

Trophic interactions in the pelagic

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

vorgelegt von

Susanna Knotz
Kiel 2006

Referent: Professor Dr. U. Sommer
Koreferent: PD Dr. M. Boersma
Tag der mündlichen Prüfung: 6. Juli 2006
Zum Druck genehmigt: 4. Januar 2007
der Dekan: gez. J. Grotemeyer

Content

Chapter 1: Community structure of marine pelagic food webs and trophic interactions.....	5
1.1. Introductory remarks.....	5
1.2. The North Sea plankton communities off Helgoland	6
Phytoplankton.....	6
Zooplankton.....	7
Ichthyoplankton.....	8
Microbial food web.....	9
1.3. Factors influencing plankton communities.....	10
Hydrography of the German Bight (Otto et al. 1990).....	10
Irradiance, temperature and salinity.....	11
Nutrients and nutrient limitation – food quantity and quality.....	11
Algal toxins and chemical defence.....	14
Anthropogenic impacts – excess nutrients, toxins, fishing, climate change.....	15
1.4. Internal factors influencing zooplankton communities	16
1.5. Overview of the following chapters.....	17
Chapter 2: Microassays for a set of enzymes in individual small marine copepods	19
2.1. Introduction.....	19
2.2. Materials and methods.....	20
2.3. Results and discussion.....	24
Chapter 3: Sublethal physiological effects of food limitation in copepods	28
3.1. Introduction.....	28
3.2. Material and Methods.....	30
Laboratory experiments.....	30
Digestive enzyme activities.....	30
Water soluble protein content of individuals.....	30
Protease zymograms.....	31
Statistics	32
3.3. Results.....	32
Mortality and survival after starvation.....	32
Hunger experiments – digestive enzyme activities and protein contents.....	33
Hunger experiments – protease zymograms.....	34
Species comparison – protease zymograms	35
3.4. Discussion.....	36
Time scales of reactions.....	37
Role of enzymes.....	39
Zymograms.....	40
Species specificity.....	41
Sexual differences.....	41
Conclusion	41
Chapter 4: Influence of food quality and quantity on egg production of <i>Acartia clausi</i> and <i>Temora longicornis</i> during a spring phytoplankton bloom.....	43
4.1. Introduction.....	43
4.2. Material and Methods.....	45
Sampling – animals and surface seawater.....	45

Abiotic environmental data.....	46
Phytoplankton determination.....	46
Dry mass, C, N and P determination.....	46
Copepod egg production.....	47
Copepod grazing selectivity.....	48
Statistics.....	50
4.3. Results.....	50
Abiotic factors.....	50
Prey community structure influencing egg production.....	51
Stoichiometric factors influencing egg production.....	57
Grazing behaviour.....	58
4.4. Discussion.....	64
Conclusion.....	68
Chapter 5: Stable isotopes as predictors of copepod reproductive success....	69
5.1. Introduction	69
5.2. Material and Methods.....	71
Seasonal Study.....	71
Sampling – animals and surface seawater.....	71
Copepod egg production	72
Stable isotope determination.....	72
Protein content and enzyme activities.....	72
Spatial study.....	73
C, N and P determination.....	74
5.3. Results.....	74
Seasonal Study.....	74
Protein and digestive enzyme activities.....	81
Spatial study.....	85
5.4. Discussion.....	86
Stable isotopes.....	86
Digestive enzymes.....	89
Conclusions.....	90
Chapter 6: General discussion: synthesis and outlook.....	92
6.1. Synthesis.....	92
6.2. Outlook.....	99
Chapter 7: Summary.....	100
Chapter 8: Zusammenfassung.....	102
Chapter 9: References.....	104
Danksagung.....	116
Curriculum Vitae von Susanna Knotz.....	117
Description of the scientific contributions to multiple author manuscripts....	120
Erklärung.....	121

Chapter 1: Community structure of marine pelagic food webs and trophic interactions

1.1. Introductory remarks

This chapter presents a short overview of the processes and interconnections in the marine food web as well as the plankton communities and typical representatives of trophic levels of the North Sea around Helgoland (Fig. 1.1). Factors that shape and change food webs in the zooplankton-phytoplankton interface are also introduced as well as the contents of the following chapters.



Fig. 1.1: Sampling station Helgoland Reede in between the islands Helgoland and Düne (54°11.3'N, 007°54.0'E) (with courtesy of FRS Helgoline GmbH&Co.KG: http://www.helgoline.de/presse_fotos.php).

Presently, we can describe plankton community structures (based on data from cruises (Krause and Radach 1980), long-term sampling (Franke et al. 2004), and Continuous Plankton Recorder surveys (Barnard et al. 2004; Beaugrand 2004), but we still do not fully understand the underlying dynamic mechanisms that drive the system. To model and to predict the structures of these communities, we need to know more about the timing of the different influencing factors. That is, we need to link individual life history dynamics with population and community dynamics. This thesis identifies external and internal factors that influence individual plankton organisms as well as community structure. This thesis was conducted as part of the "Helgoland Food Web Project" and the "GLOBEC-Germany Project".

Previously, the general view of energy flow was that of a linear food chain that directly linked primary producers via herbivorous zooplankton to carnivorous predators such as fish. However, we now know that this is a highly oversimplified picture, and that trophic interactions are much more reticulate than previously thought.

Bottom-up, competitive and top-down processes shape plankton communities. Most calanoid copepods which often dominate the zooplankton community, have an omnivorous feeding mode, during which they ingest phytoplankton, microzooplankton, and even detritus (Kleppel 1993). Many calanoid copepods prefer herbivorous microzooplankton, and in many cases, this selectivity can reduce the grazing pressure on smaller phytoplankters, such as heterotrophic dinoflagellates and other protozoans, which tend to be the most efficient herbivores (Kiørboe et al. 1996; Paffenhöfer 1998a; Sommer and Sommer 2006). Hence, we now recognize that the microbial food web is intertwined with the classical food web such that, for example, there are even fish larvae that feed directly on microzooplankton (Fukami et al. 1999). Other interactions that can cause deviations from the classical linear food chain include cannibalism (Kang and Poulet 2000) as well as chemical (Shaw et al. 1995; Teegarden 1999) and mechanical feeding deterrents (Dutz et al. 2005).

Nutritional deficiencies of phytoplankton affect the fecundity and biochemical constitution of grazers. Biochemical deficiency can be passed on to the top predators and influence their population dynamics (bottom-up control) (Malzahn 2006).

The different trophic levels are dominated by a specific assortment of organisms, which are presented in section 1.2. Hydrographical and meteorological parameters influence advection, nutrient availability, food concentration, and food quality. As a result, the structure of the pelagic community and the biomass of the community vary considerably seasonally and spatially.

1.2. The North Sea plankton communities off Helgoland

Phytoplankton

Phytoplankton is the basis of all life in marine ecosystems. In the North Sea, phytoplankton communities are dominated by *Bacillariophyceae* (diatoms) – which range in size from 5-500 μm – mainly during spring and autumn blooms. Some important representatives of diatoms are the genera *Chaetoceros*, *Thalassiosira*, *Coscinodiscus*, *Rhizosolenia*, *Odontella*, *Leptocylindrus* and *Skeletonema*

Chapter 1: General introduction

(Hoppenrath 2004). *Haptophyceae* or *Prymnesiophyceae* (*Phaeocystis*, *Chrysochomulina*, *Emiliana*) usually peak after diatom maxima. They occur in single cells of about 5 to 15 μm , although some species such as *Phaeocystis* can form colonies up to 10 mm in size. Autotrophic *Dinophyceae* (dinoflagellates) (*Ceratium*, *Gonyaulax*, *Alexandrium*, *Dynophysis*) are the main producers during the summer months (5-200 μm). During the same period, *Cryptophyceae* are abundant in coastal communities as well as in the Central North Sea (3-20 μm). *Crysophyceae* are usually represented by silicoflagellates such as *Distephanus* but only form minor components of the phytoplankton community in the North Sea. Small procaryotic *Cyanophyceae* (~ 1 μm), for example, *Synechococcus* are probably more important as previously thought (Reid et al. 1990).

