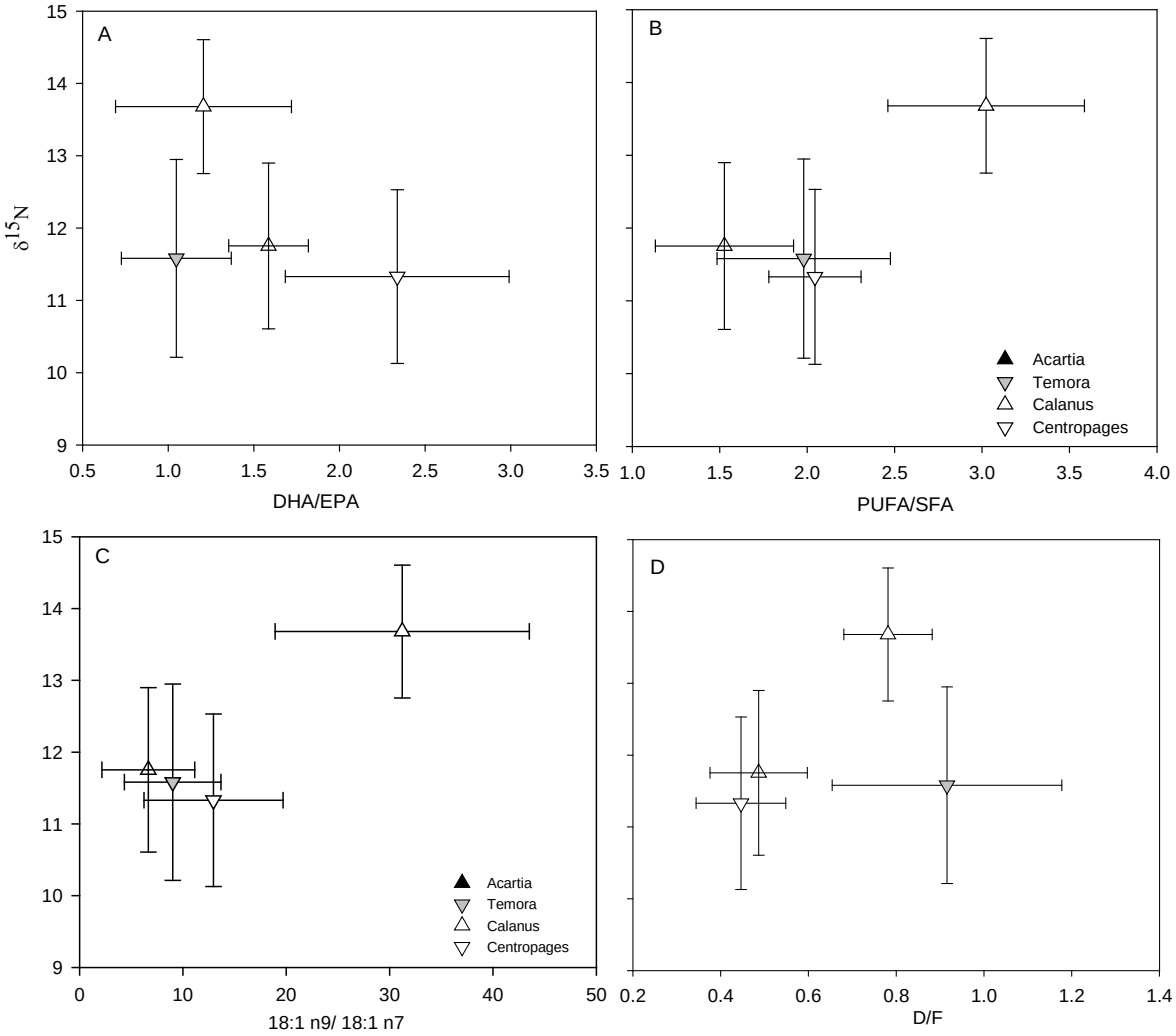

Figure 6: $\delta^{15}N$ (‰) and concentration of different fatty acid biomarkers (A) DHA/EPA, (B) PUFA/SFA, (C) 18:1n9/18:1n7 and (D) D/F expressed as % of total fatty acids for four species of copepods. Mean values for one year. Error bars indicate standard deviation.

The MDS ordination plot indicated similar groupings based on the fatty acids of the copepods (Figure 7). *Acartia* spp. and *T. longicornis* showed a similar pattern and were grouped together. *Centropages* spp. and *C. helgolandicus* were distinct from the other two copepods.

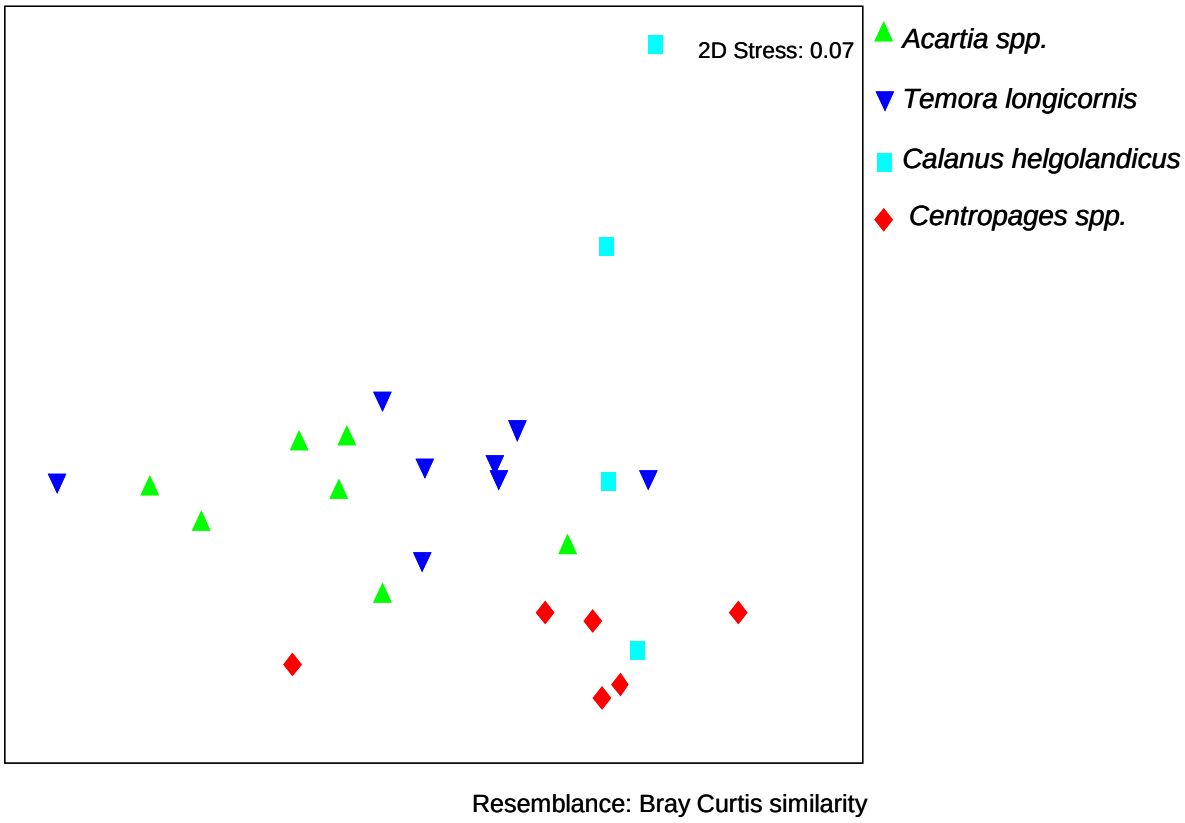

Figure 7: Multidimensional scaling ordination of a Bray-Curtis matrix calculated from untransformed fatty acids.

DISCUSSION

When investigating the trophic linkages in a complex multi-trophic system, the establishment of an appropriate baseline against which the variations of the higher trophic levels can be gauged is of utmost importance. Knowledge of the composition of the lowermost trophic levels and its quality as a food source are necessary to understand enrichment processes at higher trophic levels. Since seston fractions are commonly used as a representative for the food webs' baseline, changing proportions of autotrophic and heterotrophic components which are subject to changes in species composition, abundance and nutrient content of their medium are factors that have to be considered. Therefore, we investigated the effects the different factors have on the $\delta^{15}N$ signature of the seston and the possible effects this changing baseline signature has on the copepods.

The seston $\delta^{15}N$ signal was highly variable. The main driver of this signal seems to be the total amount of living material irrespective of whether this is autotrophic or heterotrophic, and the nutrient availability for the autotrophic fraction. From this one must conclude that apparently the $\delta^{15}N$ signal of the non-living fraction in the seston is much lower than that of the living fractions, which also precludes the computation of the theoretical $\delta^{15}N$ signals of the autrotrophic and heterotrophic fractions. The seasonal pattern displayed by the seston was largely mirrored by the copepods, whose own change in $\delta^{15}N$ followed that of the seston signal. The enrichment between the seston and the copepods, however, was unexpectedly large and in excess of the expected values for enrichment over one trophic level. Either because some other factors which affected the seston $\delta^{15}N$ signal also had an effect on the $\delta^{15}N$ signature of the copepods or, as stated above, as a result of a very low $\delta^{15}N$ signal of the detritus and/or inorganic fraction in the seston, we could not estimate the $\delta^{15}N$ signals of the potential food of the copepods properly.

In our study the range of the $\delta^{15}N$ of the seston, i.e. at the base of the food web, was larger than the 2-5‰ difference normally attributed to a one step difference in trophic levels within food webs (Post 2002). The seasonal variability in seston stable isotope signatures is commonly attributed to shifts in the species composition, with higher $\delta^{15}N$ signals usually related to a higher amount of heterotrophic organisms (Aberle et al. 2010; Agurto 2007). Our results showed that while the $\delta^{15}N$ was related to the composition of the organisms making up the bulk of the seston, there was also a strong abiotic influence on the stable isotope signal. If the seston $\delta^{15}N$ was related

only to the composition of the organisms present, the relative contributions or amounts of these fractions would account for the changes in the signal. However, as we have shown, this was not the case. Thus, stable isotope signatures of the seston observed in this study varied not only due to changes in the composition of the dominating autotrophic and heterotrophic components in the seston, but also by the non-living fraction as well as the availability of nutrients and resulting limitations in the sestonic organisms.

The stable isotope signature of phytoplankton is known to be influenced by a variety of factors, such as the CO_2 concentration, temperature, salinity, species, nutrient availability and cell size (Aberle and Malzahn 2007; Burkhardt et al. 1999; Needoba et al. 2003). The enrichment of $\delta^{15}N$ therefore varies greatly within and between phytoplankton taxa and seasons (Vuorio et al. 2006). The changes in biomass, seston composition and nutrient availability typical for spring bloom dynamics in temperate seas were also observed at Helgoland Roads during this study. Nutrient limitation in algae and nitrogen limitation in particular, has been show to affect the fractionation and enrichment of stable isotopes (Adams and Sterner 2000). Aberle and Malzahn (2007) investigated nutrient-dependent variability of isotopic fractionation and enrichment in a laboratory experiment. Their results showed that algae grown under nutrient-limitation exhibited low fractionation resulting in higher $\delta^{15}N$ signals. Because nitrogen is highly depleted right after the phytoplankton spring bloom it seems likely that the large ^{15}N enrichment of seston fractions observed during late spring and summer were the cumulative effect of higher heterotrophic fractions in the seston and ^{15}N enrichment of the seston due to low ^{15}N fractionation under nitrogen limitation.

Furthermore the nitrogen content of the algae can affect the fractionation and enrichment of $\delta^{15}N$ in the consumers. Adams and Sterner (2000) found that the $\Delta\delta^{15}N$ of the cladoceran *Daphnia magna* increased with decreasing nitrogen content of the algae. Vanderklift and Ponsard (2003) also reported a positive relationship between the $\delta^{15}N$ enrichment of consumers and the nutritional quality of the diet, measured as C:N ratio. The $\delta^{15}N$ enrichment in the consumer increased with the C:N ratio of the diet, that is to say with the decrease of the quality of the diet. Similar results have been reported from a variety of studies (e.g.Jones et al. 2004; Vuorio et al. 2006). This lends further support to the hypothesis that lower quality food, i.e. with a higher C:N ratio indicative of nitrogen limitation, leads to higher $\delta^{15}N$ enrichment. The enrichment of $\delta^{15}N$ between primary

producers and their consumers can as a consequence range from 0‰ to 8‰ (Schmidt et al. 2003).

Since a variety of factors affect the fractionation and enrichment of the $\delta^{15}N$ at the base of the food web, it is difficult to ascribe changes in the $\delta^{15}N$ signal of the seston to a definite source. When tracing dietary linkages based on the $\delta^{15}N$ of a seston baseline important information on feeding and trophic linkages may therefore become shrouded by variations in the signal.

Several studies have used mixing models for stable isotopes and applied them to determine the composition of a consumer's diet (Post et al. 2000). There are intrinsic problems involved with calculations based on a changing baseline such as the seston, in which the fractionation and enrichment depended on a great variety of factors and hence become largely unpredictable. One of the other major problems underlying this approach, however, is the vast array of potential food sources in complex ecosystems such as the marine ecosystem studied here. Additionally, consumers tend to feed on more than one food source and change their feeding strategy in relation to the food regime. The signal of e.g. the different diatom species, as well as that of the organisms making up the microzooplankton, may have varied greatly due to interspecific differences in fractionation (Aberle and Malzahn 2007; Needoba et al. 2003). Furthermore, it seems likely that the copepods fed on other dietary sources e.g. other phytoplankton fractions which were not specifically sampled in this study. In this context a tendency of copepods towards cannibalism preying upon copepod nauplii and copepodites as it has also been reported from gut-content analyses (Gentsch et al. 2009; Wesche et al. 2007) during times when autotroph production in the water column is low and zooplankton biomass at a high level would thus be a plausible explanation for the high $\Delta\delta^{15}N$ in copepods.

While the $\delta^{15}N$ signal shows the trophic level an organism feeds on, the $\delta^{13}C$ signal is habitually used to infer the dietary source of carbon. In our study, the $\delta^{13}C$ of the different copepod species were within similar ranges thus not allowing for food source differentiation based on stable carbon isotopes only. Here lies the advantage of combined stable isotope and fatty acid analysis as with the help of the fatty acid composition we were able to trace the actual dietary preferences of the copepods (Dalsgaard et al. 2003; El-Sabaawi et al. 2009; Rossi et al. 2006; Stevens et al. 2004a). The fatty acid composition of the copepods helped strengthen and further

elucidate the trophic linkages and food preferences between these consumers and their prey.

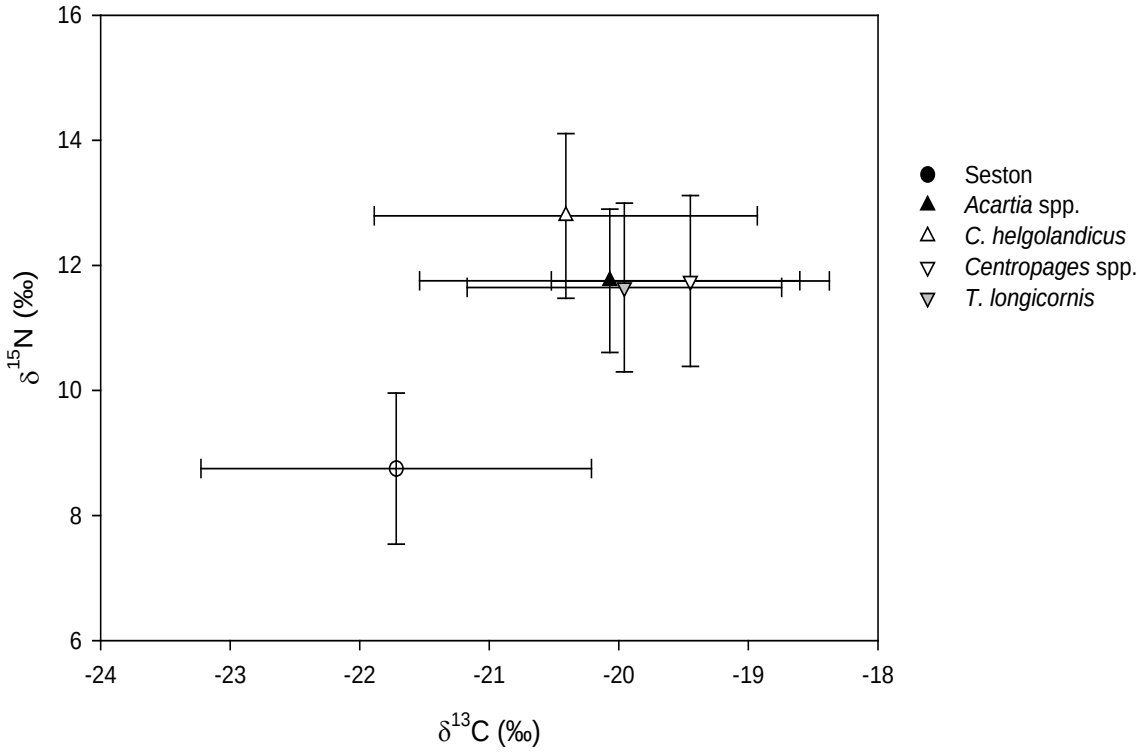

Figure 8: Isotope mixing diagram of $\delta^{15}N$ (‰) and $\delta^{13}C$ (‰) of seston and zooplankton collected at Helgoland Roads from 2007-2008.

While the annual mean $\delta^{15}N$ of *Calanus* spp. was higher than that of the other copepods sampled (Figure 8), indicating feeding on a higher trophic level, the fatty acid biomarkers also showed a high contribution of diatoms to the diet of this species. This is in accordance with observations by Meyer-Harms et al. (1999) and Harris et al. (2000) who reported a preference of *Calanus spp.* for larger diatoms and dinoflagellates. The other three copepods investigated in this study shared a similar $\delta^{15}N$ signature which is in line with observations of Agurto (2007) and Aberle et al. (2010), and could therefore be assumed to feed on the same dietary items. A closer look at the fatty acid markers, however, showed some slight differences in feeding preference. Both *T. longicornis* and *Acartia* spp. show low amounts of carnivorous fatty acid markers and both biomarkers indicate an omnivorous diet. *Centropages* spp. was richer in the carnivorous marker DHA/EPA than *T. longicornis* and *Acartia* spp. indicating a higher amount of heterotrophic dinoflagellates in the diet and hence

an omnivorous tendency. Previous studies have reported that while *Centropages* is considered an omnivorous copepod, it selectively feeds on large motile prey, including ciliates and dinoflagellates, particularly at times of high dinoflagellate biomass (Calbet et al. 2007; Saage et al. 2009). In the case of this copepod the fatty acid signatures show selective feeding on microzooplankton invisible from the stable carbon isotope signal.

In conclusion, combining the stable isotope and fatty acid biomarker approach to investigate food web interactions and trophic linkages has proven to be a powerful tool, disentangling the relative trophic position and feeding preferences of copepods at Helgoland Roads. This combination is particularly valid since sestonic stable isotope signals display such an amount of unexplained variance still. Finding a proper baseline for stable isotope studies in short living, high turnover organisms such as members of the plankton is still a major challenge for further research.

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CHAPTER II

Does the nutrient stoichiometry of primary producers affect the secondary consumer *Pleurobrachia pileus?*

Katherina L. Schoo, Nicole Aberle, Arne M. Malzahn, Maarten Boersma

ABSTRACT

We investigated whether phosphorus limitations of primary producers propagate upwards through the food web, not only to the primary consumer level but also onto the secondary consumers` level. A tri-trophic food chain was used to assess the effects of phosphorus-limited phytoplankton (the cryptophyte *Rhodomonas salina*) on herbivorous zooplankters (the copepod *Acartia tonsa*) and finally on zooplanktivores (the ctenophore *Pleurobrachia pileus*).

The algae were cultured in phosphorus-replete and phosphorus-limited media before being fed to two groups of copepods. The copepods in turn were fed to the top-predator, *P. pileus*, in a mixture resulting in a phosphorus-gradient, ranging from copepods having received only phosphorus-replete algae to copepods reared solely on phosphorus-limited algae.

The C:P ratio of the algae varied significantly between the two treatments, resulting in higher C:P ratios for those copepods feeding on phosphorus-limited algae, albeit with a significance of 0.07. The differences in the feeding environment of the copepods could be followed *Pleurobrachia pileus*. Contrary to our expectations we found that phosphorus-limited copepods represented a higher quality food source for *P. pileus*, as shown by the better condition (expressed as nucleic acid content) of the ctenophore. This could possibly be explained by the rather high C:P ratios of ctenophores their resulting low phosphorus demand and relative insensitivity to P deficiency. This might potentially be an additional explanation for the observed increasing abundances of gelatinous zooplankton in our increasingly phosphorus-limited coastal seas.

Keywords: phosphorus limitation; ctenophores; ecological stoichiometry; marine food webs; gelatinous zooplankton; trophic transfer

INTRODUCTION

Consumers habitually face the problem of nutritional imbalances, as the nutrient content of their prey does not always meet their requirements, leading to a mismatch between supply and demand (Sterner and Elser 2002). Specifically, in the relationship between primary producers and herbivores, nutrients such as nitrogen (N) and phosphorus (P) are often limiting, as there is a surplus of carbon in plants (White 1993). Carbon is of course needed as an energy source, and hence it will be respired through the food web, but as a result many consumers will have more problems meeting their nutrient requirements than their energy requirements. Furthermore, the stoichiometry of primary producers (i.e. the balance between different nutrients) is highly variable due to the changing balance between light and nutrients and often reflects the changing nutrient availability of the environment (Sterner et al. 1998). Therefore, for many consumers food may be of varying but generally poor quality. In contrast to their prey, animals are much more homeostatic (i.e. they maintain a constant body composition), and their nutrient stoichiometry should therefore be only weakly affected, if at all, by changes in the nutrient stoichiometry of their food (Elser et al. 2000a). This herbivore homeostasis has led to the prediction that food quality effects are not passed on to higher trophic levels beyond the herbivore, as the nutrient limitation of the primary producers would be buffered by the herbivorous consumer (Sterner and Elser 2002). Secondary consumers should subsequently not be strongly affected by food quality effects at the primary producer-herbivore interface. However, keeping homeostasis is not without costs, and many studies have shown that herbivores actually perform much worse in food that is limited in phosphorus (Elser et al. 2001; Urabe et al. 1997) or nitrogen (Van Nieuwerburgh et al. 2004). Thus the effect of nutrient limitation results in lower densities and potential food shortage for secondary consumers. Hence, the expectation is that the low food quality experienced by primary consumers should translate in a quantity effect on higher trophic levels (Sterner and Elser 2002).

It is, however, becoming more and more apparent that nutrient homeostasis of primary consumers is less than perfect. Herbivorous zooplankters are not always capable of maintaining a strict homeostasis when faced with changes in nutrient stoichiometry of their food source and their nutrient content changes accordingly (Malzahn et al. 2007a). Food quality effects at the bottom of the food chain can thus change the stoichiometry of the consumers and hence may be passed on through the

food chain as quality effects. As an example, carbon to phosphorus ratios of the freshwater cladoceran *Daphnia* can vary by a factor of two (Boersma and Kreutzer 2002; Plath and Boersma 2001). This implies that not only the quantity but also the quality of zooplankters as food source for their predators could be affected. Consequently, higher trophic levels could in turn be faced with food of different nutrient ratios, and the quality effect of the primary producers could be transferred higher up the food chain. This aspect has long been neglected in the literature and only very few studies have addressed the possible effects of varying phosphorus levels in primary producers on higher trophic levels (Boersma et al. 2008; Dickman et al. 2008; Frost et al. 2008; Malzahn et al. 2007a). Only recently, Malzahn et al. (2007a) showed in an experiment extending over three trophic levels that phosphorus limitation on primary producers affects the physiological condition of planktivorous fish larvae and that severe phosphorus limitations in algae can be traced to secondary consumers.

We therefore set out to further our knowledge on nutrient limitations propagating upwards through the food web in the form of food quality and investigate the effects of phosphorus limitation in primary producers on higher trophic levels. Hence, we studied an artificial tri-trophic food chain, with the ctenophore *Pleurobrachia pileus* as the secondary consumer. In contrast to fish (from the aquaculture literature) not much is known about the body composition of ctenophores. Even though the work of Kremer and co-workers is substantial when it comes to carbon, nitrogen, and biomolecules (Kremer 1977; Kremer 1982; Kremer et al. 1986; Youngbluth et al. 1988), the only study that we are aware of also including phosphorus (Borodkin and Korzhikova 1991) suggest that the C:P ratio of ctenophores can be as low as 83 (molar), and hence the phosphorus requirement of *P. pileus* should be high. Based on this we expected that P-limited copepods would be a food source of very poor quality for *Pleurobrachia pileus.*

MATERIALS AND METHODS

We designed a tri-trophic experiment consisting of a primary producer, the cryptophyte *Rhodomonas salina*, a primary consumer, the copepod *Acartia tonsa*, and a secondary consumer, the ctenophore *Pleurobrachia pileus*. We chose P as the nutrient under investigation as gradients of P-limitation are stronger in algae growing in different media, and therefore experimentally easier to manipulate. More important, however, is that P can be the limiting nutrient in coastal seas (Elser et al. 2007; Vermaat et al. 2008), and it has been observed that the phytoplankton in the German Bight is P-limited in summer (van der Zee and Chou 2005).

Primary producers

Stock cultures of the cryptophyte *Rhodomonas salina* were cultivated in f/2 medium (Guillard and Ryther 1962). The water used during the experiment was taken from the North Sea on one single occasion. The water was filtered with a sterile 0.2 µm filter and stored dark and cold until use. For the experimental treatments *R. salina* was cultured in nutrient replete medium and in medium without added P. The nutrient replete medium consisted of natural seawater enriched with f/2 nutrients, following Guillard and Ryther (1962) containing 36.3 µmol l^{-1} NaH_2PO_4. The P-limited treatment consisted of f/2-enriched seawater without the addition of any phosphorus (-P). The algae had access only to the P contained in the natural seawater at the time of filtration (1.4 µmol L^{-1}). Experimental algae were kept at 17°C under a 16:8 h light:dark (L:D) cycle.

Preliminary tests on algal growth rates under experimental conditions showed a P limitation of *R. salina* with the P-deficient medium after 4 days. Algal densities in the stock solution were determined with a CASY cell counter (Schärfe System CASY Cell Counter and Analyser System). To ensure constant food quality, new cultures of *R. salina* were inoculated daily for both treatments with a starting concentration of 0.2 $\times 10^6$ cells mL^{-1} for the f/2-treatment and 0.3 $\times 10^6$ cells mL^{-1} for the –P treatment. After the predefined growth period of four days algae were harvested at densities of approximately 1.5 $\times 10^6$ cells mL^{-1} for the f/2- treatment and 1.0 $\times 10^6$ cells mL^{-1} for the –P treatment.

Primary consumers

Copepod eggs were obtained from a laboratory culture of the calanoid copepod *Acartia tonsa*. For the production of eggs, animals were kept in filtered natural seawater (salinity 31) in a 200-L cylindrical tank on a 16:8 h L:D cycle at 18°C. The copepods were fed a mixture of the algae *Rhodomonas salina* and the heterotrophic flagellate *Oxyrrhis* sp. Eggs were siphoned off the bottom of the tank daily and stored in an airtight container in the dark at 4°C until use.

When needed, these eggs were incubated in fresh seawater in 4-litre plastic bags at a density of about 3000 individuals per litre. The hatching rate was around 25%. Copepods were first fed 24 hours after hatching; 48 hours after the addition of the eggs to the water in the plastic bags. In order to avoid changes in the phosphorus content of the algae during their incubation with the copepods, the eggs were incubated in phosphorus-free artificial seawater, adjusted to a salinity of 31 (salt: hw Marinemix, www.hw-wiegandt.de). Copepods were fed 10 000 cells of *Rhodomonas* per individual and day, which is considered to be *ad libitum* for larval stages (>1 mg C L^{-1}). Copepods were fed the same amount of algal cells for each treatment to avoid food quantity effects. To guarantee a steady supply of food at constant quality for the secondary consumer, the ctenophore *Pleurobrachia pileus*, two new bags of copepods were started each day.

The copepods grown on P-limited algae displayed a delayed development resulting in a time-lag of approximately one day when compared to the copepods reared on f/2 algae. Therefore, P- limited copepods were harvested on the eighth day after hatching, when the majority had reached the sixth naupliar stage, and f/2-copepods were one day younger. This ensured that copepods from different treatments were in the same developmental stage and had the same size. Copepods were fed to the ctenophore along a P-gradient at a concentration of 0.5 individuals mL^{-1}.This amount is above the densities reported for food saturation in ctenophores (Gibbons and Painting 1992; Greve 1972; Reeve et al. 1978), which was confirmed by the fact that some copepods were still present in all ctenophore containers 24 hours after feeding.

Secondary consumer

The ctenophore *Pleurobrachia pileus* was obtained from Helgoland Roads, North Sea (54° 11′ N, 7° 53′ O). *P. pileus* is an ambush predator, catching its motile prey in lateral filaments on its tentacles (Gibbons and Painting 1992; Greve 1970). This feeding mechanism is unselective for actively swimming prey (Fraser 1970). Individuals were transferred to flow-through tanks and kept at ambient water temperature in filtered seawater. Individuals were starved for 5 days prior to the first feeding in the experiment. Only ctenophores of the same size range (10-15 mm) were used in the experiment. For the duration of the experiment each individual *P. pileus* was kept in a separate 1-L glass bottles containing filtered seawater. Water was changed daily prior to feeding to remove most of the uneaten food organisms. *P. pileus* were fed copepods along a P-gradient, resulting in 5 treatments. The treatments were randomly assigned to 50 ctenophores, 10 replicates for each of the 5 feeding treatments. The copepods were mixed prior to being fed to *P. pileus*, resulting in the following regimes: at the extreme ends of the P-gradient the ctenophores were fed only copepods from the f/2- or the –P- treatment, respectively; three other treatment groups received 75%, 50% and 25% of the f/2-reared copepods, and 25%, 50% and 75% of –P-reared copepods to result in the amount of 500 copepods per ctenophore day^{-1}. *P. pileus* were fed daily for 9 days, after which the ctenophores were harvested and first frozen at -80°C and thereafter freeze-dried, weighed and kept in a desiccator until analysis.

Analytical procedures

For the analysis of carbon content of the algae, approximately 4×10^6 cells were filtered onto pre-combusted and washed Whatman GF/F filters. For the analysis of copepod carbon, 50 individuals were counted into tin capsules. The carbon content of the samples was measured with a Fisons

EA 1108 CHN analyser. Phosphorus was analysed as orthophosphate, after the method described by Grasshoff et al (1999), following oxidative hydrolysis. The samples were treated with an oxidation agent ($K_2S_2O_8$, H_3BO_3, NaOH in distilled water) under high pressure and at high temperature (120°C) in an autoclave to convert the phosphorus compounds to the ortho-phosphate form. Molybdate-antimony- solution (containing ammonium molybdate $(NH_4)_6Mo_7O_{24}$ x $4H_2O$, antimony potassium tartrate $K(SbO)C_4H_4O_6$ x $0{,}5H_2O$) and ascorbic acid was added

and the P-content measured photometrically. For the analysis of carbon and P-content of *Pleurobrachia pileus*, pulverised tissue homogenate was used.

Dry weight (or somatic growth) is not a useful response variable in this case for several reasons. First, as we could not work with a cohort of animals of completely equal size and age, initial weight could not be established accurately enough, and hence the variation in computed growth rates would probably be much larger than any expected effect. Secondly, dry weight in gelatinous zooplankton is mainly determined by the salt in the water of the body cavities, thus does not give an accurate estimate of the growth in biomass. Consequently, we used the carbon content per dry weight as an indicator for physiological condition. Animals with a higher C to dry weight ratio have a better physiological condition , as was observed by for example Daly (2004). Furthermore, the nucleic acid content of the animals was also established. The techniques to determine the amounts of RNA and DNA in animal tissue are well-established, and using the amount of nucleic acids RNA and DNA in the individual organisms as a measure of overall physiological condition, and as a proxy for growth, is commonly used in fisheries biology (e.g.Clemmesen et al. 2003). In many studies a ratio between the RNA and the DNA content of animal tissue is used, under the rationale that DNA content as a constitutive component of the cell should be more constant relative to RNA, which should be higher under active growth. A high RNA to DNA ratio is therefore indicative of growth and a good physiological condition. Ctenophores are known to react to adverse conditions by changes in size (Kremer 1977). This means that most likely, they lose complete cells, with both DNA as well as RNA content. Indeed, recent work by Hamer (2008) showed that the RNA:DNA ratio in the ctenophore *Mnemiopsis leidyi* was more or less constant, even after many days of starvation, whereas the concentrations of RNA and DNA were much more responsive. Therefore, in this study we used the absolute amounts of RNA and DNA in the tissue of the individuals as indicators for animal physiological condition (Ferron and Leggett 1994; Gorokhova 2003; Parslow-Williams et al. 2001). The method used for the nucleic acid analysis was modified from Clemmesen et al (2003) after the determination of the protocol for the extraction of nucleic acids from ctenophore tissue.

Freeze-dried tissue was pulverised using a pellet pestle and rehydrated in 400µl Tris-SDS buffer (Tris 0.05mol L^{-1} , NaCl 0.01mol L^{-1}, EDTA 0.01mol L^{-1}, sodium dodecyl sulphate (SDS) 0.005%) for 25 minutes. Glass beads (2mm and 0.17-

0.34mm diameter) were added and the tubes shaken in a Retsch MM 301 cell-mill for 15min. The homogenate was centrifuged (Sartorius Sigma 3-18 K; 8min, 3800g, 4°C) and 130µL of the supernatant used for analysis.

The amount of nucleic acids was determined fluorometrically in a microtiter fluorescence reader (Fluoroskan Ascent) using the fluorophor ethidiumbromide (EB). Total nucleic acids were measured first. Subsequently RNAse was added to the samples in order to digest the RNA. After the enzyme treatment (30min at 37°C) the remaining DNA was measured. The RNA fluorescence was calculated by subtracting the DNA fluorescence from the total nucleic acid fluorescence. RNA calibrations were set up each measurement day. The DNA concentrations were calculated using the relationship between RNA and DNA fluorescence described by Le Pecq and Paoletti (1966).

RESULTS

Primary producer and primary consumer

Algal molar C: P ratios varied significantly between treatments (f/2 ~ 230, -P ~ 430; ANOVA $F_{1,18}$=10.75; p<0.004; Figure 1). The results confirm that the –P growth medium really was limited in phosphorus; the natural seawater contained just enough P to enable the algae to grow. The molar C: P ratio of the copepods also showed differences between treatments, which were significant at p=0.07 (f/2 ~ 140, -P ~ 195; ANOVA $F_{1,10}$=4.06; p=0.07; Figure 1).

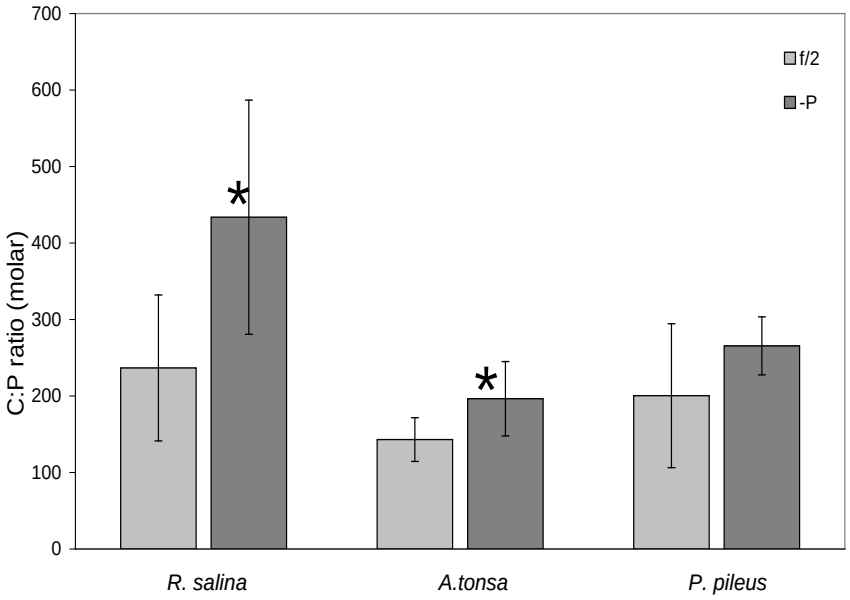

Figure 1. Molar C:P ratios of *Rhodomonas salina* (n=10), *Acartia tonsa* (n=7) and *Pleurobrachia pileus* (n=7) in a nutrient replete (f/2) and phosphorus-limited (-P) environment. Asterisk marks significant difference (p<0.05 for *R. sali*na, p=0.07 for *A. tonsa*) from the other treatment of the given species. Error bars: standard deviation.

Secondary consumer

The differences in the C:P ratios of *P. pileus* cultured on the extremes of the food gradient were not significant (Figure 1), but were significantly higher than those for their food (ANOVA $F_{1,17}$=6.60; p=0.02), which was mainly caused by the differences between f/2 copepods and ctenophores, with much lower C:P values for the copepods. As expected for the reasons mentioned above, dry weight of *Pleurobrachia pileus* was not significantly affected by the food they received (Figure 2a), nor was there a clear pattern of C: P ratios of the *P. pileus* individuals over the gradient (Figure 2c).