Zooplankton

Marine pelagic zooplankton larger than 0.2 mm is dominated by copepods around the globe. As a result, these crustaceans form an important link between primary producers and higher trophic levels in all pelagic food webs. They are the most important food source for fish larvae in the plankton. Thus, the condition of copepods, and shifts in zooplankton communities are of high economic significance for the fisheries industry (Beaugrand et al. 2003). Small copepods of 0.5 to 1.5 mm length dominate the mixed waters of the shallow North Sea. The dominant copepod species at Helgoland roads are *Acartia* sp. (mainly *A. clausi*), *Centropages hamatus*, *C. typicus*, *Paracalanus* sp. and *Pseudocalanus elongatus*, and *Temora longicornis*. Other abundant copepods in the area are *Corycaeus anglicus*, *Euterpina acutifrons*, and species of the genera *Cyclopina*, *Microsetella*, *Oithona* and *Tisbe* (Fransz et al. 1991; Greve et al. 2004). Usually, herbivorous copepods dominate the spring followed by more carnivorous copepods during the summer, and detritivorous harpacticoids later in the year (Greve et al. 2004). The main predators on copepods are fish larvae and adult planktivorous fish; however, predators also include other zooplankton such as the chaetognaths *Sagitta setosa* and *S. elegans* or the ctenophore *Pleurobrachia pileus*. The turbellarian *Alaurina composita* can have significant predatory impact on the copepod community (Greve et al. 2004). There are three regular occurring species of scyphomedusae (*Aurelia aurita*, *Cyanea lamarcki*, and *C. capellata*) and smaller hydromedusae. Three species of cladocerans (*Podon intermedius*, *P. leuckarti*, *Evadne nordmanni*) are also common in the North Sea. Since 1991 (first record), another cladoceran *Penilia avirostris* has

been found to reach high abundances during the summer months. Along with *P. avirostris* the salp *Doliolum nationalis* and the siphonophoran *Muggiaea atlantica* (Greve 1994) have become part of the Helgoland Roads zooplankton community only fairly recently (Greve et al. 2004). In the southern North Sea, euphausiids (mainly *Nyctiphanes couchii*) are only infrequently met (Lindley 1982). During the summer months, the appendicularians *Oikopleura dioica* and *Fritillaria borealis* are abundant. The only holoplanktonic polychaets are *Tomopteris helgolandicus* and *T. septemtrionalis*. An assortment of decapod, gastropod and bivalve larvae, echinoderm larvae, and larvae of cirripeds as well as a variety of polychaet larvae are meroplanktonic species that can be dominant at certain times in shallow areas of the North Sea.

The main focus of this thesis is on copepods, with special attention to the abundant species *Temora longicornis* and *Acartia clausi* (Greve et al. 2004) (Halsband and Hirche 2001) (Fig. 1.2.).

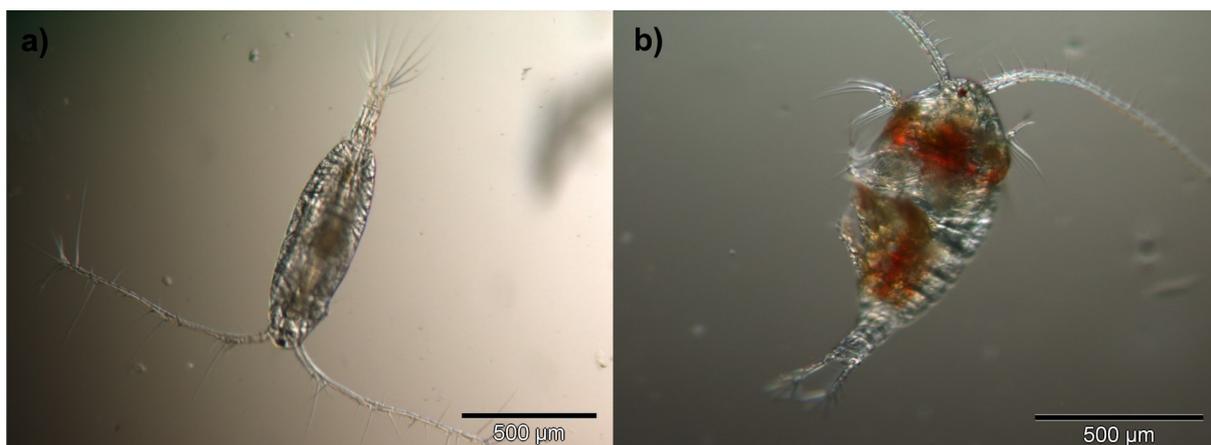


Fig. 1.2: a) *Acartia clausi* female, b) *Temora longicornis* female.

Ichthyoplankton

Many benthic and most pelagic North Sea fish species do not attach their eggs to any substrate and instead release them into the water column. Even if the eggs are demersal, this phase is usually followed by a planktonic phase of the larvae. Around Helgoland, two peaks in ichthyoplankton density occur, one early in the year during late winter and spring, and a second one in June/July (Malzahn 2006). By far the most abundant fish larvae are lesser sandeel (*Ammodytes marinus*), followed by dab (*Limanda limanda*). In the beginning, larval fish preferentially feed on small zooplankton, but also on heterotroph protozoans and even phytoplankton. However, phytoplankton does not support the successful development of fish larvae (Malzahn

Chapter 1: General introduction

2006), but can trigger digestive processes and enhance survival and growth (Cahu et al. 1998). Surviving fish larvae quickly develop to visual predators preferring larger prey with growing size. The efficiency of the food web for fish recruitment is determined in part by the number of trophic steps between primary producers and fish (Sommer et al. 2002).

Microbial food web

Picophytoplankton and bacteria often form the bulk of phytoplankton and can dominate primary production (Fogg 1995). The microbial food web plays an important role in recycling nutrients (Thor et al. 2003). The organisms of the microbial food web can provide particulate organic matter for higher trophic levels in the plankton food web. Nutrients are recycled by bacteria to a large extent, but bacteria can also accentuate nutrient limitation effects by competing with phytoplankton for nutrients in nutrient depleted conditions (Guerrini et al. 1998).