P. pileus fed a diet consisting solely of P-limited copepods (0% treatment) showed the highest values of carbon per dry weight (Figure 2b; linear regression, $y = 4.253\text{-}0.015x$; $r^2=0.36$; $p=0.001$). Furthermore, both the DNA content (µg mg DW^{-1}) (Figure 2d; linear regression, $y = 0.294\text{-}0.002x$; $r^2=0.18$; $p=0.01$), as well as the RNA content of the experimental animals (Figure 2e; linear regression, $y = 1.249\text{-}0.009x$; $r^2=0.18$; $p=0.01$) showed a significantly negative relationship with the percentage of f/2-copepods in their food. The content of RNA and DNA in individuals were highly correlated ($r^2=0.98$). These results indicate a simultaneous loss of both nucleic acids related to the amount of f/2 copepods in their diet (Figure 2 d, e), and hence the ratio between RNA and DNA concentration of the experimental *P. pileus* individuals did not show significant change over the experimental gradient (Figure 2f). The RNA content (µg mg DW^{-1}) of the animals in the starvation group (mean value 0.4 µg RNA mg DW^{-1}) was lower than that of the fed individuals. No significant correlation was found between dry weight of the animals and their biochemical composition (data not shown).

Even though the amount of explained variation in the significant relationships is modest, all physiological condition indicators point in the same direction: copepods that were grown on P-limited algae represent a food source of higher quality for *Pleurobrachia pileus* compared to those fed P-replete algae.

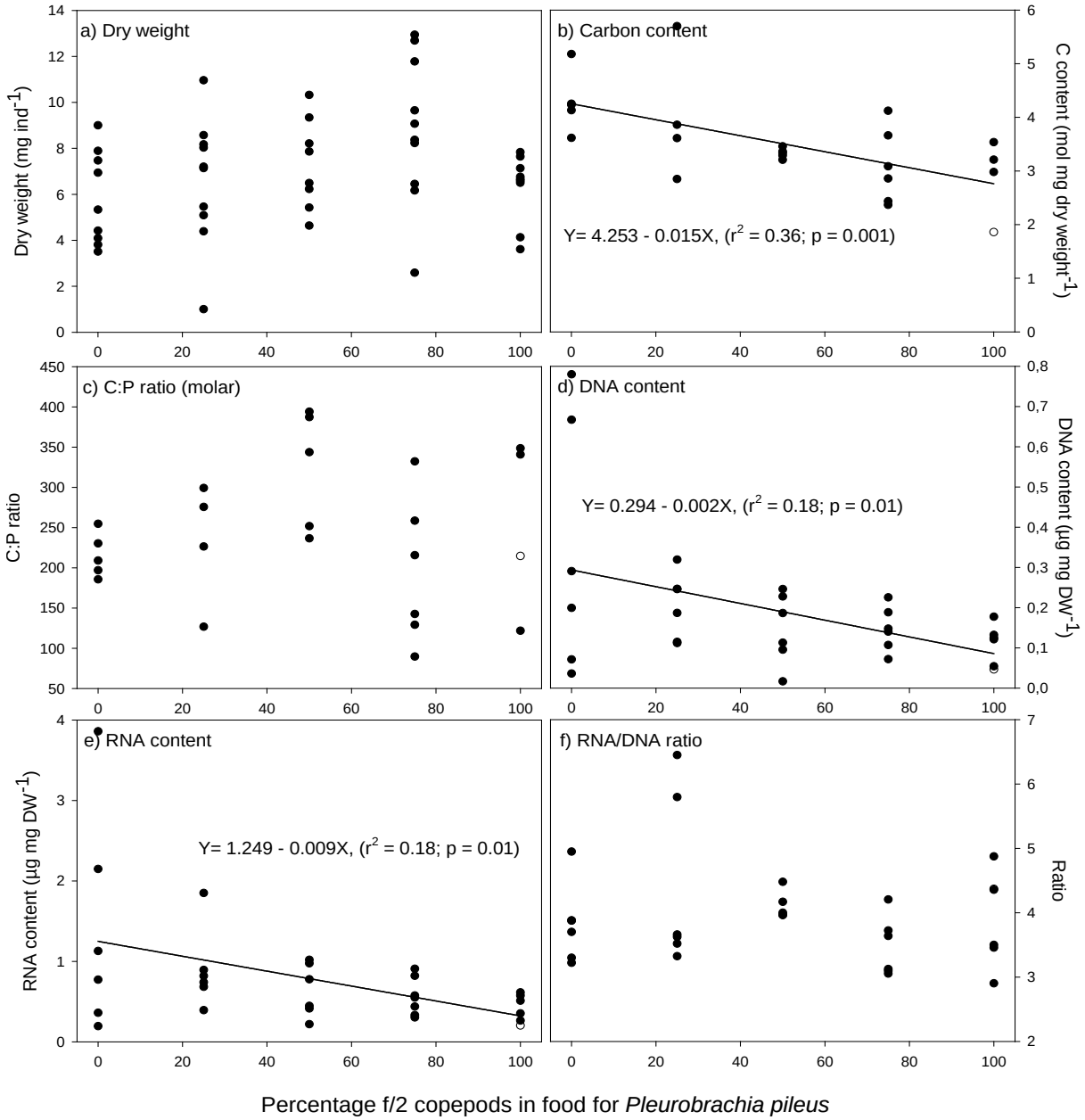

Figure 2. Reactions of *P. pileus* to food of different P-content. Regression lines indicate significant relationships between the percentage of f/2-fed copepods in the diet of *P. pileus* and the response variables. a) Dry weight of *P. pileus* after the incubation period; b) carbon content (mol per mg dry weight; c) C:P ratio (molar) of the experimental *P. pileus*; d) DNA content; e) RNA content; and f) the ratio between RNA and DNA content in *P. pileus.*

DISCUSSION

The classical picture in the current literature is that when going up in the food chain the C:P ratio of the organisms in each level is lower than that of the level below, as carbon is lost through respiration and excretion (Sterner et al. 1998). Here, we present an unusual case where the C:P ratio of the predator (*P. pileus*) actually is higher than that of the prey. As a consequence, the f/2 copepods in this study may have been a lower quality food source for the predator *P. pileus*, not because they contained too much carbon relative to phosphorus, but rather the reverse, and it does explain why we find the best physiological condition of the *P. pileus* individuals on copepods fed P-limited *R. salina*. Most likely, given the high C:P values of *P. pileus,* even the low-P copepods contained too much phosphorus. This excess in P resulted in a diet poor in carbon, making the ctenophores potentially energy-limited, rather than P-limited (see also Gaedke et al. 2002). So, the hypothesis based on the study of Borodkin and Korzhikova (1991), that due to their relatively high phosphorus content *Pleurobrachia*, or perhaps even all ctenophores, are expected to show phosphorus limitation quickly, clearly needs to be rejected. In fact, several studies (Anninsky et al. 2005; Schneider 1989) have indicated a very high content of proteins in ctenophores, which is corroborated by the low C:N values found by Kremer and co-workers (Kremer 1977; Kremer 1982; Kremer et al. 1986; Youngbluth et al. 1988), so the measurements of Borodkin and Korzhikova (1991) for *Mnemiopsis leidyi* might be considered unusual and not reflect a general pattern in ctenophores.

Virtually no information exists on the nutrient stoichiometry and physiology of ctenophores, and of *P. pileus* in particular. Hence, we can only speculate on the physiological processes that underlie the patterns that we have observed. Ctenophores are highly opportunistic predators that feed when possible but can withstand long periods of starvation, particularly in temperate regions (Greve 1972), and respiration rates go to virtually zero under starvation (Hamer, pers. com.). The fact that these mechanisms are in place suggest that carbon limitation is not uncommon in ctenophores. Furthermore, the only study that we are aware of reporting on excretion rates of carbon and phosphorus in ctenophores (Kremer 1977) reported very high turn-over rates of *Mnemiopsis leidyi* for phosphorus (20-50% d^{-1}), also suggesting that phosphorus is not the limiting nutrient in ctenophores. Based on this, we would not really expect to see an effect of the changed nutrient

stoichiometry, as obviously even P-limited copepods contain sufficient phosphorus to sustain an optimal physiological condition in *P. pileus*. We do, however, see a decrease in condition of the ctenophores with increasing phosphorus content of their prey. Boersma and Elser (2006) hypothesized that any excretion of surplus nutrients comes with a cost. Traditionally, most studies on zooplankton feeding (Darchambeau et al. 2003; DeMott et al. 1998; Sterner and George 2000; Sterner and Hessen 1994; Urabe et al. 1997), have focused on the effect of high C:P food on growth and reproduction, and explained lower performance on high C:P food with the need for the animal to rid itself of excess carbon to meet the P demand. When we follow Boersma and Elser (2006) and accept that also excreting excess phosphorus comes with a cost, we can explain the patterns found in this study. Based on our results we suggest that the phosphorus content of the f/2 copepods may have been too high and that the excretion of the excess phosphorus could come at the cost of a reduced physiological condition.

The analysis of the RNA and DNA contents in animals has been utilized as an indicator of the fitness and physiological condition of animals (Clemmesen et al. 2003; Malzahn et al. 2007b; Melzner et al. 2005). The method for the analysis of the RNA and DNA concentrations used here was adapted from Clemmesen (2003) after the best conditions for nucleic acid extraction from ctenophore tissue were determined. Both the amount of tissue used and to a more important extent the concentration of the detergent in the buffer influenced the results. Extraction of nucleic acids from the tissue of *P. pileus* proved difficult, as the yield was very low and unstable at first. By testing a range of combinations of different sample weights and buffer concentrations, a procedure enabling a stable and consistent extraction of nucleic acids was established. The most stable and reliable results were obtained with a combination of high tissue sample weights and a reduced concentration of the buffer SDS, from the 0.01% used in the protocol established by Clemmesen et al. (2003) to 0.005%. Our results show that RNA and DNA content were highly correlated (r^2=0.98). Both the DNA content as well as the RNA content was affected in parallel as a result of the different feeding environment. Ctenophores react to adverse feeding situations by decreases in body size (Kremer 1977); the underlying mechanism to this seems to be the shedding of whole body cells (Hamer 2008), reducing the total amount of cells and including the loss of RNA and DNA contained in these cells. As a result the ratio of RNA to DNA content in ctenophore

tissue is not a very useful measure to assess physiological condition, as was already expected from the fact that body size is so plastic in ctenophores.

Obviously, this experiment represented a very artificial world, with organisms that might not even meet in the field. However, our results have the power to reveal mechanisms that would not be visible in the field, and certainly may be of relevance. Having said this, ctenophores obviously do not feed exclusively on juvenile copepods as was the case in this study. Ontogenetic changes on the nutrient stoichiometry and the phosphorus content in particular have been reported by Carillo et al. (2001) and Villar-Argaiz et al.(2002). The P-content of copepods (the calanoid copepod *Mixodiaptomus laciniatus*) was observed to have changed over the course of the life cycle, with nauplii having a higher P-content than adult copepods. So the results might have been different if we had used adult copepods as prey for *P. pileus.* Furthermore, 1 L bottles are certainly fairly small for *P. pileus*, and the organisms might have had problems developing their tentacles. However, as all experiments were carried out in the same sized vessels this should not have been a problem.

Potential Implications

By feeding the copepods to the ctenophores along a gradient, we were able to show that more subtle phosphorus limitations can also have an effect on consumers and are still traceable to the next trophic level. This is of particular importance during transitional phases in the phosphorus content of seawater, such as the late phase of a phytoplankton spring bloom in temperate regions. During this time, phytoplankton becomes more and more phosphorus-limited, and as a result the zooplankters feeding on them will also most likely change their nutrient composition to some extent. In this special case, P-limited copepods represent a food source of higher quality for *P. pileus.* Interestingly, but with a great need for further study, this period of phosphorus-depletion in phytoplankton is exactly the time when we see high densities of *Pleurobrachia pileus* around Helgoland (Greve et al. 2004). It is possible that the ctenophores, being less vulnerable to the P-limited situation, are at an advantage during this time.

Currently, many coastal seas are undergoing re-oligotrophication as a result of decreasing phosphorus inputs from the rivers (Wiltshire et al. 2008). This change of the nutrient composition towards a more phosphorus-limited regime may result in food of high quality for the ctenophores. In turn, Malzahn et al. (2007a) showed that

P-deficient copepods are inferior food for zooplanktivorous larval fish, which utilize the same food source. One might speculate that this mechanism could further support the general trend towards a more gelatinous North Sea as reported by Atrill et al (2007) as a phosphorus-limited environment would favour the occurrence of ctenophores in the plankton. Furthermore, the effects of nutritional imbalances may even be increased by rising CO_2 levels, which are predicted by future climate change scenarios (IPCC 2007b). This could possibly lead to changing stoichiometric composition of primary producers, which has consequences for consumers performance (Urabe et al. 2003) and might lead to higher C:P ratios of primary producers. It has been forecast that in phosphorus-deficient systems zooplankton with high C:P ratios, such as *P. pileus*, will become more important (Gaedke et al. 2002). From this, one would predict that ctenophores will continue to grow in their importance in many coastal ecosystems.

The results presented here show that phosphorus limitations in primary producers can be traced to primary and secondary consumers. The ensuing quality effects propagate through the food web and affect higher trophic levels, albeit in a different manner than originally expected. The effects of the P-limitation, as such, on *P. pileus* were rather weak, but the changed nutrient composition of the algae affected the secondary consumer nevertheless. Contrary to our original expectations, our results show that the ctenophore *Pleurobrachia pileus* is not negatively affected by low P food and may even benefit from a diet of copepods feeding on P-deficient phytoplankton.

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CHAPTER III

The reaction of European lobster larvae (*Homarus gammarus*) to different quality food: effects of ontogenetic shifts and pre-feeding history

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ABSTRACT

The high mortality commonly observed in decapod larvae has been linked to poor nutrition. To investigate the effect of food quality on the nutritional condition of lobster larvae we established a tri-trophic food chain consisting of the cryptophyte *Rhodomonas salina*, the calanoid copepod *Acartia tonsa* and larvae of the European lobster *Homarus gammarus*. In a set of experiments we manipulated the C:N:P stoichiometry of the primary producers, and accordingly those of the primary consumer. In a first experiment *R. salina* was grown under N- and P-limitation and the nutrient content of the algae was manipulated by addition of the limiting nutrient to create a food quality gradient. In a second experiment the effect on lobster larvae of long- and short-term exposure to food of varying quality during ontogenetic development was investigated. The condition of the lobster larvae was negatively affected even by subtle N- and P-nutrient limitations of the algae. Further, younger lobster larvae were more vulnerable to nutrient limitation than older ones, suggesting an ontogenetic shift in the capacity of lobster larvae to cope with low quality food. The results presented here might have far-reaching consequences for the survival of lobster larvae in the field, as in the light of future climate change and re-oligotrophication of the North Sea, lobster larvae might face strong changes in temperature and nutrient conditions thus altering their condition and growth significantly.

Keywords: Ecological stoichiometry; nutrient limitation; trophic interactions; marine foodwebs

INTRODUCTION

The European lobster, *Homarus gammarus*, is found around the rocky island of Helgoland in the North Sea, where its fishery has always been of great importance. Stocks have, however, declined steeply in the last 50 years. The annual catch has declined from about 80,000 lobsters in the 1930s to only a couple of hundred individuals in the 2007 fishing season (Schmalenbach 2009). Among the possible reasons for the collapse of the lobster population are habitat destruction, unsustainable fishing pressure, pollution and competition with the edible crab *Cancer pagurus*. Unfortunately, the lobster stocks have not recovered significantly in spite of new fisheries legislation and restocking programs (Schmalenbach et al. 2009). The globally observed trend of rising sea surface temperatures is also clearly visible in the North Sea (Edwards and Richardson 2004; Franke et al. 1999; Wiltshire et al. 2008). Therefore, Schmalenbach and Franke (Schmalenbach and Franke 2010) investigated the impact of this changing temperature regime on *H. gammarus*. Increased mean temperatures, particularly during winter, accelerate the embryonic development of larvae of the European lobster, resulting in an earlier larval release (Schmalenbach and Franke 2010). Schmalenbach and Franke (2010) give two reasons why this forward shift should have a negative effect on larval lobster survival. Earlier hatching means hatching into a colder environment. This causes higher intrinsic mortality as well as higher predation risk as the larvae spend much more time in the vulnerable pelagic stage. On the other hand, Schmalenbach and Franke (2010) speculate that the timing of larvae release should have been such that the larvae were confronted with optimal feeding conditions before, and thus a shift forward might also lead to a worse feeding environment, especially if these temperature-related shifts are different for prey organisms. Thus, the larvae may be released into the water column at a time when the conditions, such as temperature and food availability, are sub-optimal for their further development. While Schmalenbach and Franke give convincing support for the temperature hypothesis, they did not investigate the effects of food. Under the current temperature regime the lobster larvae at Helgoland are likely to hatch at a time when nutrient concentrations in the water column available for phytoplankton are low. Furthermore, the ongoing re-oligotrophication of the Southern North Sea (van der Zee and Chou 2005; Wiltshire et al. 2008) has caused and probably will continue to cause a decrease in concentrations of such essential nutrients as nitrogen and phosphorus. As recent

evidence has shown that the nutrient content of phytoplankters may affect growth and development not only of herbivores but also of secondary consumers (Boersma et al. 2008; Malzahn et al. 2010; Schoo et al. 2010) this may impose additional stress on successful recruitment of the lobster population. Unfortunately, we do not know anything about how the quality of their food, in terms of nutrient content, affects the lobster larvae.

As the nutrient ratio of primary producers is strongly dependent on light and the nutrient availability of the surrounding seawater, this ratio is subject to natural variations (Dickman et al. 2008; Elser et al. 2002). In the relationship between primary producers and herbivores, nutrients such as nitrogen (N) and phosphorus (P) are often limiting, as there is a surplus of carbon in plants (White 1993). Therefore, for many herbivorous consumers food may be of poor quality at times of nutrient limitation. In contrast to their prey, animals are able to maintain a relatively stable homeostasis (i.e. they maintain a constant ratio of carbon to nutrients). The focus in the study of nutritional quality aspects in aquatic ecosystems has therefore been on the primary producer-herbivore interface, in particular on the link between algae and microcrustaceans (Gulati and DeMott 1997). Herbivores have developed several mechanisms to deal with the excess carbon in their diet, ranging from increased activity (Plath and Boersma 2001) and respiration (Darchambeau et al. 2003; Malzahn et al. 2010) to changes in the digestion of carbon (DeMott and Tessier 2002). Whatever the mechanisms used, getting rid of the excess carbon comes at a cost to the herbivore and often results in decreased growth and reproduction (Boersma 2000; Malzahn et al. 2007a).

Herbivore homeostasis has led to the prediction that food quality effects are not passed on to trophic levels beyond the herbivore, as the nutrient limitation of the primary producers would be buffered by the herbivorous consumer. Even if this should not be the case the nutrient imbalances facing secondary consumers should be small compared to those at the primary consumer-herbivore interface. Recent studies have, however, shown that homeostasis in herbivores is less than perfect, meaning that their quality as food to consumers is subject to variations (Malzahn et al. 2007a; Malzahn et al. 2010; Schoo et al. 2010), and potentially passed through the food chain.

Given the present temperature regime, where lobster release their larvae in a period of low nutrient availability, the timing of larval release might be even less favourable

under the predicted rise in temperatures (IPCC 2007a). Hence we set out to investigate one of the consequences of the potential uncoupling of larval release and optimal food conditions in a stoichiometric context by subjecting lobster larvae to different quality food in a laboratory based experiment. The primary producer *R. salina* was grown under nutrient replete (f/2) conditions and under N- or P-limitation (-N and –P, respectively), and the nutrient-content subsequently manipulated to create a nutrient-gradient. These algae were fed to the calanoid copepod *Acartia tonsa*, which in turn served as food for the lobster larvae. A second experiment was designed to test the effect of a P-limitation gradient on lobster larvae of different ages and pre-feeding histories, thereby investigating the vulnerability to food quality changes throughout the planktonic life of the lobster larvae and in relation to the larval condition. As copepods are not perfectly homeostatic, and lobster larvae are relatively fast growers, we hypothesise that we will see an effect of the nutrient condition of the algae transferred up the food chain by the herbivorous copepods.

MATERIALS AND METHODS

Experiment 1

In order to test how different levels of N- and P-limitation affect freshly-hatched lobster larvae, algae were grown under nutrient-limited conditions before being pulsed with the missing nutrient. Copepods reared on the different algae were in turn fed to the lobster larvae.

Phytoplankton

A stock culture of *R. salina* was cultivated in enriched seawater (f/2) according to Guillard and Ryther (1962). All algal treatments were grown under a 16:8 h light:dark (L:D) regime at a constant temperature of 18°C. Natural seawater was used for the experiments, which was filtered using a sterile 0.2 µm filter and stored cool and dark until use. The seawater for the nutrient-replete f/2 treatment was enriched with the full set of nutrients as described by Guillard and Ryther (1962). This contains 36.2 µM NaH_2PO_4 and 882 µM $NaNO_3$. The water for the growth medium of the two limitation treatments was also enriched according to the f/2 recipe, but without the addition of the limiting nutrient (N or P respectively). By enriching the seawater with all of the other nutrients (i.e. N or P, vitamins, metals) in excess, the growth of the algae and the rapid limitation with respect to the missing nutrient were assured. The algae had access only to the P and N contained in the natural seawater at the time of filtration and became limited with respect to the missing nutrient after a growth period of 4 days.

Algal densities in the stock solution were measured with a CASY cell counter (Schärfe System CASY Cell Counter and Analyser System). To ensure constant supply and food quality new cultures of *R. salina* were inoculated daily for every treatment with a starting concentration of 0.2×10^6 cells L^{-1} for the f/2 treatment and 0.3×10^6 cells L^{-1} for the –N and –P treatments. Algae were harvested after a growth period of 4 days at densities of 1.5×10^6 cells L^{-1} for the f/2 treatment, 0.65×10^6 cells L^{-1} for the- N and 1.0×10^6 cells L^{-1} for the –P treatment.

For the P-addition treatments the P-limited algae were pulsed with two different concentrations of P, in the form of NaH_2PO_4; 1.8 µM for the P1 and 7.3 µM for the P2 treatment, respectively. Preliminary tests had shown that the P-limited *R. salina* were unable to take up more than the 20% (relative to the concentration of 36.2 µM

P in the f/2 medium) additional P and indeed reached a saturation point. The algae were then incubated for 15 minutes, centrifuged and washed with artificial seawater to remove any excess dissolved phosphorus not taken up by the algal cells. This resulted in a P-content gradient raging from –P (completely limited) to P1 and P2, the P-pulsed algae.

Uptake of dissolved nitrogen by N-limited *R. salina* cells is not as rapid; prior tests had shown that while the N-limited *R. salina* take up the dissolved nitrogen and change their C:N ratio accordingly, this process takes several hours. Therefore the full f/2 amount of dissolved nitrogen (Na_2NO_3, concentration 882 µM) was added to the N-limited algal cultures and the algae harvested 5 (called N1, hereafter) and 10 hours (called N2) after the nutrient addition. The uptake process was stopped by washing the algae with nutrient-free artificial seawater before feeding the suspension to the copepods.

Zooplankton

Copepod eggs were obtained from a culture of the calanoid copepod *Acartia tonsa*. The copepods for egg production were kept in filtered natural seawater (salinity ~32) in a 200 L tank at 18°C under a 18:6 light:dark regime. The copepods were fed a mixture of the algae *R. salina* and the heterotrophic flagellate *Oxyrrhis* sp. Eggs were siphoned off the bottom of the tank daily and stored in an airtight container in seawater at 4°C until use. No eggs older than 3 months were used in the experiments. Hatching rate was about 20%.

The copepod eggs were incubated at a density of about 2000 individuals L^{-1} in artificial seawater (salt: hw Marinemix, www.hw-wiegandt.de) adjusted to a salinity of 32. Copepods were first fed 24 hours after hatching; 48 hours after the addition of the eggs to seawater. The copepods were fed 50 000 cells of *R. salina* per individual and day (corresponding to ~ 3 mg C L^{-1} d^{-1}), irrespective of the nutrient treatment. This concentration is considered *ad libitum* for juvenile copepods. Copepods were fed the different algae treatments for 8 days. Since the copepods fed on P limited algae showed a slower growth and development, the copepods fed f/2 were hatched a day later. This was necessary to ensure that all the copepods fed to the secondary consumers were of the same age and size class. To ensure a constant food quality for the secondary consumers for every day of the feeding experiment new copepod cultures were started for each of the 7 nutrient treatments.

The copepods were transferred to fresh artificial seawater daily prior to feeding in order to avoid remineralisation of the nutrient-limited algae by waste products of the copepods. The artificial seawater was stored at 18°C and constantly aerated.
Copepods were passed over a sieve and transferred to fresh artificial seawater prior to being fed to the lobster larvae.

Lobster larvae

Freshly hatched larvae of the European lobster *Homarus gammarus* were collected from the lobster rearing facility at the Biological Station, Alfred-Wegener-Institute, on Helgoland. Larvae from different females were collected to minimize batch difference and possible maternal effects. The larvae were carefully sorted and transferred to individual 40 mL beakers containing seawater. Twenty larvae were randomly assigned one of the 7 nutrient regimes (f/2, -P, P1, P2, -N, N1, N2). An additional 20 larvae were assigned to a starvation group. The lobster larvae were fed 50 copepodites per individual and day. Preliminary tests had shown that this concentration represents *ad libitum* feeding conditions. Feeding in a 24 hour rhythm ensured that the animals had access to food both during the day and during the night, when they are normally found foraging for food in the field (Mehrtens et al. 2005). The water in the beakers was changed daily prior to feeding. The lobsters were kept at a 16:8 light: dark regime under indirect light in a temperature-controlled room at 15°C. This temperature resulted in a time frame of approximately five days until first moulting (Branford 1978; Templeman 1936). On the fifth day after hatching the lobster larvae were sampled, rinsed in distilled water and frozen at -80°C until analysis.

Experiment 2

In the second experiment lobster larvae were exposed to a P-gradient in their diet either from hatch onward or after first having been reared to second or third larval stage on a diet of *Artemia* sp.. This experiment thereby examines the effects of pre-feeding history and possible ontogenetic shifts in the responses of larvae exposed to food of varying but low quality.

Phytoplankton

In the second experiment, algae were grown under nutrient replete conditions (f/2), as well as under P-limited conditions (–P, hereafter). The culture conditions were the same as described for Experiment 1 above. In order to test for more subtle nutrient limitation effects, two additional C:P ratios were included in the P-limitation gradient, resulting in 5 different P-limitation treatments. The P-pulses to the P-limited algae consisted of 2.5%, 5%, 10% and 20% of the full f/2 P-content. This means that a pulse of 0.9 µM, 1.8 µM, 3.62 µM and one of 7.3 µM, respectively, was added to the P-limited algae to change their stoichiometry. The algae were then incubated, washed and fed to the copepods, as described or Experiment 1.

Zooplankton

Copepods for the second experiment were treated the same way as those in Experiment 1, described above. There were five different nutrient treatment groups, equivalent of the five different algal P-treatments from P-limited to the 7.3 µM added treatment, as well as a f/2 food group.

Lobster larvae

In order to test the effects of long-term and short-term exposure as well as the effect of ontogeny to food with varying P-content, two parallel experiments were run. In the first, lobster larvae were reared on the P-limited copepods from hatch onwards, and in the second experiment lobster larvae were reared on nauplii of the brine shrimp *Artemia* sp. (cysts from Sander's Brine Shrimp Company) until the first and second moult (i.e. reaching stage 2 and stage 3, hereafter named A2 and A3, respectively), before being exposed to P-limited copepods. An additional starvation treatment was added to both experiments. This experimental design allows taking into account the pre-feeding history and the ontogenetic shifts in the responses of the larvae when exposed to food of different quality at different stages in their life cycle.

The lobster larvae to be reared on the copepod diet from the beginning were again collected shortly after hatching and randomly assigned a treatment and a sampling date, i.e. whether they were to be sampled in first or second stage.

The larvae reared on *Artemia* sp. nauplii, until second or third stage were also collected shortly after hatching and transferred to a rearing tank, constructed after Hughes et al. (1974), consisting of a cylindrical container equipped with a water circulator and overflow device. The circulation of the water ensures a high incidence of encounter between lobster larvae and their food as well as reducing cannibalism. The overflow device also enables the effective removal of partially eaten food and other waste products, ensuring high water quality.

The larvae were kept in the rearing tanks at 16°C water temperature in dim light and fed fresh *Artemia* sp. nauplii twice a day. Development was checked daily and only freshly moulted larvae were introduced into the experiment.

Analytical procedures

We analyzed the stoichiometry and fatty acid composition of each of the three levels of the food chain. For the analysis of the primary producer *R. salina* approximately 4 × 10^6 cells were filtered onto pre-combusted and washed Whatman GF/F filters. For each of the stoichiometric analyses of the copepods 75 individuals were counted into tin capsules; for the analysis of the fatty acid content the copepods were collected on GF/F filters.

As parameters to assess the reaction of the *H. gammarus* larvae to the different prey we used dry weight as well as the ratio between RNA and DNA and C:N:P ratios of the animals.

For the analysis of carbon, nitrogen and phosphorus of *H. gammarus*, pulverised tissue homogenate of freeze-dried whole larvae was used. For the analysis of fatty acid content and the RNA:DNA ratio of the larvae, whole freeze dried animals were used. In order to analyse both parameters from a single animal, the lipids were extracted in a first step, after which the RNA and DNA content was measured. This method was adapted from Peschutter (Peschutter 2008). Tests conducted prior to this experiment showed no effect of the lipid extraction with dichlormethane-methanol on the nucleic acid content and composition of the lobster larvae.

The carbon and nitrogen content of the samples was measured with a vario MICRO cube CHN analyser (Elementar Analysensysteme, www.elementar.de).

Phosphorus was analysed as orthophosphate, after the method described by Grasshoff et al. (1999), following oxidative hydrolysis. The samples were treated with an oxidation agent ($K_2S_2O_8$, H_3BO_3, NaOH in distilled water) under high pressure and at high temperature (120°C) in an autoclave to convert the phosphorus compounds to the orthophosphate form. Molybdate-antimony- solution (containing ammonium molybdate $(NH_4)_6Mo_7O_{24}$ x $4H_2O$, antimony potassium tartrate K(SbO) ($C_4H_4O_6$ x $0,5H_2O$) and ascorbic acid was added and the P-content measured photometrically.

The fatty acids were measured as fatty acid methyl esters (FAMEs). Lipids were extracted from the samples by dichloromethane:methanol (2:1 vol:vol) at -80°C for 48 hours. After the initial extraction step the lobster larvae were removed from the solvent-solution and freeze-dried to enable the subsequent analysis of the RNA and DNA content. The filters containing the algae or copepods were removed from the solvent and discarded. The water-soluble fractions were removed by washing with 0.88% KCl buffer, followed by centrifugation. The aqueous phase was removed, and the organic remainder was evaporated using nitrogen gas. The esterification was done using methanolic sulphuric acid at 70°C for 30 min. The FAMEs were washed from the methanolic sulphuric acid using n-Hexane. Excess n-Hexane was evaporated using nitrogen gas. All chemicals used were gas chromatography (GC) grade. FAMEs were analyzed by gas chromatography using a Varian CP 8400 gas chromatograph equipped with a DB-225 column (J&W Scientific, 30-m length, 0.25-mm inner diameter [ID], 0.25-mm film). The injector temperature was set to 250°C. The column oven was set to 80°C, which was heated to 150°C at a rate of 30°C min^{-1} after injection , then to 170°C at 6°C min^{-1}, and finally to 220°C at 1.5°C min^{-1}, which was held for 21 min. The complete measurement ran for 61.60 minutes. The carrier gas was helium at a constant pressure of 12 PSI. The flame ionization detector was set to 300°C. Injection of the 1 µl aliquots of the samples was done in a split-less mode. FAMEs were quantified using calibrations set up for each fatty acid separately and a known amount of C 23:0 was added at the first step of the preparation as an internal standard.

The technique to measure RNA and DNA content of animal tissue is well established and commonly used especially in fisheries science to determine the overall physiological condition and growth (e.g. (Buckley 1984; Clemmesen 1993; Malzahn et al. 2003). A high RNA to DNA ratio is indicative of a good overall condition and

high growth rates, as the DNA content of the cell should be constant while RNA should increase under active growth conditions. Lately the technique has also been successfully applied to determine the condition of crustaceans (e.g. (Rosa and Nunes 2004; Rosa and Nunes 2005; Wagner et al. 1998). The method used for the nucleic acid analysis was modified from Clemmesen et al. (2003).

Freeze-dried larvae were pulverised and rehydrated in 400 µl Tris-SDS buffer (Tris 0.05mol L^{-1} , NaCl 0.01mol L^{-1}, EDTA 0.01mol L^{-1}, sodium dodecyl sulphate (SDS) 0.01%) for 30 minutes. Glass beads (2 mm and 0.17-0.34 mm diameter) were added and the tubes containing the samples shaken in a Retsch MM 301 cell-mill for 15 minutes. The homogenate was centrifuged (Sartorius Sigma 3-18 K; 8min, 3800g, 4°C) and 130 µl of the supernatant used for analysis. The amount of nucleic acids was determined fluorometrically using the fluorophor ethidiumbromide (EB) in a microtiter fluorescence reader (Labsystems, Fluoroskan Ascent).

The total amount of nucleic acids in the sample was measured first, before the addition of RNAse to digest the RNA. The remaining DNA was measured after the enzyme treatment and the RNA fluorescence calculated by subtracting the DNA fluorescence from the total nucleic acid fluorescence. The DNA concentrations were calculated following Le Pecq and Paoletti (1966) using the RNA calibrations which were set up daily prior to the measurements.

Statistical Analysis

The nutrient ratios C:N, C:P and N:P of the primary producer *R. salina* from both experiments as well as fatty acid ratios were analysed as dependent variables in a one-way ANOVA with ‘nutrient treatment’ as the factor. The copepod stoichiometric measurements and fatty acid ratios were also analysed in a one-way ANOVA with ‘nutrient treatment’ as factor.

For the first experiment the stoichiometric measures of the lobster larvae, as well as their fatty acid ratios, RNA:DNA ratios and dry weight were analysed as the dependent variables in a one-way ANOVA, with ‘nutrient treatment’ as the factor.

The stoichiometric measures and nucleic acid ratios (RNA:DNA) of the Lobster larvae from the second experiment were analysed as the dependent variables in a one-way ANOVA in which the ‘nutrient treatment’ was used as factor. Linear regression analyses were used to test for the effect of food nutrient content on the condition of the larvae.

Tukey's HSD test was used as the post hoc test in all cases. All statistical tests were carried out with Statistica (STATISTICA 7, StatSoft) and Prism software (Graph Pad Prism 3.0).

RESULTS

Experiment 1

Phytoplankton

The algae grown under N- and P- depleted and nutrient replete conditions showed significant differences in their nutrient stoichiometry (Figure 1A-C) and fatty acid profiles (Figure 2A and Table 2). The C:P ratio of the P-limited algae was significantly different from that of all the N-limited and nutrient-replete treatments (Figure 1A). The C:N ratios of the algae grown under N-limited conditions were significantly higher compared to those grown under P-limited or nutrient-replete conditions (Figure 1B). The nutrient additions resulted in a C:nutrient gradient and created C:P (P1 and P2) and C:N (N1 and N2) ratios that were between those of the nutrient replete and the corresponding limited treatment (Figure 1). The nutrient-pulsed treatments were significantly different from each other and from the corresponding nutrient-limited treatments (Table 1). For the N:P values significant differences were detected among the P-limited treatments only (Figure 1C).

Table 1: Experiment 1 - Summary of all analyses of variance (ANOVA).

Organism	Factor		F	*df (df error)*	*p*
R. salina	C:P	Treatment	84.33	6 (28)	<0.001
	C:N	Treatment	47.61	6 (28)	<0.001
	N:P	Treatment	56.38	6 (28)	<0.001
A. tonsa	C:P	Treatment	4.22	6 (19)	<0.01
	C:N	Treatment	0.46	6 (19)	0.83
	N:P	Treatment	2.65	6 (19)	0.05
H. gammarus	C:P	Treatment	3.05	6 (39)	<0.05
	C:N	Treatment	7.20	6 (39)	<0.005
	N:P	Treatment	2.77	6 (39)	<0.05
	RNA:DNA	Treatment	17.22	6 (34)	<0.001
	Dry weight	Treatment	1.29	6 (81)	0.27

The fatty acid profiles of the algae showed significant differences between the nutrient treatments, with higher fatty acid content found in the limited algae (Figure 2A). The concentrations not only of total fatty acid content but particularly of the unsaturated fatty acids and the n6 fatty acids were significantly higher in the limited algae (Table 2). Algae from the N-limited treatments also contained significantly higher amounts of saturated fatty acids that the algae from the other nutrient treatments (Figure 2A). The n3:n6 ratio, however, was highest in the f/2 treatment. The addition of nutrient pulses to the limited algae (N1 and N2, P1 and P2) did not change the fatty acid content of the algae, the concentrations remained the same as in the nutrient limited –N and –P algae.