Bacteria and nanoflagellates reach high abundances in coastal waters of the North Sea ranging from 2.7 to 4.5 x 10⁶ cells mL⁻¹. In frontal systems, even higher numbers can be encountered (Van Duyl et al. 1990). Bacterioplankton shows seasonal and geographical variations related to phytoplankton development and temperature (Billen et al. 1990; Gerdts et al. 2004). Bacterial numbers and community structure stay relatively stable from October to March. Community shifts arise during March just before numbers go up, while the highest community variability occurs from April to July (Gerdts et al. 2004). Numbers are mainly controlled by HNF (Zubkov et al. 2001; Beardsley et al. 2003) and bacteriophages (Wichels et al. 2002). Heterotrophic flagellates and other protozoans are competitive grazers as well as an additional food source for copepods. Different copepod species exhibit variable impacts on ciliate communities (Gismervik 2006). Copepod grazing on flagellates can stimulate bacterial growth (Van Wambeke et al. 1996) as well as phytoplankton development by reducing numbers of the efficient microzooplankton herbivores (Kjørboe et al. 1996; Maar et al. 2002). Cladocerans and doliolids supported growth of bacteria by removing bacterivorous HNF more than copepods, which mainly feed on larger phytoplankton and not controlling HNF, which feed on bacteria (Katechakis et al. 2002). Non loricate ciliates such as *Strombidium* and *Lohmanniella* and loricate forms such as *Tintinnopsis* dominate the ciliate fauna in the North Sea. The heterotrophic dinoflagellate *Noctiluca scintillans* can form extensive blooms that feed on detritus, phytoplankton, and zooplankton (Fock and Greve 2002).

1.3. Factors influencing plankton communities

Hydrography of the German Bight (Otto et al. 1990)

The German Bight is a very shallow part of the North Sea with an average depth of about 20 m and where tidal currents lead to a mixing of different water masses. Temperature differences between summer and winter are extreme in the continental coastal waters as compared to areas that are influenced by North Atlantic water masses. Zooplankton distribution in the North Sea differs between three main water bodies: Atlantic water masses, central North Sea water mass, and coastal water masses (Krause 1995).

The overall current in the North Sea is anti-clockwise. Thus, water in the German Bight moves from west to east and northwards along the Frisian coast. Water masses are moved around by tidal currents. Two amphidromic points are centres of tidal strengths in the German Bight. One is located at the Eastern tip of the Dogger Bank and the other near the entry of the Southern Bight. Atlantic water flows in through the English Channel and the northern pathway, mainly during autumn and winter. A strip of mixed coastal water is separated from the usually stratified central North Sea waters by a slightly changing transitional zone which encompasses the sea around Helgoland. The main outflow of North Sea water is northward bound flowing along the Norwegian coast.

The German Bight is also influenced by large freshwater masses. Frontal zones can develop in areas where water masses of different density meet, that is, water masses with different temperatures and/or salinity. Three types of frontal zones can occur in the German Bight (Otto et al. 1990) after (Krause et al. 1986): Tidal mixing fronts forced by tidal friction mixing, wind stress and temperature as along the Frisian coast; river plume fronts along the Danish-German coastline of Jutland between freshwater and oceanic water; and upwelling fronts west of Helgoland where bottom water advected from the central North Sea is forced upward by easterly wind stress. Where two water masses meet a strong horizontal gradient in salinity and or temperature is apparent.

The main nutrient input into the German Bight is from the freshwater systems of the Elbe, Weser and Ems rivers. As a result, a gradient exists from high coastal concentrations towards the lower nutrient concentrations of the central North Sea (Raabe et al. 1997). The nutrient distribution and cycling in the North Sea is

Chapter 1: General introduction

hydrographically influenced (Brockmann et al. 1990). The transitional zone of a shallow water frontal system contains the highest nutrient values and turnover rates compared to the adjacent stratified and mixed waters. Hence, the waters in this zone trigger the growth of phytoplankton in the frontal areas (Maguer et al. 2000).

Irradiance, temperature and salinity

In the temperate zones of the globe, seasonally variable irradiance mainly plays a role for primary production, which in turn influences all other trophic levels. With increasing light in spring phytoplankton blooms develop. Biochemical compounds of phytoplankton can vary extremely between photoperiod and dark phase (Granum et al. 2002). The light regime (intensity and spectrum) can alter algal nutrient requirements (Wynne and Rhee 1986), which can affect grazers (Hessen et al. 2002). In response to low light conditions, the chlorophyll-a contents of algae are increased to increase the light harvesting ability of the algae (Geider 1987). With such an increase in chlorophyll-a, the demand for nitrogen increases, which in turn increases the risk of nitrogen-limitation.

The metabolic processes of poikilothermic marine invertebrates are correlated with temperature. Hence, energy demands increase with increasing temperature due to rising metabolic activity. Food ingestion of copepods increases with rising temperature (Kiørboe et al. 1982), as does egg production (Halsband and Hirche 2001; Holste and Peck 2006). Higher temperatures increase food limitation thresholds by increasing the energy demand of marine crustaceans, as well as influencing individual and population growth (Savage et al. 2004). Sub-populations of the same species occurring over a wide geographical range such as the calanoid copepod *Centropages typicus* are adapted to different optimal temperatures in areas of different latitudes (Halsband-Lenk et al. 2002).

Nutrients and nutrient limitation – food quantity and quality

Food is the single most important biological (bottom-up) factor that determines copepod success, as it affects developmental times, weight and egg production of copepods (Hirche et al. 1997; Rey-Rassat et al. 2002). Copepods respond to increased food availability with increased ingestion linearly up to the incipient limiting level (Mayzaud and Poulet 1978; Mayzaud et al. 1992).

Apart from the quantity of the food, the quality of food can also play a major role. First and foremost, the spectrum of the available particles is of importance. Ciliates (Bonnet and Carlotti 2001) or heterotrophic dinoflagellates (Klein Breteler 1980)

provided in conjunction with phytoplankton can enhance the fecundity and viability of copepods. Heterotrophic dinoflagellates produce essential fatty acids that are absent in their phytoplankton food, thereby upgrading the food quality for copepods (Klein Breteler et al. 1999; Veloza et al. 2006). One of the other main quality determining factors is the nutrient content of the food (algae). Phytoplankton organisms depend on an assortment of nutrients in the water to grow. The most important nutrients besides carbon (C) are nitrogen (N), phosphorus (P) and iron (Fe). Silica (Si) is also important for diatoms and other silica shell forming phytoplankton such as silicoflagellates. Globally, nitrogen and iron probably play the largest roles, but recently a growing role for phosphorus has been acknowledged for the open water marine environment (Brockmann et al. 1990; Downing et al. 1999). Phosphorus even seems to be widely limiting phytoplankton in oceans (Sañudo-Wilhelmy et al. 2004). According to Redfield (1958), the general ratio between carbon, nitrogen, and phosphorus is 106:16:1 (C.N.P) (Redfield 1958). However, this ratio tends to vary between species, and can have even larger variation within a species due to the physiological state of individuals. In general, phytoplankton is usually severely N-limited at a N:P ratio of < 5 , and P limited when this ratio is over 100. Replete conditions are indicated by ratios in the range of 5-19 (Geider and La Roche 2002). Nutrient deficiency changes the growth behaviour and biochemical constituents of phytoplankton. Phytoplankton exponential growth ceases upon nutrient limitation, which leads to a stationary phase and senescence. During a phytoplankton bloom massive biochemical changes occur that can be related to the nutrient availability (Morris et al. 1983; Morris et al. 1985). Nutrient limited cells may be larger and denser than cells grown under optimal nutrient conditions. The protein content of nitrogen depleted phytoplankton is decreased while phosphate limited algae rather produce more carbohydrates and lipids (Kilham et al. 1997). Phosphate deficiency leads to lower DNA levels (phosphate is a major compound of DNA and RNA), while protein and chlorophyll-a (chl-a) contents remain similar. Nitrogen limitation affects protein and chl-a contents of cells (Granum et al. 2002). Therefore, a higher protein:DNA or chl-a:DNA ratio indicates phosphate rather than nitrogen limitation (Berdalet et al. 1996). Nutrient deficient phytoplankton produces and releases more polysaccharides (Penna et al. 2000). In nutrient limited phytoplankton bulk lipids increase and are dominated by storage and membrane lipids formed of

Chapter 1: General introduction

triacylglycerols (TAG) (Roessler 1990). TAG contains mostly monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) with no nitrogen. Nitrogen and phosphate depletion can reduce absolute and proportionate levels of polyunsaturated fatty acids (PUFA) and decrease the sterol content in diatoms, eliciting negative effects on copepod development (Klein Breteler et al. 2005). However, higher levels of essential PUFAs have been found in phosphorus limited phytoplankton compared to phosphorus sufficient algae (Müller-Navarra 1995; Malzahn 2006).