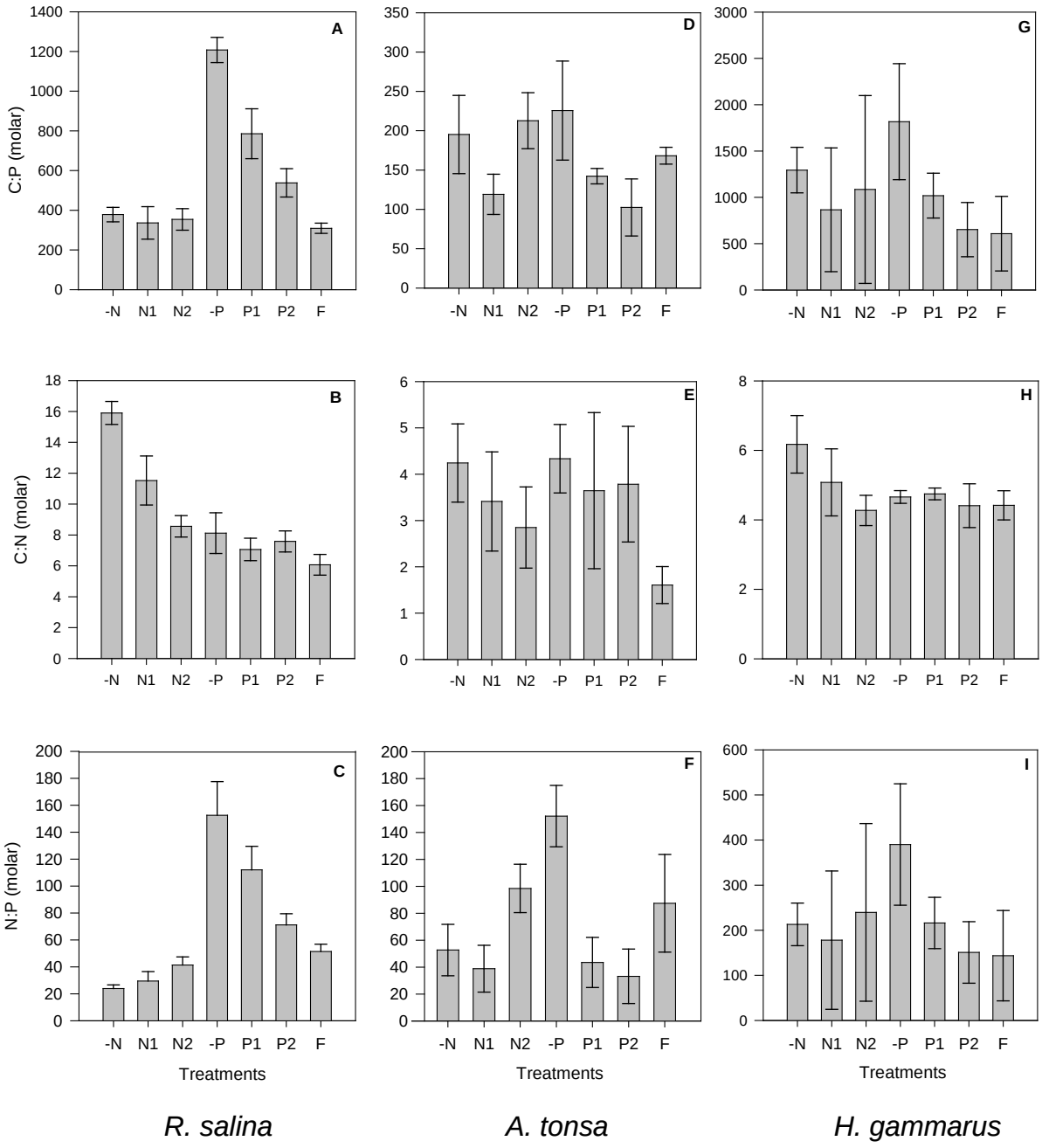

Figure 1: Stoichiometric composition of *R. salina* (A,B,C), *A. tonsa* (D, E, F) and *H. gammarus* (G, H, I) from Experiment1. Error bars are standard deviation. Note different scales of axes between organisms.

Zooplankton

The stoichiometry of the copepods also showed strong differences between treatments (Figure 1D-F). The C:P ratios differed significantly depending on the nutrient treatment of their diet (Figure 1D), as did the N:P ratios (Figure 1F, Table1). The C:N ratios of the copepods showed no significant differences (Figure 1E).

The fatty acid profiles of the copepods showed significant differences between treatments (Table 2). The copepods fed limited algae contained significantly higher total concentrations of fatty acids and higher concentrations of unsaturated and n6 fatty acids (Figure 2B). N-limited copepods also contained higher concentrations of saturated fatty acids. The highest rate of n3:n6 fatty acids was again observed in the f/2 treatment.

Lobster larvae

The nutrient composition of the food had significant effects on the lobster larvae (Figure 1G-I). The stoichiometric parameters were affected by the nutrient limitation and the larvae suffered from a decrease in condition as a result of the nutritional imbalances of their food. The different nutrient treatments of the primary producers were still visible in the lobster larvae, as the C:P (Figure 1G), C:N (Figure 1H) and N:P (Figure 1I) ratios of the larvae were significantly different depending on their nutrient treatment (Table 1).

The highest RNA:DNA ratio was found in the food chain with the highest P-pulse, P2 (Figure 3); this ratio was higher than that of the animals in the nutrient-replete (f/2) food chain ($p=0.026$; Tukey HSD test). Generally the RNA:DNA ratios of the N-limited treatments were lower than those from the P-limited treatments. The starvation group differed significantly from the RNA:DNA ratio of the P-pulsed treatments (P1, $p=0.039$, and P2, $p<0.001$; Tukey HSD) as well as from the nutrient-replete treatment ($p=0.003$, Tukey HSD test). No significant differences were detected between the starvation group and the RNA:DNA of the N-limited treatments.

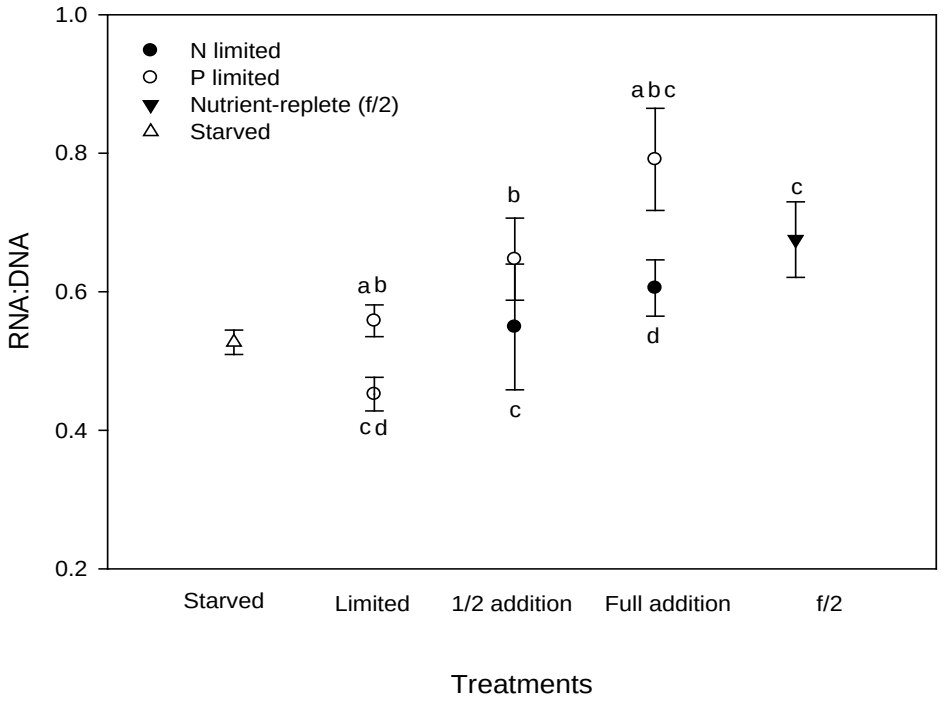

Figure 2: Experiment 1: Mean lobster RNA:DNA for each experimental treatment. 'Limited' corresponds to the –N and –P treatments, 'full addition' to the N2 and P2 treatments. Error bars are standard deviation. Significant differences ($p<0.05$) between treatments are indicated with different letters.

Table2: Experiment 1 - Summary of analysis of variance (ANOVA) for fatty acid (FA) sum parameters for the organisms.

Organism	Factor		df (df error)	p
R. salina	Total fatty acids	Treatment	6 (28)	<0.01
	Saturated fatty acids	Treatment	6 (28)	<0.001
	Unsaturated fatty acids	Treatment	6 (28)	<0.01
	Saturated: Unsaturated FA	Treatment	6 (28)	<0.001
	n3 fatty acids	Treatment	6 (28)	0.53
	n6 fatty acids	Treatment	6 (28)	<0.001
	n3:n6 fatty acids	Treatment	6 (28)	<0.001
A. tonsa	Total fatty acids	Treatment	6 (23)	<0.05
	Saturated fatty acids	Treatment	6 (23)	<0.05
	Unsaturated fatty acids	Treatment	6 (23)	<0.05
	Saturated: Unsaturated FA	Treatment	6 (23)	<0.05
	n3 fatty acids	Treatment	6 (23)	0.09
	n6 fatty acids	Treatment	6 (23)	<0.05
	n3:n6 fatty acids	Treatment	6 (23)	<0.05
H. gammarus	Total fatty acids	Treatment	6 (32)	<0.05
	Saturated fatty acids	Treatment	6 (32)	<0.05
	Unsaturated fatty acids	Treatment	6 (32)	<0.05
	Saturated: Unsaturated FA	Treatment	6 (32)	0.17
	n3 fatty acids	Treatment	6 (32)	0.07
	n6 fatty acids	Treatment	6 (32)	<0.05
	n3:n6 fatty acids	Treatment	6 (32)	<0.05

The fatty acid profiles of the lobster larvae showed significant differences in the concentrations of fatty acids between the treatments (Figure 2C).

The dry weight of the Lobster larvae did not differ between the treatments (ANOVA: $F(7, 94)=1.1680$, $p=0.33$).

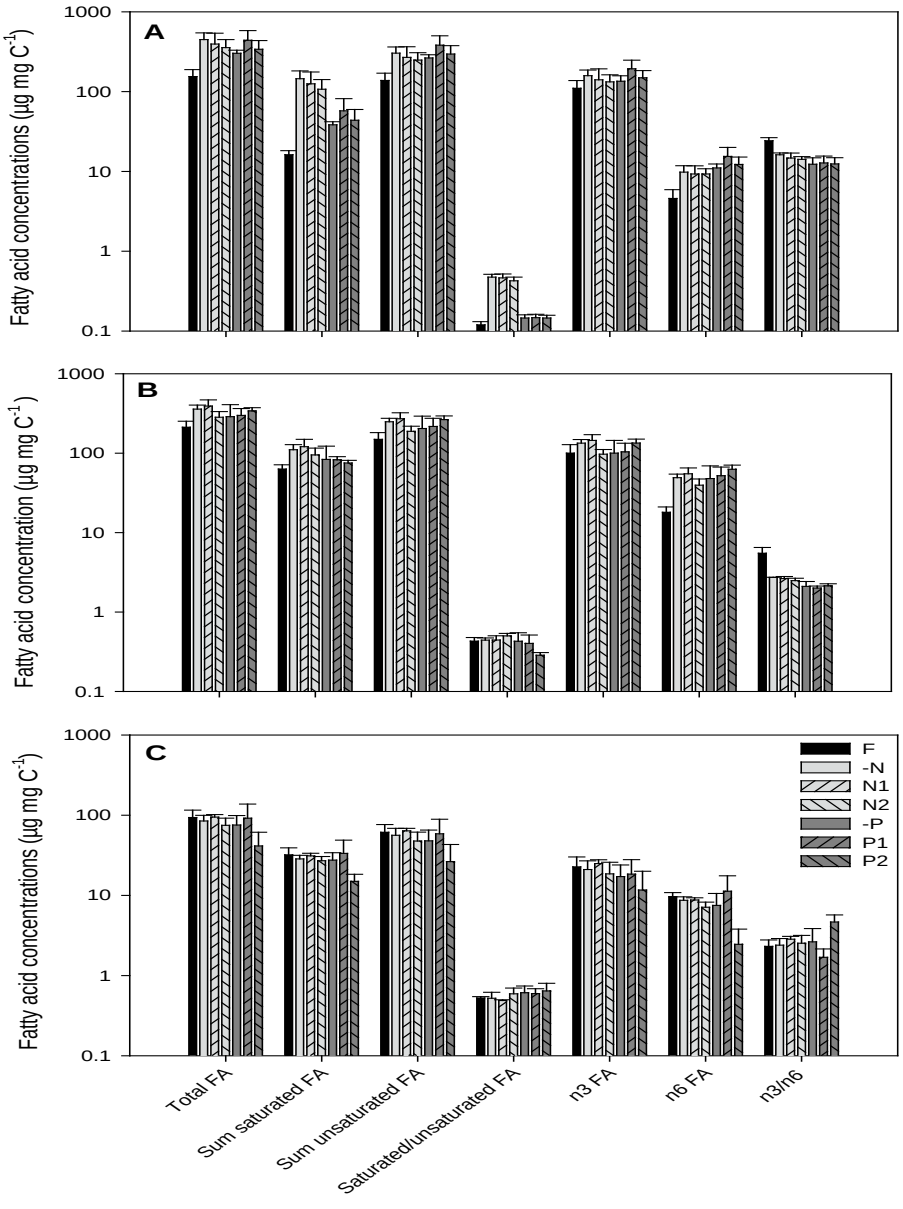

Figure 3: Experiment 1- Fatty acid sum parameters for *R. salina* (A), *A. tonsa* (B) and *H. gammarus* (C). Error bars are standard deviation.

Experiment 2

Phytoplankton and zooplankton

The stoichiometry of the *R. salina* showed significant differences with respect to their P-content between the nutrient treatments. The C:P gradient in the algae could be traced to the primary consumer (Figure 4; linear regression: y=0.445x-9.467, r^2=0.61, p<0.001) and resulted in significant differences in the C:P ratios of the copepod *A. tonsa* between treatments.

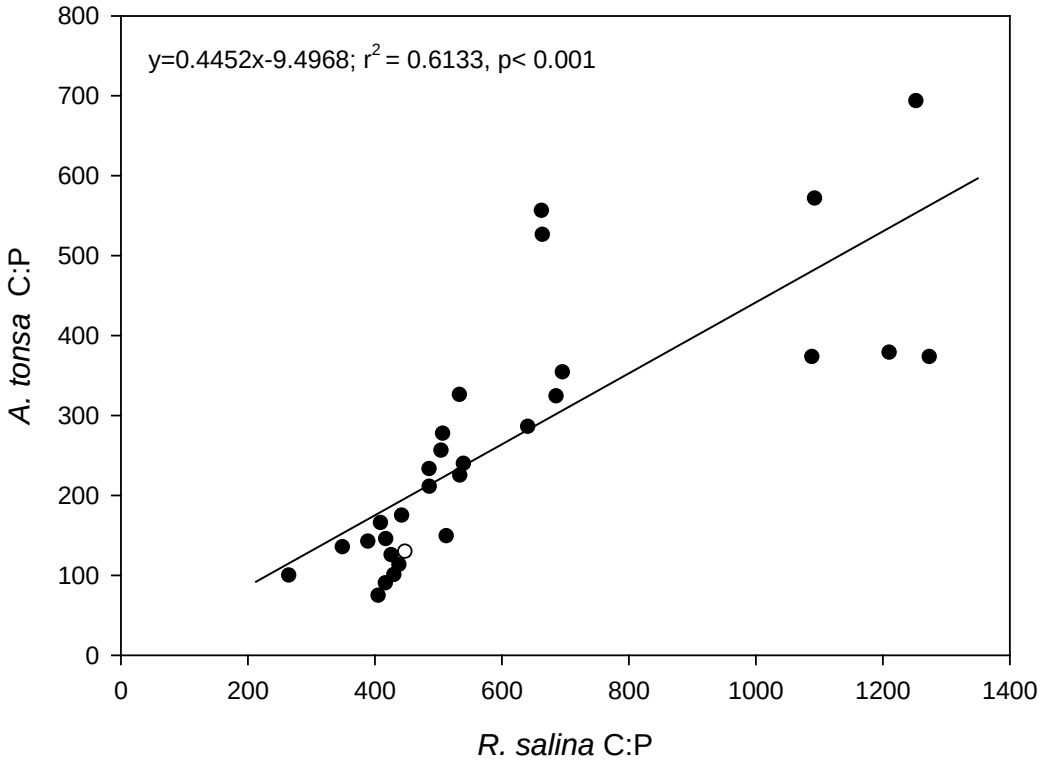

Figure 4: Experiment 2 - Correlation of the C:P stoichiometry of *R. salina* and *A. tonsa* along the algal P-limitation gradient.

Lobster larvae

The condition of the lobster larvae was significantly affected by the C:P stoichiometry of their food (Figure 5). The P-limitation gradient of the food was visible in the C:P ratio of the first stage lobster larvae (ANOVA: $F(5,36)= 4.56$, $p<0.01$; Figure 5A), but not in the A2 (ANOVA, $p=0.33$; Figure 5B) and A3 (ANOVA, $p= 0.61$; Figure 5C) larvae. No C:P values are available for the stage 2 lobster larvae of the continuous copepod food treatment.

Most larval stages showed a negative correlation between the RNA:DNA ratio of the larvae and the P-content of the diet was observed. The low P-content of their diet, i.e. food with high C:P ratios, resulted in larvae with lower condition, as expressed by the low RNA:DNA ratio. The larvae which were reared on the differently fed copepods from the beginning (stage 1) showed the most significant correlation (Figure 5A; linear regression, $y= -0.0007x+0.8309$, $r^2=0.6634$, $p<0.001$). The lobster reared through stage 2 on the different copepods showed the same pattern, with lowest condition in the animals fed the highest C:P diet (Figure 5b; linear regression, $y= -0.0002x+0.4957$, $r^2=0.2048$, $p=0.059$). The lobster larvae reared on *Artemia* sp. until reaching the second stage (A2) also reacted to the lack of P in their food (Figure 5B; linear regression $y= -0.0002x+0.4901$, $r^2=0.2031$, $p=0.027$).

The reaction of the stage 1 lobster larvae differed significantly from that of the stage 2 larvae. The slope for stage 1 (Figure 5A) was significantly different from the slope for stage 2 (linear regression analysis, $p<0.01$) and the slope for stage A2 (linear regression analysis, $p<0.001$). No difference was detected in the strength of reaction to the lack of P in the food between the second stage larvae reared on the copepods from the beginning on and those first fed on *Artemia* nauplii. The slopes were not statistically different from each other (linear regression analysis, $p>0.05$).

Only the lobster larvae reared to the third stage on the *Artemia* diet (A3 larvae) showed no reaction to the P-gradient of the copepod diet (Figure 5C; linear regression, $y= 0.00007x+0.6421$, $r^2=0.006$, $p=0.7$).