Hence, the underlying biochemical compounds, and their bioavailability should not be neglected when evaluating effects of nutrient depletion (Geider and La Roche 2002; Anderson et al. 2004) as the analysis of elemental ratios does not normally suffice to recognize biochemical limitations (Anderson and Hessen 1995). Distinct fatty acids and amino acids have been recognized to be limiting for zooplankton (Kleppel et al. 1998; Anderson and Pond 2000). Different phytoplankton species and other organisms differ in their fatty acid profiles per se (Reitan et al. 1997). The most important fatty acids that have been identified to limit copepod growth and reproduction include eicosapentaenoic acid (20:5(n-3); EPA) and docosahexaenoic acid (22:6(n-3); DHA). These fatty acids are often limiting in the field (Jónasdóttir 1994; Jónasdóttir et al. 1995; Arendt et al. 2005), but amino acids have also been mentioned as limiting factors for zooplankton reproduction (Guisande et al. 2000; Guisande et al. 2002).

Coming back to nutrients, grazers normally display homeostasis in their C:N:P stoichiometry despite large stoichiometric changes in their prey (Koski 1999; Pertola et al. 2002). Grazers can adjust their feeding strategy to the nutrient limitation of their prey (Mitra and Flynn 2005). Compensatory grazing was observed in *Daphnia magna* fed with phosphate limited algae (Plath and Boersma 2001) and in *Acartia tonsa* feeding with 480% higher rates on low quality dinoflagellate *Karenia brevis* as compared to high quality *Rhodomonas lens* (Prince et al. 2006). It has been hypothesized that by feeding more, more energy is used for nutrient uptake, which counterbalances mineral imbalances (Plath and Boersma 2001). Moreover, selective digestive processes are predicted to decrease the assimilation of a superfluous element and to increase the release rate of the element (Darchambeau 2005). Indeed, copepods and other zooplankton grazers did release excess nutrients that were immediately taken up by slightly nitrogen limited phytoplankton (Katechakis et

al. 2002). High C:N ratios of prey often cause lower egg production rates in copepods (Checkley 1980b). Lower C:N ratios have similar effects as a temperature increase when food concentration is not limiting (Ambler 1986), but nitrogen deficient phytoplankton can also have an opposite effect on copepod fecundity by increasing egg production rates (Augustin and Boersma 2006).

Despite the relative homeostasis of copepods mineral changes can be passed on to the herbivore levels (Van Nieuwerburgh et al. 2004; Malzahn 2006), but mostly indirectly. The fatty acid composition of copepod grazers and their eggs reflects that of their food, so that changes in selective feeding during seasons can be followed (Peters et al. 2006). Even typical bacterial fatty acids that were transferred via ciliates can be traced (Ederington et al. 1995). Different signatures of algae species can be traced via herbivores up to fish larvae (Reitan et al. 1997; Pedersen et al. 1999). Therefore, characteristic fatty acid profiles can be used as food type or trophic markers throughout the food web (Dalsgaard et al. 2003). Thus, nutrient limitation that changes the fatty acid profiles of prey organisms leaves its traces throughout the food web.

Algal toxins and chemical defence

Algae produce biochemicals (toxins, feeding deterrents) that suppress grazing but they can also inhibit competitors with allelochemicals (Legrand et al. 2003). Nutrient deficiency triggers or increases toxin production in such different algal species as *Chrysochromulina polylepis* (Johansson and Granéli 1999), *Pseudonitzschia seriata* (Fehling et al. 2004), different *Alexandrium* species (Frangópulos et al. 2004) and *Prymnesium parvum* (Granéli and Johansson 2003). In *Alexandrium funduyense*, toxin production ceased upon nitrogen limitation but increased when phosphate and nitrogen were limiting (John and Flynn 2000). Several copepod species are able to discriminate between toxic and non-toxic strains of *Alexandrium* and select for non-toxic alternatives (Teegarden 1999). *Phaeocystis* is grazed by copepods mainly in its colonial form but only to a low extent. Copepods select against the haptophyte during peak abundance and the algae seems to be unsuitable for copepod nutrition (Klein Breteler and Koski 2003) Instead, during blooms they prefer the accompanying heterotroph protozoan fauna (Gasparini et al. 2000; Tang et al. 2001; Koski et al. 2005).

Chapter 1: General introduction

For many years diatoms in general were considered as first rate food for copepods. Copepod populations usually develop well following springtime diatom blooms (Gowen et al. 1999). Recently, however, evidence was presented that diatoms may be low quality food for copepods (Ban et al. 1997). The low quality was ascribed to toxic aldehydes that are produced by the diatoms upon grazing, thus arresting embryonic development (Miralto et al. 1999; Ianora et al. 2004). Others presented evidence that alternative food in naturally occurring diatom concentrations encountered in the field could probably compensate deleterious effects of diatoms (Irigoien et al. 2002). Grazing experiments with mixed food items indicated that copepods could indeed compensate negative effects caused by putative toxic phytoplankton including diatoms (Colin and Dam 2002). Recently, negative diatom effects on egg production and hatching success could clearly be ascribed to nutritional deficiency of diatoms rather than toxicity (Jones and Flynn 2005). Even a small proportion of good dinoflagellate food had beneficial effects on fecundity and vitality of copepods.

Anthropogenic impacts – excess nutrients, toxins, fishing, climate change

Coastal zones of the sea are strongly influenced by human activity. Besides the higher natural inflow of organic matter and inorganic nutrients transported by rivers, waste water including additional high amounts of organic and inorganic compounds and toxic substances from land runoff and atmospheric emission affect life in coastal zones. Fishery and oil industry also affect life in the oceans, especially in a heavily used shelf sea as the North Sea. Anthropogenic nutrient input does affect the primary production level without directly affecting the herbivorous zooplankton trophic level (Micheli 1999). However, additional nutrient inputs can shift primary producer communities to inedible or low quality organisms (Müller-Navarra et al. 2004). Fisheries reduces grazing pressure on mesozooplankton but may support the development of jellyfish or other alternative predators.

New species that drift into the area may suddenly find a suitable environment to establish a stock population (Greve et al. 2004). Temperature influences zooplankton communities from such different seas as Black Sea, North Sea, and Baltic Sea in similar ways (Niermann et al. 1998). In the past 100 years the average global temperature has risen by 0.6 °C. This shift has already lead to pronounced effects on a vast assortment of terrestrial and marine species, their range and abundance, and

timing of events in their life cycles (Root et al. 2003). The authors found a considerable range shift polewards and a time shift towards earlier starts of events such as migration, flowering, or egg laying during spans of 10 years in 80 % of the species or species groups treated in the studies. Community shifts due to climatic change (Hays et al. 2005) can lead to increasing trophic mismatch situations (Cushing 1990; Edwards and Richardson 2004; Richardson and Schoeman 2004). The German Bight is a frontier zone for some northern as well as for some southern species. Southern species are better adjusted to higher temperatures. Thus, their offspring survival rate may increase, while northern species may find less suitable conditions for their survival. In the waters around Helgoland, the average salinity has increased and the average sea surface temperature has risen by 1.13°C over the last 4 decades, which have shifted the onset of the spring diatom bloom to a later date potentially due to increased winter grazing (Greve et al. 2004; Wiltshire and Manly 2004).

1.4. Internal factors influencing zooplankton communities

The antennules of copepods are equipped with mechanosensors and chemosensors (Bundy and Paffenhöfer 1993). In principle they can remotely detect hydromechanical as well as chemical signals in the water. Chemoreception is not necessary for the perception of prey in the far field (Bundy et al. 1998). Copepods can differentiate hydromechanical signals of prey from predators and discern these from background signals of the ambient flow (Hwang and Strickler 2001). Motility of prey can be a positive selective force (DeMott and Watson 1991). A general model for predators with different feeding modes and prey motility or sinking was developed to calculate distance perception and differentiation between prey and predator signals (Kjørboe et al. 1999; Kjørboe and Visser 1999). The model predicts the predation risks of differently behaving copepods with a set of different prey (Viitasalo et al. 1998). The mechanosensory warning system of copepods habituates quickly under high turbulence; copepods can avoid unnecessary flight movements and improve their foraging success in turbulent water (Hwang et al. 1994). Copepods do feed selectively. They can sense large cells remotely but can also handle prey smaller than 10 µm (Price et al. 1983). Copepods adjust their feeding mode to the cell sizes they encounter: small cells are ingested by regular flapping of the mouthparts, which is occasionally interrupted by combing the feeding appendages,

Chapter 1: General introduction

while larger cells are individually handled (Vanderploeg and Paffenhöfer 1985; Price and Paffenhöfer 1986). Copepods can reject cells of "bad taste" with some wide sweeping movements. They can manipulate cells (chop-stick feeding), for example remove spines from *Chaetoceros* sp., align chain forming diatoms to fit into their mouth (banana-like feeding) or squeeze the contents out of dinoflagellates with strong cell walls (R. Strickler, personal communication). Besides chemosensory detection of potential mating partners, it was unclear whether copepods use chemosensors on their antennules to detect food in their vicinity, and not just after they have captured it. Electrophysiological experiments on isolated antennules of female *Temora longicornis* have shown that copepods can definitely sense cell free algal homogenates and several amino acids with their antennules (Schütte 2006). Chemosensory abilities enable copepods to select for taste and therefore quality of prey (DeMott 1986; Paffenhöfer 1998b). Food concentration and feeding history influence selective grazing behaviour (Donaghay and Small 1979; DeMott 1989) and the acclimation of the digestive system (Hassett and Landry 1983; Landry and Hassett 1985; Roche-Mayzaud et al. 1991; Mayzaud et al. 1992). Digestive physiology and its underlying genetic control is a potential factor that determines selective feeding behaviour (Sotka 2003). Copepods can digest a great variety of possible substrates (Mayzaud and Mayzaud 1981). The set of digestive enzymes and their portion in the organism can help to determine their nutritional (Kumlu 1997) or trophic status (Jones et al. 1997). High enzyme activity can denote the importance of the substrate for the analysed animal but also the deficiency or difficult digestibility of the available natural substrate. Therefore, interpretation of digestive enzyme studies can be ambiguous. Digestive enzyme activities can change during ontogeny, which suggests different food niches for each developmental stage (Hirche 1981; Johnston 2003). Similarity between phylogenetic related species can overrule nutritional differences (Chan et al. 2004). Digestive enzymes are released into the water and can even override the bacterial extracellular enzymatic activity (Vrba et al. 2004). For larger crustaceans, enzyme studies on faecal pellets have been carried out to survey changes in the digestive system over a longer period with minimal animal disturbance (Cordova-Murueta et al. 2003).

1.5. Overview of the following chapters

The aim of this study is to identify internal physiological factors that influence the adaptability of different copepod species to different food niches within the same

Chapter 1: General introduction

environment. The focus is on the digestive enzyme system and the grazing behaviour of copepods and the potential of these internal factors to react to food quantity and quality changes. This study also examines the possible influence of general nutritional factors on copepod reproduction in the field. The influence of copepod and seston stoichiometry and stable isotopes on reproduction are evaluated from a spring survey at Helgoland and compared with the conditions in a temporal study at Helgoland and a spatial study within the German Bight.

The two following chapters concentrate on the methodology to evaluate internal copepod physiology. Chapter 2 presents methods to measure digestive enzyme activities and the water soluble protein content on an individual basis. The methods are tested on differently sized small North Sea copepods and accompanied by measurements of lipid and elemental content. Data for different dominant copepod species of the North Sea are compared. Chapter 3 presents a method to determine protease isozyme patterns in individual copepods is presented. Intra-specific versus inter-specific and environmentally caused variability of isozyme production and the possible ecological significance for copepods is also discussed.

Chapter 4 discusses the influence of grazing activity, food quality, quantity and the physical environment on copepod fecundity and physiology during spring, a time of strongly changing parameters. The main focus of the chapter is on elemental quality. Chapter 5 concentrates on a temporal and spatial study on the influence of trophic level and digestive physiology on copepod fitness and fecundity. Chapter 6 presents a synthesis of the results and discusses their possible meaning for increasing model predictability.

Chapter 2: Microassays for a set of enzymes in individual small marine copepods

Fluorogenic assays for a set of 5 enzymes which are involved in digestion and food utilization (alanine and arginine aminopeptidase, lipase/esterase, chitinase, and beta-glucosidase) were optimised to measure activities of these enzymes in the same extracts of individual small North Sea copepods. The enzyme activities of *Acartia clausi*, *Centropages typicus*, *Corycaeus anglicus*, *Paracalanus parvus*, and *Temora longicornis* showed distinct species specific activity patterns, but also high intra-specific variability. Protein, lipids, carbon and nitrogen (C, N) were determined with micro-scale assays in individual copepods or in batches of 10 to 50 animals. Water soluble protein contents ranged from 16 to 38%, and lipid contents from 2.4 to 5.5% of dry mass. The molar C:N ratios were between 4.1 and 4.5. The presented microassays provide suitable tools for studying physiological reactions of copepods and other small pelagic crustaceans in response to variable environmental conditions.

2.1. Introduction

Copepods hold key positions in pelagic food webs and contribute significantly to the transfer of matter and energy between trophic levels. However, the detailed functions of particular species or developmental stages within pelagic food webs are still largely unknown due to the variety of possible trophic interactions.

The biochemical utilization of food is facilitated by a set of digestive enzymes that are synthesized in the midgut region of copepods (Arnaud et al. 1980; Brunet et al. 1994). The activities of such enzymes reflect the potential to digest different organic matter and may indicate adaptations to different food sources (Rodriguez et al. 1994; Jones et al. 1997; Le Vay et al. 2001). Even though digestive enzymes have been measured extensively in copepods since the 1970's (Boucher and Samain 1974; Mayzaud and Conover 1976; Mayzaud and Poulet 1978), the sensitivity of the enzyme assays was low, and hence mostly applicable to pooled samples of up to several hundred copepods or to larger animals (Johnston and Freeman 2005). Accordingly, information on developmental stages or species was difficult to obtain, while information on individual copepods was not available. However, this information is crucial in interpreting physiological conditions and trophic interactions.