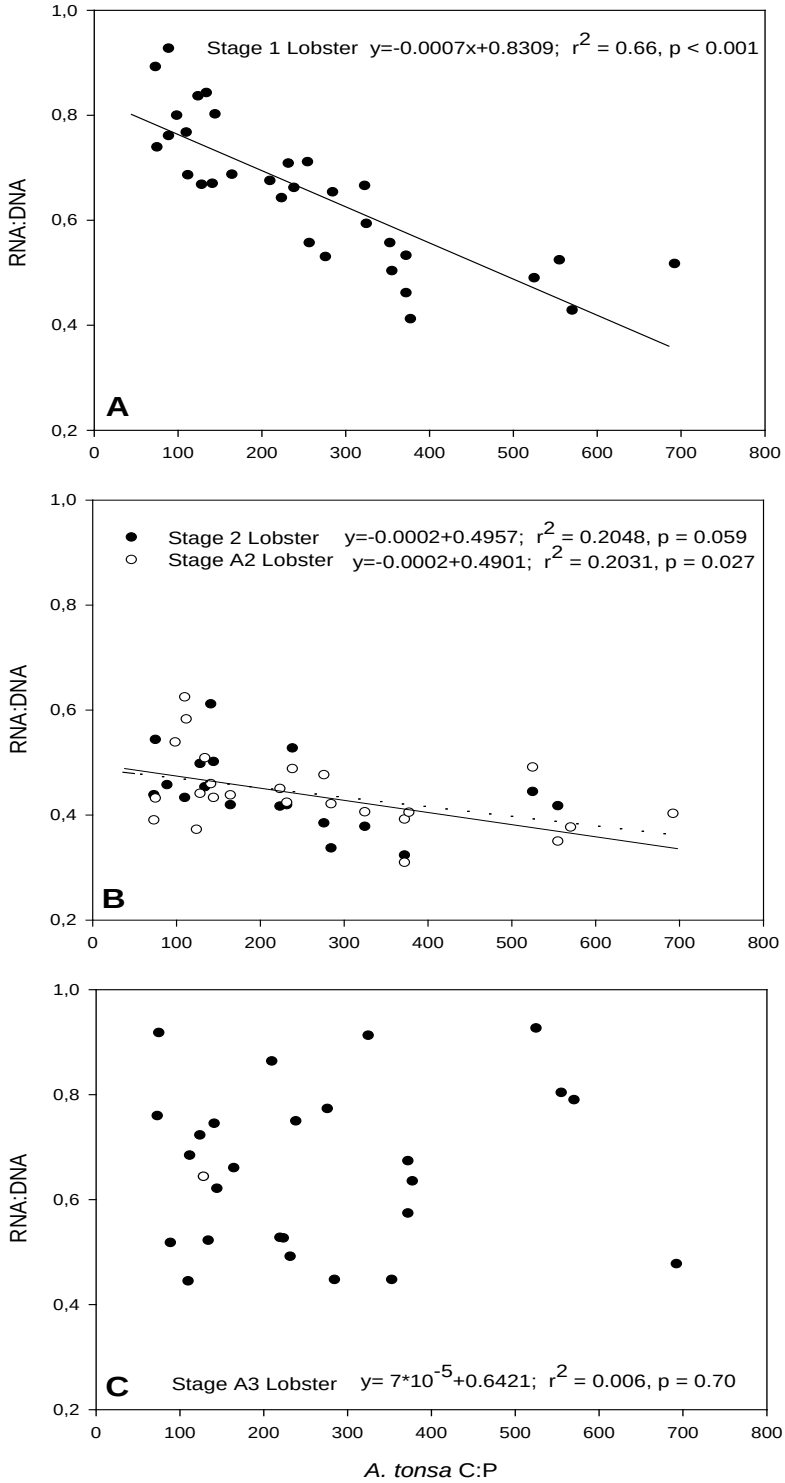

Figure 5: Experiment 2 - Lobster RNA:DNA and food C:P. Correlations of lobster RNA:DNA ratio and the C:P content of their copepod food source. A) Stage 1, B) Stage 2 (lobsters from both pre-feeding treatments are shown, solid line represents linear regression for stage 2 lobsters, dashed line for stage A2 lobsters) , C) Stage 3.

DISCUSSION

The aim of this study was to investigate the effect of various low quality foods during ontogeny of lobster larvae. The first three larval stages are the most vulnerable stages in the life history of the lobster due to a high predation risk on meroplanktonic larvae (Schmalenbach and Franke 2010). The larvae undergo rapid growth and three moults before reaching the fourth stage, which is the first juvenile stage. During this fourth stage the metamorphosis to a benthic life style occurs in the larvae of the European lobster. Rapid progress through the larval stages is in the best interest of the larvae, as juveniles are less at danger from predation and stage-specific mortality (Schmalenbach and Franke 2010). This rapid larval growth requires abundant food of high quality, which is often described through the stoichiometry, as the C:nutrient ratio of the food (Elser et al. 2000b). It has been observed that the stoichiometry of the consumer can be used to draw conclusions about the allocation of the nutrients contained in it to certain biological compounds such as proteins or phospholipids (Elser et al. 2000b; Sterner and Elser 2002). Rapidly growing animals, such as the lobster larvae in this experiment, have a high demand for both N and P (Elser et al. 2000b; Hessen et al. 2007; Vrede et al. 2002) and are therefore particularly vulnerable to nutrient limitations.

The fact that the lobster larvae showed some of the stoichiometric patterns displayed by their food, namely the elevated C:N and C:P ratios in all of the nutrient limited food chains, is further indication that homeostasis is relaxed rather than strict in both the copepods, who did not buffer the imbalance of the algal stoichiometry, and in the lobster larvae themselves. The limitation signals, including the more subtle ones of the nutrient-pulsed treatments, propagated all the way to the secondary consumer and the lobster larvae can be matched to their food source. The stoichiometric constraints presented to the first stage lobsters by their food had a detrimental effect on the condition of the larvae. The lowest condition of all treatments in the first experiment, expressed as the RNA:DNA ratio of the larvae, was observed in the N-limited food chain.

The nutrient-pulsed food chains with their lowered C:nutrient ratios resulted in higher condition of the larvae, strengthening the argument of direct nutrient limitation, rather than indirect biochemical limitation. Whether the direct effects of nutrient limitation (Boersma 2000; Hessen 1992; Plath and Boersma 2001; Sterner et al. 1993; Urabe et al. 1997) or the accompanying biochemical changes, such as

changing fatty acid composition (DeMott and Müller-Navarra 1997; Müller-Navarra 1995b), are responsible for the quality effects on consumers is a topic under debate. Nutrient-limited algae usually contain a different fatty acid profile, often lacking in essential fatty acids (Müller-Navarra 1995a; Reitan et al. 1994). A lack of particular biochemical compounds, such as certain essential fatty acids, can potentially lead to similar limitation effects as the lack of mineral nutrients as such. Since the addition of the missing nutrient to the larvae grown under nutrient-limitation changed only the stoichiometry of the pulsed algae, the ensuing effects observed in the food chain were solely due to the added nutrients themselves and not related to the biochemical composition of the algae, which remained virtually the same (Boersma 2000). Therefore, we conclude that lobster larvae were suffering from direct effects of mineral limitation rather than from biochemical limitations (Boersma 2000; Malzahn et al. 2007a; Malzahn et al. 2010). Furthermore, the animals fed the nutrient-replete f/2 food chain had a better condition (higher RNA:DNA ratio) than the animals on the fully limited diets, which indicates that the nutrient content of the f/2 diet played a more important role than the superior fatty acid composition of the limited diets.

Rapid growth in lobster larvae is strongly dependent on the larva's ability to synthesise proteins. Mehrtens (2008) observed an increment of approximately 160 µg of protein per individual larva over the course of four days, while Torres et al. (2002) reported even higher increases of around 300 µg per individual (which may have been due to the higher temperatures in the experimental set-up of Torres et al. (2002) resulting in faster growth rates of the larvae). As proteins are an N-rich compound (around 17% N according to Sterner and Elser 2002) the larvae would have a high demand for N from their diet (Hessen et al. 2007). The N-limited food chain obviously did not satisfy the requirements and hence the larval growth and condition were negatively affected. In addition to the protein synthesis in order to grow, lobster larvae also need to produce a new carapace after each ecdysis. The exoskeleton of lobsters consists of calcium carbonate in combination with chitin, a structural polysaccharide rich in N ($C_8H_{13}NO_5$). Therefore it can be assumed that lobster larvae have a high N demand and that their growth and development will be negatively affected under N-limited conditions.

Although the addition of N via the pulsed food improved the condition of the larvae, the RNA:DNA ratio of the N-limited food chains were still lower than the condition observed in the P-food chain. But P-limited conditions were far from optimal for the

larvae since the condition of the lobsters improved noticeably with decreases in the C:P ratio of the food, indicating a great demand for P in the larvae and a strong effect of P-concentration in the food. The reaction of the first stage larvae to the low P food was the same in both the first and second experiments, resulting in lower RNA:DNA ratios in larvae fed higher C:P food. The more subtle P-limitation gradient in the second experiment was still traceable to the secondary consumer and affected the condition of the larvae. The second stage lobster larvae also showed a reaction to the low food quality, although the reaction of the freshly-hatched stage 1 larvae was stronger. Interestingly the effect of the low quality food was virtually the same in both sets of second stage larvae, regardless of whether the larvae had been reared on copepods with differing food quality or on *Artemia* nauplii. In general, the RNA:DNA ratio of the second stage larvae was lower than that of the first stage larvae. This indicates that these larvae were still vulnerable to the effects of lower quality food after the first moulting.

The third stage larvae of the lobster represent the last pelagic stage in the lobster life cycle before the metamorphosis to the benthic fourth stage, the juvenile, takes place (Cobb and Whale 1994; Ennis 1995). Although the third larval stage is feeding in the water column and has not yet changed its diet to one more suited for benthic environments (Charmantier et al. 1991; Ennis 1995), the larvae in this experiment were not affected by the low P-content of the copepods. This is possibly due to the fact that these larvae have been reared on *Artemia* sp., which is considered a high quality food source, and have hence had the opportunity to store enough nutrients and energy to buffer the effect of the low P copepods. Since the metamorphosis to the post-larval stage involves another moulting and the associated growth spurt, it is unlikely that the larvae are no longer affected by the low P in their diet. It has however been reported that an ontogenetic change from rapid growth to one more focussed on maintenance and structure can shift the animals' requirements from high-P to high-N food (Elser et al. 1996; Elser et al. 2000b). The metamorphosis to the juvenile stage involves changes in the structure of the carapace and the appearance of larger pincers, potentially creating an increased requirement for N-rich food for the production of structural proteins (Hessen et al. 2007).

The importance of N and P for animal growth has been the subject of several studies lately (Acharya et al. 2004; Elser et al. 2003; Elser et al. 1996; Malzahn et al. 2007a; Vrede et al. 2004) , however, the investigations on the effects of limitation of one or

the other of these elements have focused mainly on herbivorous primary consumers. Because both N and P are crucial for the synthesis of protein and hence the growth rate of an organisms, animal growth and development is likely to suffer under nutrient-limited conditions. Although N provides the main building block for proteins (see Sterner and Elser 2002), growth is most tightly coupled to P. Elser et al. (1996) described the link between P and the growth of an organism in the Growth Rate Hypothesis. Numerous studies have since investigated this link and have discovered it to fit for most organisms (Carrillo et al. 2001; Elser et al. 2003; Elser et al. 2006; Hessen et al. 2007; Main et al. 1997). In accordance with this hypothesis, the severe reaction of the freshly hatched lobster larvae to the low P-food in our study may indeed have its source in the causal relationship between growth and P (Elser et al. 2003). The lack of P in the diet reduced the animals' ability to build RNA, which slowed growth and resulted in the low condition of the larvae.

The above results indicate a change in growth rate during ontogeny, and related changes in nutrient requirements for the different larval stages (Elser et al. 2006). The third stage larvae in this study showed no reaction to the lowered quality of their diet. This could be due to their pre-feeding history and a possible reserve acquired during the first two stages. Additionally, the ability of the larvae to cope with nutrient imbalances may have improved during ontogeny. During larval development alterations in anatomy (appearance of additional appendages etc.) and behaviour (eg. phototaxis, swimming) are coupled to physiological changes (Schmalenbach and Buchholz 2010). One of the most important of these involves the ability of older lobster larvae to osmoregulate (Charmantier et al. 2001). As this capability emerges during ontogeny it is likely that other regulatory mechanisms such as homeostasis also progress during development.

In the light of climate change alterations in the thermal conditions are considered to have inevitable consequences for plankton dynamics leading to regime shifts, changes in trophic relations and temporal asynchronies (McGowan et al. 2003; Smol et al. 2005). Since 1962 an increase in sea surface temperature of 1.5°C (Wiltshire et al. 2008) has been recorded at the Helgoland long-term sampling station in the North Sea (Helgoland Roads, 54°11'03''N, 7°54'00''E) where the increase in temperature, especially during the winter months (Franke et al. 1999), has been greater than average. With regard to the lobsters such a winter warming in the North Sea might alter larval recruitment since under laboratory conditions an increase of 3°C during

winter relative to normal surface temperatures throughout embryonic development resulted in larvae hatching about 50 days earlier, shifting forward from mid-June to mid-April (Schmalenbach and Franke 2010). Thus, the freshly hatched lobster larvae will be faced with temperatures which are still too low to be conducive to their rapid larval development, thereby lengthening the time spent as larvae and increasing the danger of mortality through stage-specific mortality and predation (Schmalenbach and Franke 2010). Furthermore, it has been shown that *H. gammarus* larvae cannot successfully undergo the transformation to the juvenile stages at temperatures below 14°C (Schmalenbach and Franke 2010), which are not usually attained in the North Sea around Helgoland until mid-June (Wiltshire et al. 2008; Wiltshire and Manly 2004). With regard to feeding conditions, an earlier appearance of the lobster larvae in the water column would on the one hand fall into a period of high zooplankton prey availability right after the phytoplankton spring bloom, whose timing has been proven to remain rather constant despite the given temperature increases at Helgoland Roads (Wiltshire et al. 2008). When it comes to food quality, however, the post-bloom period is characterized by a complete nutrient depletion thus leading to stoichiometric constrains of consumers. Results from the present experiment have shown that decreases in the nutrient content, and thereby food quality, negatively affect the lobsters' condition. Apart from warming-induced changes in the occurrence in lobster larvae, alterations in food quality resulting in nutritional imbalances of consumers might even be potentiated by ongoing re-oligotrophication in the North Sea (van der Zee and Chou 2005). Thus, the combined effects of global warming and the re-oligotrophication of the North Sea might further threaten the recovery of lobster stocks around Helgoland.

In conclusion, our experiments show that even subtle nutrient limitations can be traced through three trophic levels and affect the consumers. The effects observed seem to be related to direct nutrient limitation, suggesting that the stoichiometry rather than biochemical components such as fatty acids determine the quality of food for consumers. Fast growing life stages seem particularly vulnerable to the effect of nutrient limitation, although animals appear better equipped to deal with suboptimal conditions as their development progresses. The interplay of food quality with other factors such as temperature and phenological changes and their combined effects for plankton communities require further investigations.

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CHAPTER IV

Food quality affects the condition of larval European lobster at low quantities

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ABSTRACT

The issues of food quality and food quantity have long been regarded as crucial for trophic interactions. Most of the studies have focussed on the interaction between primary producers and herbivorous consumers. Since recent studies have shown that homeostasis in primary consumers is not strict and limitation effects have been traced from the bottom of the food chain to higher trophic levels, the question of how these higher consumers are affected by low quantity and low quality food requires clarification. In this study a tri-trophic food chain was designed, consisting of the algae *Rhodomonas salina*, the copepod *Acartia tonsa* and freshly hatched larvae of the European lobster *Homarus gammarus*. The lobster larvae were presented with food of two different qualities (C:P ratios) and four different quantities to investigate the combined effects of food quality and quantity. Our results show that the quality of food has an impact on the condition of lobster larvae even at very low food quantities. Food of higher quality (more balanced C:P content) resulted in higher condition of the lobster larvae regardless of the quantity of food.

Keywords: ecological stoichiometry; food quality; food quantity; *Homarus gammarus*

INTRODUCTION

The quality and quantity of food available to consumers influences development, growth and reproduction and thereby shapes trophic interactions and food web dynamics. In stoichiometric terms food quality is defined by the ratio of carbon to nutrient content of the food (Sterner and Elser 2002). In the aquatic environment, herbivorous consumers are often faced with low quality food as plants contain high amounts of C and relatively low amounts of P and N, making the latter nutrients potentially limiting (Sterner and Elser 2002; White 1993). Furthermore, the nutrient content of aquatic primary producers undergoes changes and reflects that of its surrounding environment (Sterner et al. 1998). Herbivores therefore regularly face imbalanced food in terms of energy and nutrients, which negatively affects their growth, development and reproduction (Sterner and Elser 2002). Under low quality food conditions (high C:nutrient content) animals are faced with an excess of carbon in their food. Among the mechanisms herbivores have developed to cope with this surplus of carbon are increased respiration (Trier and Mattson 2003), potentially through an increase in activity (Plath and Boersma 2001) or excretion (Darchambeau et al. 2003). Whichever mechanism is employed, it comes at a cost to the animal, usually resulting in reduced condition and fitness. Furthermore, under low nutrient conditions growth and reproduction can be nutrient limited, as both N and P are required for production (Færøvig and Hessen 2003; Sterner et al. 1993; Sterner and Hessen 1994). Therefore, low food quality at the plant-herbivore interface is likely to affect the quantity of the food of carnivores as the fitness of herbivores is negatively affected, and thus population densities of herbivore prey for higher trophic levels is lower.

It has been hypothesized that the effects of nutrient limitation are important at higher quantities of food only, because at low quantities the energy limitation due to a lack of energy (carbon) would mask the effects of mineral limitation (Sterner 1997; Sterner and Robinson 1994). While carbon is needed for biomass and energy, mineral nutrients are mostly required for structural components. If the amount of available food is too low to provide the organism with sufficient energy for growth, the metabolic energy requirements for maintenance must be met first, theoretically, as no new tissue is synthesised, only requiring carbon. Therefore, at low quantities, food quality effects should not be present (Sterner 1997). In contrast, at higher food

quantities food quality should play an important role, as growth is no longer C-limited and mineral nutrients will be needed for production (Sterner 1997).

The studies conducted on the interaction of food quality and food quantity have so far concentrated on the primary producer-herbivore interface (Gulati and DeMott 1997). One of the main reasons for this focus is the stoichiometry theory, which assumes that the consumers maintain homeostasis to a large extent (i.e. a constant ratio of carbon to nutrients) (Elser et al. 2000a; Sterner and Elser 2002). Negative effects of low quality are therefore thought to be buffered by the primary consumers and should not affect higher trophic levels. Recent studies have shown, however, that the homeostasis in herbivores is far from perfect and that the effect of low quality food can be traced to higher trophic levels via the herbivores (Boersma et al. 2008; Malzahn et al. 2010; Schoo et al. 2010). Hence, the question of how higher trophic levels react to food of simultaneously low quality and low quantity requires further study and is relevant to our understanding of trophic interactions and food web dynamics.

Here we investigate the effect of differing qualities (C:P ratios) and quantities of food on larvae of the European lobster, *Homarus gammarus*. The lobster is an important top predator around Helgoland in the North Sea, and plays a crucial ecological role in maintaining the species diversity of the local community (Schmalenbach and Franke 2010). The population numbers have been in steady decline since the 1940s, making this lobster community particularly vulnerable. By constructing a laboratory based three-trophic food chain in which the quality of the food was manipulated separately from the quantity we aim to disentangle the potentially confounding effects of these two factors.