Chapter 2: Microassays for a set of enzymes in individual copepods

In this study, we adapted sensitive enzyme assays previously used to detect enzymatic activity in water samples (Hoppe 1983; Oosterhuis et al. 2000; Sastri and Roff 2000) to measure enzyme activities in individual copepods. The catalytic potential in different species may provide additional information on the utilization of food that is preferably eaten by these animals or help to explain the dynamics of nutrient uptake. Beyond that, we analysed general nutritive parameters such as lipid and water soluble protein content of the animals as well as their C and N content. As test organisms, we used the most abundant pelagic North Sea copepods around Helgoland known for their carnivorous, omnivorous and more herbivorous feeding modes (Kleppel 1993). In this study we concentrated on copepods to establish the analytical methods. However, the analytical procedure will be suitable for a wide range of small pelagic crustacean or their developmental stages.

Table 2.1: List of studied species: abbreviations, feeding preferences, distribution (Turner, 1984; Kouwenberg, 1993; Krause, 1995; Mauchline, 1998).

Species	Abbr.	Feeding mode	Distribution
<i>Corycaeus anglicus</i>	Ca	carnivorous	neritic, warm to cold temperate, Atlantic and Pacific
<i>Acartia clausi</i>	Ac	omnivorous	oceanic to neritic, warm to cold temperate, Atlantic and Pacific
<i>Centropages typicus</i>	Ct	omnivorous	oceanic, marine, warm to cold temperate, Atlantic
<i>Temora longicornis</i>	Tl	omnivorous	neritic, marine to estuarine, warm to cold temperate, Atlantic,
<i>Paracalanus parvus</i>	Pp	most herbivorous	oceanic to neritic, worldwide, warm to cold temperate, Atlantic

2.2. Materials and methods

The copepods, *Acartia clausi*, *Centropages typicus*, *Corycaeus anglicus*, *Paracalanus parvus* and *Temora longicornis* (Table 1) were sampled in autumn 2003 off Helgoland (54°11N, 7°54E, North Sea, German Bight). Females were selected, transferred to aquaria (1 L, 15 ± 1 °C) and fed with a mixture of flagellates *Rhodomonas sp.*, *Isochrysis sp.* and *Oxyrrhis marina* (Klein-Breteler 1980). The aim of standardising the feeding condition of all copepods was to measure species peculiarities rather than effects due to nutritional differences. After two days of feeding females were selected, shortly rinsed with deionised water, blotted dry and frozen at -80 °C.

Chapter 2: Microassays for a set of enzymes in individual copepods

Cephalothorax lengths and widths were measured in 20-30 live individuals under a dissection microscope using a video analysis system (analysis, Soft Imaging System).

Water soluble protein content of individual females was measured with the bicinchoninic acid assay (BCA, Pierce Ltd.) (Smith et al. 1985). The method was adapted for the use in 96-well microplates. Individuals were ground in reaction cups with a micropestle in 55 μL deionised water, while being cooled on ice. The extracts were centrifuged for 10 min (15000 g, 4°C). Fifty μL of supernatant were mixed with 250 μL test kit reagents and incubated for 1 h at room temperature. The microplates were read at 550 nm (Dynatech MR 7000). Bovine serum albumin (BSA, 1 to 5 μg per well) was used as standard.

Table 2.2: List of enzymes, substrates, and assay conditions.

Enzyme	Substrate	Source	Buffer system	pH
Chitobiase	4-Methylumbelliferyl N-acetyl-beta-D-glucosaminide	Sigma 2133	Mcllvaine	5
Beta-glucosidase	4-Methylumbelliferyl beta-D-glucoside	Sigma 3633	Mcllvaine	5
Esterase/lipase	4-Methylumbelliferyl butyrate	Fluka 19362	Tris/HCl	7
Alanine aminopeptidase	L-Alanine-4-methyl-7-coumarinylamide-trifluoroacetate	Fluka 05198	Tris/HCl	7
Arginine aminopeptidase	L-Arginine-7-amido-4-methylcoumarin hydrochloride	Sigma 2027	Tris/HCl	7

Total lipids were measured with the sulphophosphovanillin method (Zöllner and Kirsch 1962). A commercial test kit (Merckotest 3321) was adjusted for the use in 96-well microplates. Copepods (10 to 35 individuals per replicate) were boiled for 10 min in 60 μL concentrated sulphuric acid in stoppered glass vials. After cooling to room temperature, 30 μL of the solution were transferred into microplate wells. Samples received 300 μL of phosphovanillin reagent (8 mmol L^{-1}) and blanks were prepared with 300 μL of phosphoric acid (11.9 mol L^{-1}). Standards (1.2 to 6 μg serum lipids per well) were treated alike. A gravimetric control of copepod lipids could not be done due to the extreme low amount of material. The optical density (OD) was read at 530 nm. A lipid extraction step was not necessary as preliminary experiments showed that the yield did not improve after extraction with chloroform/methanol.

Chapter 2: Microassays for a set of enzymes in individual copepods

Carbon, nitrogen and dry mass (dm) were analysed in pools of 25 to 50 freeze dried females with a CHN analyser (Fisons Instruments EA 1108). Acetanilide (Thermo Quest, 338 36700) served as standard.

Enzyme activities were measured in individual females. These were homogenized with a micropestle in 200 μL of ice cold Tris/HCl buffer (0.1 mol L^{-1} , pH 8) and centrifuged for 10 min at 15000 g and $4 \text{ }^\circ\text{C}$. Extracts of a single animal were used for the analysis of two protein degrading exopeptidases (arginine and alanine aminopeptidase), lipid hydrolysing esterase/lipase, and two carbohydrases (chitobiase and beta-glucosidase). Assays were run at $25 \text{ }^\circ\text{C}$ with 5 to 40 μL of sample. Stock solutions of the substrates (Table 2.2) were prepared in ethylene glycol monomethylether (5 mmol L^{-1}).

Table 2.3: Morphometric and nutritive data (mean \pm SD); Sp. = species, Cs = cephalosome, dm = dry mass; Protein = water soluble protein; * 25 to 50 individuals per replicate; ** 10 to 35 individuals per replicate.

Sp.	Dry mass* ($\mu\text{g ind}^{-1}$)	Cs length ($\mu\text{m ind}^{-1}$)	Cs width ($\mu\text{m ind}^{-1}$)	Carbon* (% dm)	Nitrogen* (% dm)	Molar C:N*	Lipid** (% dm)	Protein (% dm)
Ca	4.62 ± 0.04 (n=3)	636 ± 33 (n=20)	272 ± 11 (n=20)	39.8 ± 1.0 (n=3)	10.6 ± 0.3 (n=3)	4.36 ± 0.01 (n=3)	3.9 ± 0.8 (n=5)	38 ± 9 (n=20)
Ac	6.02 ± 0.37 (n=4)	772 ± 31 (n=92)	250 ± 10 (n=92)	44.0 ± 0.3 (n=4)	12.3 ± 0.3 (n=4)	4.19 ± 0.07 (n=4)	5.5 ± 1.0 (n=21)	24 ± 8 (n=24)
Ct	37.86 ± 1.57 (n=4)	1208 ± 55 (n=33)	504 ± 20 (n=33)	43.9 ± 0.7 (n=4)	12.5 ± 0.2 (n=4)	4.10 ± 0.03 (n=4)	2.4 ± 0.4 (n=4)	19 ± 5 (n=24)
TI	30.35 ± 4.79 (n=3)	824 ± 92 (n=20)	491 ± 40 (n=20)	45.0 ± 0.3 (n=3)	12.1 ± 0.1 (n=3)	4.33 ± 0.01 (n=3)	4.5 ± 0.2 (n=4)	16 ± 4 (n=30)
Pp	6.62 ± 0.26 (n=2)	n. d.	n. d.	44.4 ± 1.3 (n=2)	11.5 ± 0.4 (n=2)	4.50 ± 0.29 (n=2)	5.0 ± 1.2 (n=4)	16 ± 8 (n=24)

Substrate concentrations in the assays were $100 \mu\text{mol L}^{-1}$ in a total volume of $500 \mu\text{L}$ Tris/HCl (0.1 mol L^{-1}) or citrate-phosphate buffer (McIlvaine 1921). Fluorescence was measured at 360 nm (excitation) and 450 nm (emission) for 10 to 60 min with a Kontron SFM 25 device. Blanks were run in parallel. The rate of autolysis was tested for each substrate at all given assay conditions and subtracted from the assay-results. Standard curves were prepared with 4-beta-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC). The effect of pH on MUF and AMC fluorescence was determined. Enzyme activities were calculated in relation to the average water soluble protein content of either species and were presented as specific activities ($\text{nmol h}^{-1} \text{ mg}_{\text{prot}}^{-1}$). The linearity of the assay was tested with extracts of *A. clausi* and

Chapter 2: Microassays for a set of enzymes in individual copepods

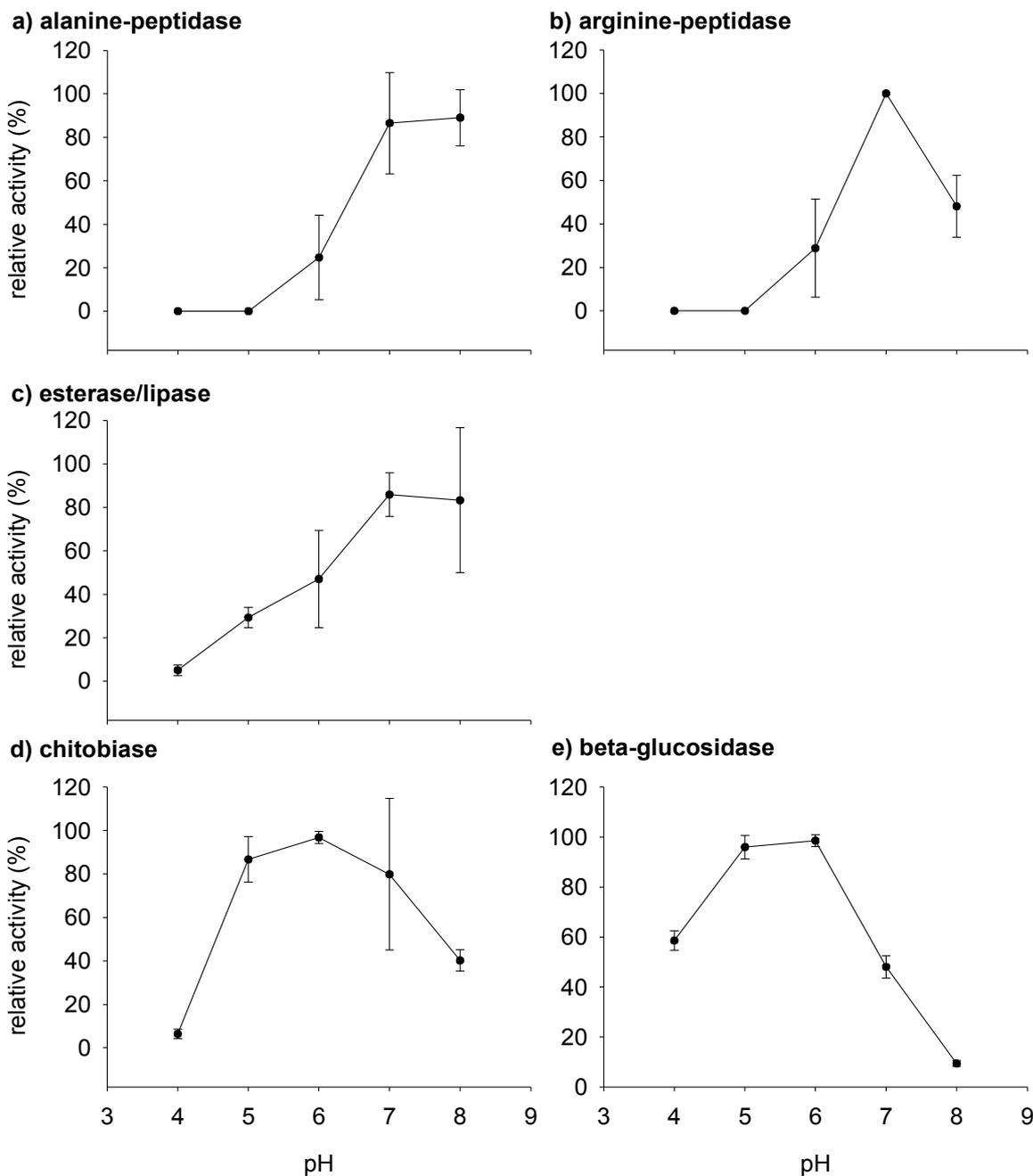


Fig. 2.1: pH-profiles of all enzymes measured with extracts of *T. longicornis* females ($n = 3$; means \pm SD).

T. longicornis. The fluorescence increased linearly between 2 and 8% of a copepod as extract in the final assay preparation ($y=0.79*x+0.0001$, $r^2=0.98$, $p=0.01$). The pH-profiles of all enzymes were determined between pH 4 and pH 8 with extracts of *T. longicornis* females. Lipase/esterase and peptidase showed maximum activities at pH 7 and carbohydrases at pH 5. The standard assays for these enzymes were run at the respective pH of maximum activity (Table 2.2).

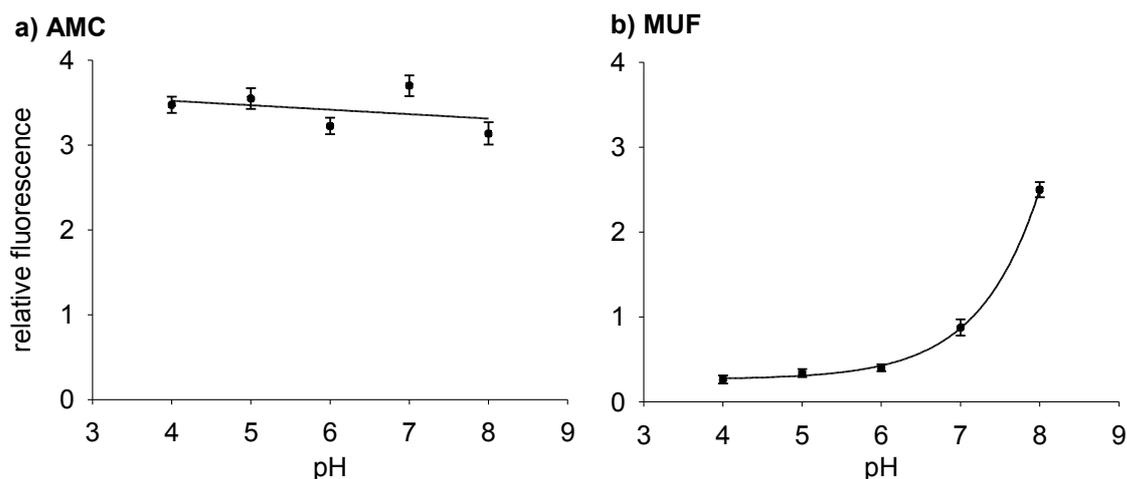


Fig. 2.2: Effects of pH on the fluorescence of a) 7-amino-4-methylcoumarin (AMC); linear regression and b) 4-beta-methylumbelliferone (MUF); exponential regression ($n = 4$; means \pm SD).