MATERIALS AND METHODS

In order to investigate the effect of low and high quality food on a secondary consumer, a three-trophic food chain was established, consisting of algae (*Rhodomonas salina*), copepods (*Acartia tonsa*) and lobster larvae. The algae and the copepods were reared under nutrient-replete and phosphorus-limited conditions in order to manipulate their nutrient ratio and hence their quality. Copepods of the two resulting different qualities (C:P ratios) were then fed to the lobster larvae at different concentrations, simultaneously exposing the larvae to differences in the quality and the quantity of their food, (see also Malzahn et al. 2010) for more detail on analytical procedures).

Phytoplankton

A stock culture of *R. salina* was cultivated in enriched seawater (f/2) according to Guillard and Ryther (1962). For the experiment, all algal treatments were grown under a 16:8 h light: dark (L:D) regime at a constant temperature of 18°C. The seawater used for the growth media in the experiments was filtered using a sterile 0.2 µm filter and stored cool and dark until use. The seawater for the nutrient-replete f/2 treatment was enriched with the full set of nutrients as described by Guillard and Ryther (1962). The water for the growth medium of the P-limited treatment was also enriched according to the f/2 recipe, but without the addition of the limiting nutrient P. The algae thus had access only to the P contained in the natural seawater at the time of filtration and previous tests of the carrying capacity showed that the algae became severely limited with respect to the missing nutrient after a growth period of 4 days.

Algal densities in the stock solution were measured with a CASY cell counter (Schärfe System CASY Cell Counter and Analyser System). To ensure a constant supply and food quality, new cultures of *R. salina* were inoculated daily for both treatments with a starting concentration of 0.2×10^6 cells L^{-1} for the f/2 treatment and 0.3×10^6 cells L^{-1} for the –P treatment. Algae were harvested after the predetermined growth period of 4 days at densities of 1.5×10^6 cells L^{-1} for the f/2 treatment and 1.0×10^6 cells L^{-1} for the –P treatment. For the feeding experiment the algal food concentrations were normalized to the same levels for both qualities prior to being fed to the copepods.

Zooplankton

Copepod eggs were obtained from a culture of the calanoid copepod *A. tonsa*. The copepods for egg production were kept in filtered natural seawater (salinity ~32) in a 200 L tank at 18°C under a 18:6 L:D regime. The copepods were fed a mixture of the algae *R. salina* and the heterotrophic flagellate *Oxyrrhis* sp. Eggs were siphoned off the bottom of the tank daily and stored in seawater under anaerobic conditions in the dark at 4°C until use. No eggs older than 3 months were used in the experiments.

The copepod eggs were incubated at a density of about 3000 individuals L^{-1} in artificial seawater (salt: hw Marinemix, www.hw-wiegandt.de) adjusted to a salinity of 32. Hatching occurred within 24 hours. Hatching rate was approximately 20%. Copepods were first fed 48 hours after the addition of the eggs to seawater, when the majority had reached the second naupliar stage. The copepods were transferred to fresh artificial seawater daily prior to feeding in order to avoid remineralisation of the nutrient-limited algae by waste products of the copepods. The copepods were fed 50.000 cells of *R. salina* per individual and day (corresponding to ~ 3 mg C L^{-1} d^{-1}), irrespective of the nutrient treatment. This concentration is considered *ad libitum* for juvenile copepods. Copepods were fed the different algae treatments for a total duration of 8 days. To ensure a constant food quality for the secondary consumers for every day of the feeding experiment new copepod cultures were started for each of the nutrient treatments. Since the copepods fed on P limited algae displayed slower growth and development, the copepod cultures fed f/2 were started one day later. This delay was necessary to ensure that all the copepods fed to the secondary consumers were of the same age and size class. Copepods were passed over a sieve and transferred to fresh artificial seawater prior to being fed to the lobster larvae to ensure the lobsters were fed only the copepods.

Lobster

Freshly hatched stage 1 larvae of the European lobster *Homarus gammarus* were collected from the lobster rearing facility at the Biological Station on Helgoland, Alfred-Wegener-Institute for Polar- and Marine Research (AWI). We used young larvae, as they have been shown to be particularly sensitive to differences in feeding environment (Mehrtens 2008). Larvae from different females were collected to minimize batch difference and possible maternal effects. The larvae were carefully sorted and transferred to individual 40 mL cylindrical glass containers containing

sterile filtered natural seawater. The water in the beakers was changed daily prior to feeding. The lobsters were kept at a 16:8 light: dark regime under indirect light in a temperature-controlled room at 15°C, resulting in a time frame of approximately five days until first moulting (Branford 1978; Havinga 1929).

Twenty larvae were randomly assigned one of the quality and quantity treatments. Copepods were fed to the lobster larvae along a density gradient. There were four quantity treatments for each of the two qualities. The highest food concentration consisted of 60 copepodites per lobster larva and day. This amount was ad libitum in preliminary tests (Schoo, unpublished data).To test the effect of different food quantity, the food concentration was reduced stepwise to result in 4 different food quantity treatments (60, 30, 15 and 7 copepodites per lobster larva and day) per food quality (-P and f/2 copepods, respectively). An additional 20 lobster larvae were assigned to a starvation group. First feeding took place within 12 hours of hatching. The larvae were provided with food once a day, which ensured that the animals had access to food both during the day and during the night, when they are normally found foraging for food in the field (Mehrtens 2008).

On the fifth day after hatching the lobster larvae were sampled, rinsed in distilled water and frozen at -80°C until analysis.

Analytical procedures

The stoichiometry of each of the three levels of the food chain was analyzed. For the analysis of the primary producer *R. salina* approximately 4×10^6 cells were filtered onto pre-combusted and washed Whatman GF/F filters. For each of the analyses of the copepods 75 individuals were counted into tin capsules. As parameters to assess the reaction of the *H. gammarus* larvae to the different prey we used the RNA: DNA and C:N:P ratios of the animals. For the analysis of carbon, nitrogen and phosphorus of *H. gammarus*, pulverised tissue homogenate of freeze-dried whole larvae was used. For the analysis of the RNA: DNA ratio of the larvae, whole freeze dried animals were used.

The carbon and nitrogen content of the samples was measured with an Elementar vario MICRO cube CHN analyser (Elementar Analysensysteme, www.elementar.de). Phosphorus was analysed as orthophosphate, after the method described by Grasshoff et al. (1999), following oxidative hydrolysis. The samples were treated with an oxidation agent ($K_2S_2O_8$, H_3BO_3, NaOH in distilled water) under high

pressure and at high temperature (120°C) in an autoclave to convert the phosphorus compounds to the ortho-phosphate form. Molybdate-antimony-solution (containing ammonium molybdate $(NH_4)_6Mo_7O_{24}$ x $4H_2O$, antimony potassium tartrate $K(SbO)C_4H_4O_6$ x $0{,}5H_2O$) and ascorbic acid was added to the solute before the P-content was measured photometrically.

The technique to measure RNA and DNA content of animal tissue is well established and commonly used especially in fisheries science to determine the overall physiological condition and growth (e.g. Buckley 1979; Clemmesen 1993; Malzahn et al. 2003). A high RNA to DNA ratio is indicative of a good overall condition, as the DNA content of the cell should be constant relative to RNA, which should increase under active growth conditions. Lately the technique has also been successfully applied to determine the condition of crustaceans (e.g. Ikeda et al. 2007; Rosa and Nunes 2005). The method used for the nucleic acid analysis was modified from Clemmesen et al. (2003).

Freeze-dried larvae were pulverised and rehydrated in 400 µl Tris-SDS buffer (Tris 0.05 mol L^{-1}, NaCl 0.01 mol L^{-1}, EDTA 0.01 mol L^{-1}, sodium dodecyl sulphate (SDS) 0.01%) for 30 minutes. Glass beads (2 mm and 0.17-0.34 mm diameter) were added and the tubes containing the samples shaken in a Retsch MM 301 cell-mill for 15min. The homogenate was centrifuged (Sartorius Sigma 3-18 K; 8 min, 3800 g, 4°C) and 130 µl of the supernatant used for analysis. The amount of nucleic acids was determined fluorometrically using the fluorophor ethidiumbromide (EB) in a microtiter fluorescence reader (Fluoroskan Ascent).

The total amount of nucleic acids in the sample was measured first, before the addition of RNAse to digest the RNA. The remaining DNA was measured after the enzyme treatment and the RNA fluorescence calculated by subtracting the DNA fluorescence from the total nucleic acid fluorescence. The DNA concentrations were calculated following Le Pecq and Paoletti (1966). RNA calibrations were set up daily prior to the measurements.

Statistical analysis

The C:P ratio of the primary producer *R. salina* and the copepod *A. tonsa* were analysed with a one-way ANOVA (StatSoft Statistica 7), using the C:P ratio as the dependent variable and the nutrient treatment (f/2 and –P, respectively) as the independent variable.

A linear regression analysis was performed to test the effect of quality (C:P ratio of the food) and quantity (amount of carbon in the diet) on the response of the lobster larvae, expressed by the RNA:DNA ratio of the larvae. After a test for homogeneity of slopes, an ANCOVA was carried out to test for the effects of both quality and quantity of the food on the condition of the lobster, with 'condition of the lobster larvae', expressed by the RNA:DNA ratio, as the dependent, 'Quality' as the categorical and 'Quantity' as the co-variable.

RESULTS

The differently manipulated growth media resulted in two algae cultures of very different quality. P-limited algal cultures yielded much lower densities than the nutrient-replete algae. The C:P ratios of the algae grown in the nutrient-replete f/2 growth medium were significantly different from those of the –P algae (Figure 1; f/2 ~ 230, -P ~1180; ANOVA: $p<0.001$).

The same pattern was visible in the primary consumer, where the copepods fed the f/2 diet had a C:P ratio of ~100, while the C:P ratio of –P copepods was elevated at ~230 (Figure 1). These differences were statistically significant at $p<0.005$. Thus the low quality (high C:P) of the algae was still visible in the copepods, which were unable to maintain complete homeostasis.

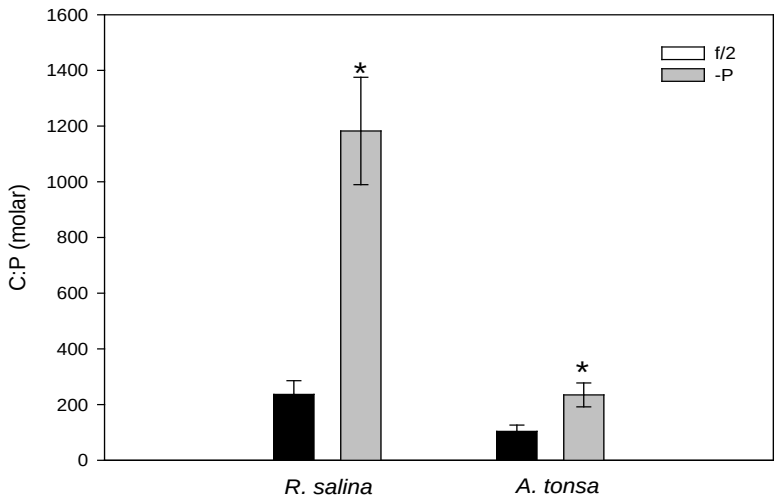

Figure 1: Food quality (C:P ratio) of the primary producer *R. salina* and the primary consumer *A tonsa*. Stars mark significant differences between treatments.

An effect on the lobster larvae of both quality and quantity of food was visible in all treatments. The higher food quality resulted in a higher condition of the lobster larvae under all of the experimental food quantity treatments. The RNA:DNA values of the lobster larvae were positively related to food quantity (expressed as C in the food, Figure 2) for both high (linear regression: $y = 0.0106x + 0.966$, $r^2 = 0.58$; $p<0.001$) and low (linear regression: $y = 0.0079x + 0.8447$, $r^2 = 0.34$; $p< 0.05$) food qualities. Copepods reared on P-limited algae contained ~0.36 µg C on average, compared with ~0.5 µg C in copepods reared on f/2 algae. Food quantity is expressed

as the amount of carbon available to the lobster larvae in the different treatments. The lower quality of food also mattered to the condition of the larvae at high quantities. While the condition of the lobster larvae increased with increasing food quantity, the condition of the larvae in the low-quality P-limited food chain was lower throughout. The lobster larvae from the starvation treatment differed from the fed lobsters and was higher than the condition of the larvae fed the lowest amounts of P-limited food. Significant differences in condition (RNA:DNA) were observed between lobsters from the starvation treatment and the two lowest P-limited food qualities, as well as between the starved larvae and the two highest f/2 food quantities

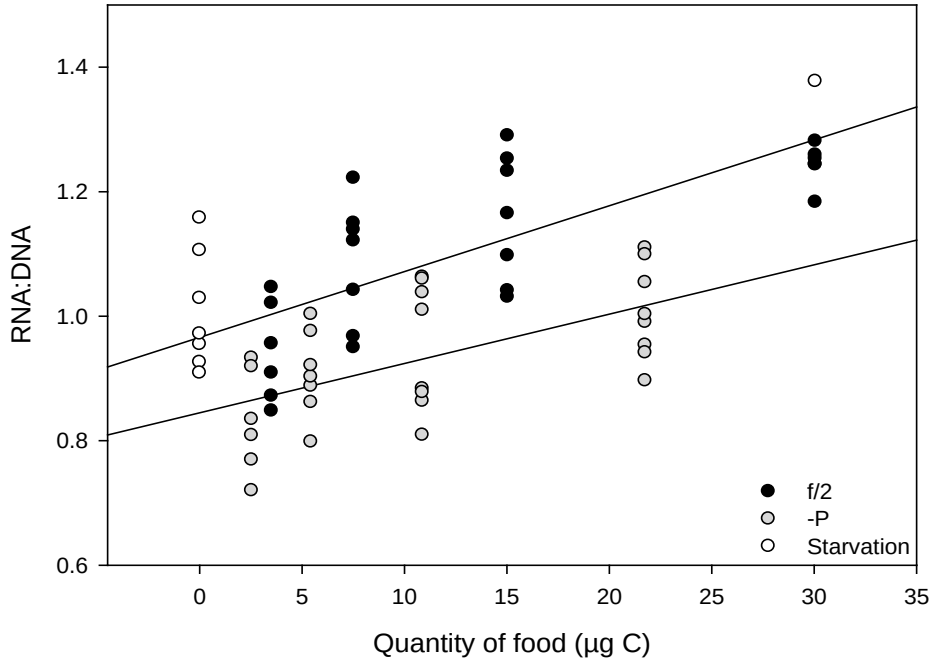

Figure 2: RNA:DNA ratio of the lobster larvae and food quantity expressed as the amount of carbon in the diet.

The reactions of the lobster larvae to the different qualities of food (as shown by the regression slopes) were not significantly non-parallel, meaning that the food quality affected the larvae irrespective of the food quantities. This is true even for very low food quantity. As the quality and the quantity of the food were independent and the assumption of homogeneity of the regression slopes was met, an Analysis of Covariance (ANCOVA) was run. The ANCOVA showed highly significant effects for food quantity ($p<0.001$) and quality ($p<0.001$), indicating that the condition of the lobster larvae is affected by both quality and quantity.

DISCUSSION

This is the first study into the combined effect of food quality and food quantity on a secondary consumer that we are aware of, building on the previous work of Boersma and Kreutzer (Boersma and Kreutzer 2002). The effect of low quality food, in terms of C:nutrient ratios, on higher trophic levels has recently received some attention and we now know that the nutrient-limitation in primary consumers can be traced to secondary consumers (Malzahn et al. 2007a; Malzahn et al. 2010; Schoo et al. 2010). The quality of the food played a significant role at all levels of food quantity. Considering the effect of food quantity on the larvae, the lowest larvae condition was observed not in the starvation group, which received no food at any point, but in the animals having received the lowest food quantities in the experiment. This difference between the feeding and the non-feeding animals can be explained by the metabolic cost of feeding. The metabolic rate of fasting animals is much lower compared to that of feeding animals, as is the rate of respiration under fasting conditions (Jensen and Hessen 2007; Lampert 1977; Sigsgaard et al. 2003). As soon as the animal feeds, however, the respiration rate increases. This increase above the basic metabolic rate associated with feeding is known as the specific dynamic action (SDA,Jensen and Hessen 2007; Sigsgaard et al. 2003). In the present experiment it appears that the metabolic costs associated with feeding at low quantities of food are actually higher than the energy gained by feeding. The lower food quantities in our experiment did not provide the lobster larvae with enough energy to compensate for the energy expenditure of feeding and accumulation and hence resulted in lower condition of the larvae.

Although the copepodites had reached the same development stage in both the P-limited and the nutrient replete f/2 treatments, a difference in the carbon content of the copepods from the different food treatments was observed, potentially further exacerbating the nutrient imbalance. Even though there was not enough energy to be gained for the lobster larvae at the lowest of our experimental food concentrations, the quality of the food ingested was not without effect. The P-deficient diet resulted in lower condition of the animals even at the lowest food concentrations. In this respect our results are in line with observations by Boersma and Kreutzer (2002) and Kilham et al. (Kilham et al. 1997) who discovered negative effects of low quality food for *Daphnia* even at low C quantities. In their study the authors found quality-dependent shifts in the threshold food concentration required for growth in *Daphnia,*

with higher amounts of low quality food required for growth. This is in contrast to the predictions by Sterner and Robinson (Sterner and Robinson 1994) and experiment-based findings by Rothhaupt (1995), where food quality was found to be of negligible importance at low quantities of food. The mineral limitation in our study had an effect on the lobsters' condition at all food quantities. The lobster larvae in this experiment were freshly hatched and had high P requirements to support their fast growth (Sterner and Elser 2002). The high growth rate requires large amounts of P-rich RNA for protein translation and synthesis (Elser et al. 1996), making these young and rapidly growing animals particularly vulnerable to nutrient limitations. The requirements of juvenile stages with their high growth rate probably differ from those of adult animals, which are likely to invest their resources into maintenance and reproduction (Sterner and Robinson 1994; Villar-Argaiz et al. 2002). Therefore the demands and responses of the animals and the repercussions to the population will probably vary depending on the ontogenetic development of the animal.

While our food chain represents a laboratory based experiment under artificial and highly controlled conditions, it is certainly relevant to the natural conditions prevailing in the aquatic environment. In previous experiments we have observed lower densities of primary producers and herbivorous consumers grown under low-quality food conditions, as well as a slower development in the copepods (Schoo, unpublished data, see alsoMalzahn et al. 2010). Under experimental conditions we were able to adjust for these differences. In the field, further factors, such as a heterogeneous composition of the prey in terms of age and developmental stage, would come into play, further complicating the issue. Additionally, rising temperatures in the North Sea are likely to affect the larval development, leading to an earlier release in lobster larvae (Schmalenbach and Franke 2010) and thereby making the larvae even more vulnerable.

A co-limitation in food quality and food quantity is likely to occur at times, even for higher trophic levels, since homeostasis in herbivorous consumers is not as strict as previously assumed. Since the fitness, survival and reproductive output, and hence to a certain extent the quantity of these consumers is determined by the quality of the food available to them, food quality and food quantity are interrelated. In the field, however, a wide range in diets, including many different prey items or a selective feeding strategy may lessen the impact of quality and quantity limitations by

providing a more balanced diet. There is, however, a great need for further studies on the combined effects of food quality and quantity on higher trophic levels.

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GENERAL DISCUSSION

Herbivorous consumers play a pivotal role in aquatic food webs. They are the link between primary producers and higher trophic levels and as such their nutrient content and quality as food determines the transfer of nutrients and energy through the food web. Traditionally, herbivores have been considered homeostatic, and hence it was assumed that they presented a food source of constant quality to their consumers who should therefore not be affected by nutrient imbalances at the base of the food web. The results presented in this thesis show that nutrient limitation at the base of the food web affects primary consumers, which are unable to completely buffer the nutritional imbalances provided to them in their food. The herbivores' reaction to the lack of specific nutrients manifested itself in lower growth rates and survival as well as in a change of their nutrient composition. While the herbivores were more constrained with respect to their nutrient content than their prey, the effect of the high C:nutrient levels of their food was visible. The imbalance in primary producers can therefore be transferred to the higher consumers feeding on the herbivores. In the experiments conducted during this thesis the nutrient-limitations of the primary producers were traced to secondary consumers, which were affected by this nutritional deficit. The reactions of the consumers varied, apparently depending on the life stage and species-specific nutrient-requirements. Because of the prevalent assumption that herbivores maintain strict homeostasis there is a distinct lack of ecological research and data about the effect of nutritional imbalances on higher consumers. From an ecological point of view this may be understandable, but not if one considers the plethora of papers available in the aquaculture literature, showing that actually secondary consumers are quite vulnerable to changes in the quality of their food. Despite this, very little is known about the effects, the symptoms and consequences of nutrient imbalances on higher trophic levels in natural or semi-natural systems. Although fish for example is very often considered food limited in natural systems (Cushing 1974; Cushing 1990), this limitation almost exclusively refers to quantity and not to the quality of the food. Hence, the main aim of this thesis was to contribute new insights to the effects of nutrient limitations in the aquatic food web.

Food quality in higher trophic levels

In order to compare the reactions of different secondary consumers exposed to nutrient imbalances at the base of the food web, I have collected data from some of the few studies that do exist on the effects of nutrient imbalances on higher trophic levels in the aquatic environment. The data used in this overview originate from the experiments carried out during my thesis and similar studies conducted by Malzahn (Malzahn et al. 2007a; Malzahn et al. 2010) and Boersma (Boersma et al. 2009).

Because the measures of condition used in this approach, the RNA:DNA ratio in the consumers tissue, varied greatly (see Manuscripts II and III, as well as Boersma et al. 2009; Malzahn et al. 2007a; Malzahn et al. 2010) it was not possible to compare these values directly between studies. To enable a comparison between the reactions of the different organisms to nutrient imbalances I therefore used a measure of sensitivity based on the changes observed in the consumers' condition relative to the condition on the food which is considered optimal, much in the same way this was done by for example Hairston et al. (1999).

The relative vulnerability of an organism to nutrient limitation is determined by its requirements for that particular element. Organisms that need to maintain a high nutrient content, i.e. low carbon:nutrient ratios, are more susceptible to poor food quality when the carbon content of their food increases (Elser et al. 2000b; Sterner and Schulz 1998). The emphasis on P in this study is due to the fact that P is often the limiting nutrient in coastal seas and lakes (Elser et al. 2007; van der Zee and Chou 2005). Furthermore the variation in the C:P ratio of P-limited autotrophs is much larger than the ratio observed in the same autotrophs under N-limited conditions. In the case of phosphorus a low C:P ratio of the animals' tissue points to a high demand for P in order to maintain that balance. Following this argument we would expect animals with high C:P ratios being not susceptible to suffer from P-limitations.

In this thesis, I found that the lobster larvae were the most sensitive to changes in food quality despite having a relatively high C:P ratio. This ratio is probably partly due to the high amount of C contained in the exoskeleton of these crustaceans. There are no measurements I am aware of for the relative contribution of the carbon in the carapace to the whole body carbon in lobster larvae. Data from a recent study by Arnold et al. (2009) show that the carapace of stage 1 larvae weighs around 0.3 mg, which would mean a contribution of about 20% to the total dry weight of the lobster

larvae. Assuming that the carapace consists mainly of $CaCO_3$ this is a rather large contribution to the total C of the animal. It seems likely that the body tissue as such, when analysed without the carapace, would have a much lower C:P content, suggesting a high P requirement for the lobster larvae. Potentially, the main reason for the high sensitivity of the lobster larvae to changes in their food quality lies in their high growth rate, leading to a high demand for phosphorus for the production of RNA (Elser et al. 1996). Larval stages undergoing ontogeny display rapid growth and development (Sterner and Elser 2002). It has been observed that rapidly growing animals have low biomass C:P ratios, which is attributed to the increased allocation to P-rich RNA supporting the protein synthesis required for fast growth (Elser et al. 2003; Elser et al. 2000b). Similar observations of high P-requirements in larval stages are available for copepods (Villar-Argaiz et al. 2002). The authors noted that the P-content of copepods decreased during ontogeny, with the highest C:P values recorded in the adult. This indicates that there are variations in the susceptibility to nutrient limitation within species as well as between species, due to changes in the nutrient requirements throughout the life cycle. As can be seen from Chapter 5 (Manuscript 4), even within the three larval stages of lobster that were studied the youngest larvae were most vulnerable to P–limitations. In general, fast growing larvae and juveniles are therefore much more vulnerable to nutrient limitation, and a lack of P at this particular life stage can have consequences for recruitment and population dynamics.

While the sensitivity of the rainbow trout *Oncorhynchus mykiss* to low quality –P food was very low, larval herring *Clupea harengus* were more strongly affected. This seems peculiar, as the relative change on food quality experienced by both fish species was very similar. The fish were, however, fed a different diet, according to their habitats. The freshwater trout were fed a diet of *Daphnia*. The *Daphnia* showed strong differences in their C:P ratio between P+ and P- treatments and the C:P ratio in the nutrient limited *Daphnia* was around 230. The Baltic herring were fed copepods (*Acartia tonsa*), whose C:P ratio under P-limited conditions was 350 (Malzahn et al. 2007a). Our explanation for the different reactions is that even the P-limited *Daphnia* provide larval fish with enough P to meet its requirements. The P-limited copepods, however, did not contain enough P for the larval herring, negatively affecting the condition of the fish. Copepods and cladocerans are the most important herbivorous primary consumers in marine and freshwater habitats,

respectively, and as such the most prominent food for planktivorous fish. The fact that the C:P imbalance is higher in the marine copepods than in the freshwater herbivore *Daphnia* indicates that marine fish might be more likely to be P-limited in their growth (see alsoBoersma et al. 2008). This is in contrast to the general view that lakes are more likely to be P-limited while marine systems are mostly thought to be more susceptible to N-limitation, and indicates the need for detailed descriptions of such limitations. It will never be that whole systems are limited by single nutrients, but rather single species or functional groups only.

The two gelatinous zooplankters, the cnidarian *Gonionemus vertens* and the ctenophore *Pleurobrachia pileus*, were the least sensitive to changes in their food quality of all the animals compared here. Jellyfish do not contain bones, scales or other P-rich structures and therefore do not require high amounts of P for their construction. Instead the organs of the jellyfish are supported by the mesoglea, which is an almost cell-free substance consisting of glycoproteins and carbohydrates. The biomass of jellyfish is consequently rich in carbon. This is in further supported by the high C:P values observed in these organisms (see Manuscript II and Malzahn et al. 2010). It seems that jellyfish are therefore more likely to be carbon limited than nutrient limited. Due to their low demand for P it is possible that the jellyfish are at an advantage during times of P-limitation in the aquatic food web. Following the spring bloom in temperate waters the quality of algae and their herbivorous consumers is depleted in nutrients, resulting in food of high C:nutrient content. This is usually considered disadvantageous for higher trophic levels and most consumers would suffer from this imbalance as the quality of their food decreases (Boersma et al. 2008; Boersma et al. 2009; Malzahn et al. 2007a). The jellyfish, however, may profit from food which suits their requirements, providing them with a diet rich in carbon. Furthermore, fish larvae, which feed on similar prey items, are vulnerable to P-limitation in their food (Malzahn et al. 2007a). Fish are therefore at a disadvantage relative to jellyfish. This situation is further exacerbated by predation on fish eggs and larvae by jellyfish (Purcell and Arai 2001) and the general reduction of fish stocks by overfishing, which effectively reduce the competitive pressure of fish on jellyfish (Lynam et al. 2006).

Food quality versus quantity

Thus far, we have seen that food quality does impact the condition and probably also growth and reproduction at higher trophic levels, and that nutrient limitation at the nutrient-primary producer interface gets transported higher up into the food web. At the same time, it is known that there can be a numerical response of the herbivores to low quality food, *i.e.* the population densities of herbivores are lower when feeding food with high to C:P ratios (Seidendorf et al. 2010). So, nutrient limitation of primary consumers leads to both a decrease of the quantity as well as the quantity of the food of secondary consumers. It is difficult to estimate the relative impact of these two mechanisms on these organisms, as there is virtually no information on this at all. Based on Chapter 5 describing the effects of food quality and quantity effects on larval lobster we could make some prediction. From Figure 2 in that Chapter (Manuscript 4) one can estimate that the difference in condition (RNA/DNA ratio) between the P-limited food and the non-limited food is around 0.15. Given the slope of the regression lines in Figure 2 (around 0.01 RNA/DNA per µg C) the food quality effect can be translated to a quantity effect of around 15 µg C. So, if we for the moment assume that the non-limited food chain would have sustained 30 µg C of copepod biomass, the question now is whether the P-limited food chain would be able to produce more or less than 15 µg C. Malzahn et al (2010) showed for the copepod *A. tonsa* that the developmental rates of copepods grown on P-limited algae are indeed lower than those on non-limited algae, at the same time, however, the densities of the cohorts grown on the different algal food were not significantly different from each other. This implies that we can expect that the effect of quality is larger than the effect of quantity for higher trophic levels such as the lobster larvae in Chapters 4 and 5.

Future implications

In light of possible future scenarios it is likely that the issue of food nutrient imbalance in the marine environment will worsen. Phosphorus concentrations in the North Sea have declined drastically in the last decades (e.g. Wiltshire et al. 2008) and it is predicted that this trend of re-oligotrophication will continue. This will lead to higher C:P ratios in the phytoplankton, will affect the quality and quantity of herbivorous consumers and thus have consequences for the higher trophic levels. Furthermore the predicted rise in CO_2 concentrations in the seas as a result of

increasing anthropogenic CO_2 emission to the atmosphere will provide primary producers with additional carbon, increasing the carbon:nutrient imbalance and lowering their quality as a food source (e.g.Loladze 2002). Thus nutrient imbalances in the aquatic system will become more frequent and more severe. So, how is the system going to react?

First of all larval stages will be most directly affected by this nutritional imbalance. Due to their high demand for P to support the rapid growth during ontogeny larval stages are very vulnerable. Furthermore, it has been speculated that jellyfish will become more important in the North Sea in the future (Attrill et al. 2007; Boersma et al. 2007), and in the marine environment in general (Gibbons and Richardson 2009; Mills 2001; Richardson et al. 2009). It is possible that apart from the factors overfishing and competitive advantage of jellyfish over fish, jellyfish could become the top predators in systems which are to date dominated by fish due to their relative imperviousness to P-limitation (e.g. Lynam et al. 2006).

Outlook

It seems clear that we know very little about how the nutrient imbalances at the base of the food web affect the higher trophic levels. While it is becoming more apparent that the nutrient limitation effects in primary producers are propagated through the food web the reactions of the top consumers vary. The results presented here indicate that there is no simple pattern and that the observed effects need to be put in a wider context of the organisms' life history.

Further studies on a wide range of higher trophic levels are crucial to our understanding of aquatic ecosystem functioning and disentangling the food quality versus food quantity effects in particular is an issue that needs to be thoroughly addressed when conducting future research in the field of ecological stoichiometry.

SUMMARY

This thesis investigates the effects of nutrient limitation in primary producers on higher trophic levels. To this aim the nutrient conditions and their effect on primary consumers in the North Sea were examined. Furthermore experiments were carried out to test the effect of nutrient limitations on tri-trophic food chains in controlled laboratory conditions.

Despite the prevailing assumption that the herbivorous primary producers maintain strict homeostasis, thus buffering any nutrient imbalances at the base of the food web and providing their consumers with food of a high and constant quality, their nutrient stoichiometry and fitness was affected by the nutrient content of their food. The nutrient deficiency of the primary producers can thus be passed on through the food web and affect higher trophic levels.

In a set of laboratory based experiments the nutrient contents of primary producers was manipulated to change their C:nutrient ratio and thereby their food quality. Primary consumers were reared on these producers and in turn were the food source for a higher consumer.

The ctenophore *Pleurobrachia pileus* was exposed to copepods with manipulated C:P ratios along a gradient. The reaction of the ctenophore to food with a high C:P ratio, generally considered to be food of lower quality, was unexpected. *P. pileus* was negatively affected by nutrient replete food with a low C:P ratio and displayed higher levels of fitness when feeding on food with a high carbon:P ratio. This ctenophore is therefore more likely to be energy limited than nutrient limited. A possible explanation for this is the high body C:P ratio observed in *P.pileus*. The results are discussed in light of possible future scenarios in the aquatic environment.

Freshly hatched larvae are very vulnerable and the mortality during this stage is particularly high. Therefore the effect of food with different C:N:P ratios was tested on larvae of the European lobster *Homarus gammarus.* Larvae were exposed to food with varying nutrient content either throughout their development or for the duration of one stage. The results confirm that the youngest larval stages are the most susceptible to a lack of nutrients in their diet, which had strong negative effects on their condition. The effect of P-limitation in particular was observed to change during ontogeny, with older larval stages being less affected.

As nutrient-limitations reduce the fitness and reproductive output of primary consumers, this translates into a quantity effect for higher trophic levels by reducing

the amount of food available to them. The combined effects of nutrient quality and nutrient quantity on a top predator were investigated in an experimental set-up. The consumer was negatively affected by the lack of nutrients in its diet even at very low nutrient quantities, indicating that quality of the food is more important than the quantity. Disentangling these two effects, the quality and quantity of food, remains a great challenge for future ecological studies in the marine environment.

These findings have potentially far-reaching consequences for the trophic interactions and population dynamics of marine organisms.

ZUSAMMENFASSUNG

Diese Dissertation befasst sich mit Nährstofflimitationen bei Primärproduzenten und ihrer Auswirkungen auf höhere trophische Ebenen. Dazu wurden die Nährstoffbedingungen der Nordsee und ihre Auswirkungen auf Primärkonsumenten untersucht. Zusätzlich wurden Laborexperimente durchgeführt um unter kontrollierten Bedingungen die Auswirkungen von Nährstofflimitationen auf drei-trophische Nahrungsketten zu erforschen.

Es ist eine weit verbreitete Annahme, dass herbivore Primärkonsumenten eine strenge Homöostase aufrechterhalten, wodurch sie sämtliche Unausgewogenheiten in der Nährstoffzusammensetzung ihres Futters puffern können. Somit würden sie eine gleichbleibend hohe Qualität als Nahrung für die nächst-höhere trophische Ebene bieten. Dennoch konnte in der vorliegenden Arbeit gezeigt werden, dass sowohl Stöchiometrie als auch Kondition dieser Primärkonsumenten beeinträchtigt wurden. Der Nährstoffmangel in Primärproduzenten kann also durch das Nahrungsnetz übermittelt werden und höhere trophische Ebenen beeinflussen.

In einer Reihe von Experimenten wurde die Nährstoffzusammensetzung von Primärproduzenten manipuliert um das Kohlenstoff- zu Nährstoff-Verhältnis und somit ihre Qualität zu verändern. Primärproduzenten wurden mit diesem qualitativ veränderten Futter aufgezogen und dienten ihrerseits als Nahrung für Sekundärkonsumenten.

Die Rippenqualle *Pleurobrachia pileus* wurde mit Copepoden gefüttert, deren C:P Verhältnis entlang eines Gradienten verlief. Die Reaktion der Qualle auf das Futter mit dem hohen C:P Verhältnis, das eigentlich als Futter von schlechterer Qualität angesehen wird, war überraschend. *Pleurobrachia pileus* wurde negativ beeinflusst von Futter mit ausgeglichenem Nährstoffgehalt und reagierte besser, wenn das Futter ein hohes Verhältnis von Kohlenstoff zu Phosphor aufwies. Dies bedeutet, dass die Qualle eher Energie- als Nährstofflimitiert ist, was möglicherweise im hohen CP-Verhältnis im Gewebe der Qualle begründet liegt. Die Ergebnisse werden im Zusammenhang mit den vorhergesagten Veränderungen in der aquatischen Umgebung, wie z.B. dem erhöhten Vorkommen von Quallen und der Oligotrophierung des marinen Systems diskutiert.

Frisch geschlüpfte meroplanktonische Larven sind sehr verletzlich und die Mortalität während dieser Lebensphase ist besonders hoch. Deswegen wurde der Effekt von Nahrung mit unterschiedlichen C:N:P Verhältnissen auf Larven des europäischen

Hummers *Homarus gammarus* untersucht. Larven wurden mit Futter unterschiedlicher Nährstoffzusammensetzung gefüttert. Dies wurde für den Zeitraum ihrer gesamten Entwicklung oder über die Dauer eines einzelnen Entwicklungsstadiums durchgeführt.

Die Ergebnisse bestätigen, dass die jüngsten Larven am anfälligsten für Nährstoffmangel sind und ihre Kondition davon stark beeinflusst wird. Die Reaktion auf P-Limitationen änderte sich während der ontogenetischen Entwicklung wobei ältere Stadien weniger stark beeinträchtigt wurden.

Da Nährstofflimitation die Kondition und die Reproduktion von Primärkonsumenten negativ beeinflusst, führt dies durch Reduktion der zu Verfügung stehenden Futtermenge zu einem Quantitätseffekt bei höheren trophischen Ebenen. Die Wirkung von Quantitäts – und Qualitätseffekten wurde in Kombination auf einen Top-Prädatoren in einem Experiment mit Hummerlarven untersucht. Die Larven wurden durch die geringe Menge an Nährstoffen sogar bei sehr geringer Futtermenge beeinträchtigt. Das ist ein Hinweis, dass die Futterqualität eine entscheidendere Rolle spielt als die Quantität.

Eine der großen Herausforderungen für zukünftige ökologische Untersuchungen im marinen Bereich wird es sein, die relative Bedeutung von Qualität und Quantität aufzuschlüsseln. Die Ergebnisse der vorliegenden Studie sollten weitreichende Konsequenzen für das Verständnis von trophischen Interaktionen und der Populationsdynamik mariner Organismen haben.

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ERKLÄRUNG

Hiermit erkläre ich, dass die vorliegende Dissertation, abgesehen von der Beratung meiner Betreuer, selbstständig von mir angefertigt wurde und dass sie nach Form und Inhalt meine eigene Arbeit ist. Sie wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Diese Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der deutschen Forschungsgemeinschaft entstanden. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

Katherina Schoo