2.3. Results and discussion

The copepod species selected for our study overlap widely in their area of distribution and are abundant in the North Sea. However, they differ distinctly in feeding habits and in size. The smaller species *A. clausi*, *C. anglicus* and *P. parvus* weighed 4.6 to 6.6 μg (dry mass) while the dry mass of *T. longicornis* and *C. typicus* amounted to 30-38 μg (Table 2.3). Due to the small size of copepods the measurements of enzyme activities and storage products require optimised methods such as micro-scale extraction procedures, sensitive substrates, and optimum assay conditions.

Enzyme activities were highest at neutral to slightly acidic conditions: the exopeptidases and esterase/lipase at pH 7 and 8 (Fig. 2.1a, b, c), and the carbohydrases at pH 5 and 6 (Fig. 2.1d, e). These activity optima correspond with the neutral to slightly acidic pH that was determined *in vivo* in the gut of *Calanus helgolandicus* (Pond et al. 1995). The fluorescence of AMC remained constant between pH 4 and 8 while the fluorescence of MUF increased exponentially above pH 7 (Fig. 2.2). Accordingly, activities measured with MUF substrates at higher pH appear larger than they are. This effect must be compensated for by applying appropriate standards.

In all species activities of arginine aminopeptidase were higher than activities of alanine aminopeptidase (Fig. 2.3a, b). Both activities were closely correlated ($r^2=0.64$, $n=34$, $p<0.00001$) which may indicate overlapping substrate specificity or co-expression of both enzymes. Since phytoplankton contains less protein than zooplankton, herbivores have to increase their catalytic ability to cover their nitrogen demand from proteins. Apparently, aminopeptidase activities increased

Chapter 2: Microassays for a set of enzymes in individual copepods

with the degree of herbivory. *T. longicornis* and *P. parvus* are considered more herbivorous than *C. typicus* and *A. clausi*, while *C. anglicus* is a carnivore (Turner 1984; Kouwenberg 1993; Mauchline 1998). *C. anglicus* showed the lowest and *P. parvus* and *C. typicus* the highest amino-peptidase activities. Therefore, the exopeptidases analysed here seem as suitable for the interpretation of zooplankton feeding modes as shown previously for endopeptidases such as trypsin (Rodriguez et al. 1994; Jones et al. 1997; Le Vay et al. 2001).

MUF-butyrate, was hydrolysed at rates of $1200 \text{ nmol h}^{-1} \text{ mg}_{\text{prot}}^{-1}$ in *C. anglicus* to $5000 \text{ nmol h}^{-1} \text{ mg}_{\text{prot}}^{-1}$ in *T. longicornis* (Fig. 2.3c). These particularly high activities of esterase/lipase clearly reflect the high potential of all species to utilize lipids. None of the species from the North Sea studied here store significant amounts of lipids (Tab. III). Therefore, these species highly depend on the immediate and rapid utilization of alimentary fatty acids, and thus, on high esterase/lipase activities. Besides their nutritive value, lipids are important compounds of egg yolk and thus crucial for reproductive success. All copepods used in this study were adult females which were able to reproduce. Accordingly, high esterase/lipase activities may fuel vitellogenesis in reproducing females by utilizing alimentary lipids (Gatten et al. 1980).

Crustaceans express two forms of chitinolytic enzymes that are involved in moulting or digestion (Peters et al. 1999). Since adult copepods do not moult, they most likely express exclusively digestive chitinase. It hydrolyses oligomers of N-acetylglucosamines (NAG) derived from chitin degradation to NAG monomers. Total activities of chitinase ranged between 90 and $930 \text{ nmol h}^{-1} \text{ mg}_{\text{prot}}^{-1}$. Surprisingly, species considered more carnivorous showed low chitinase activities, while the more herbivorous species expressed elevated activities. Therefore, herbivorous copepods may be capable of utilising diatom chitin by elevated chitinase activities as suggested for Antarctic krill (Saborowski and Buchholz 1999).

Beta-glucosidase hydrolyses terminal beta-D-glucose from various polysaccharide sources such as cellulose or laminarin and is involved in many metabolic processes. Accordingly, beta-glucosidase should exhibit high activities in all studied species. However, we found a wide range of activities from as low as $15 \text{ nmol h}^{-1} \text{ mg}_{\text{prot}}^{-1}$ in *C. anglicus* to $1100 \text{ nmol h}^{-1} \text{ mg}_{\text{prot}}^{-1}$ in *C. typicus*. Therefore, we have to consider that additional enzymes with wide specificities may complement beta-glucosidase activity, e.g. galactosidases or alpha-glucosidases.

Chapter 2: Microassays for a set of enzymes in individual copepods

The sensitivity of the lipid assay was not high enough to analyse individual copepods, but required batches of 15 to 40 specimens. Crude extracts measured with serum lipid standards provided with the test kit probably overestimate total lipid values (Barnes and Blackstock 1973; Båmstedt 1975). However, Alonzo et al. (2000) showed that the amount of total lipids measured by the sulfosphovanillin reaction closely correlated with the fluorescence based measurement of neutral and polar lipids in *Paraeuchaeta antarctica*. Our analysis showed very low lipid values in all species ranging from 2.4 to 5.5% of dry mass (Table 2.3). Polar species, in contrast, may accumulate as much as 73% lipids (Båmstedt 1986). Apparently, none of the analysed copepods were capable of storing significant amounts of lipids, which was confirmed by low C:N-ratios (Table 2.3). These species are not exposed to extended periods of food limitation. They seem to be adapted to rapid utilization of alimentary lipids facilitated by high esterase/lipase activities.

In contrast to their lipid contents, all species were rich in protein, but showed considerable inter-specific variation. Water soluble protein contents ranged from 16% in *P. parvus* and *T. longicornis* up to 38% of dry mass in *C. anglicus* (Table 2.3). Total protein concentrations of 60% of dry mass were measured in *T. longicornis* in spring and summer in Norway (Evjemo et al. 2003), while 20% protein of dry mass were measured in cultured *T. longicornis* (Oosterhuis and Baars 1985). Individual differences in nutritional history may cause such intra-specific variations (Båmstedt 1988).

There is strong evidence that proteolytic activity in crustacean larvae decreases when carnivorous feeding increases during ontogeny (Le Vay et al. 2001). In contrast, herbivorous fish species showed highest ratios of amylase to protease activity, while the most carnivorous species had high proteolytic activities (Hidalgo et al. 1999). Johnston and Freeman (2005) showed that different species of crabs express complex suits of digestive enzymes and that the relative activities of enzymes indicate different species-specific dietary niches. Accordingly, it is important to evaluate a set of enzymes to better interpret physiological characteristics and to distinguish them from nutritional effects.

In conclusion, this work is an important step forward in understanding the physiological reactions and ecological functions of copepods within a complex and ever changing environment as it enables us to analyse important biochemical

Chapter 2: Microassays for a set of enzymes in individual copepods

parameters in individual animals. The range of fluorogenic substrates can be extended to identify further important enzymes in the copepods' digestive physiology. The next step now is to investigate, whether the patterns found here are inherent properties of the species, or are dependent on the feeding conditions at the time. If copepods do change their enzymatic tools throughout a season, we might be able to use enzyme activities to infer the feeding modes of the animals *in situ*.

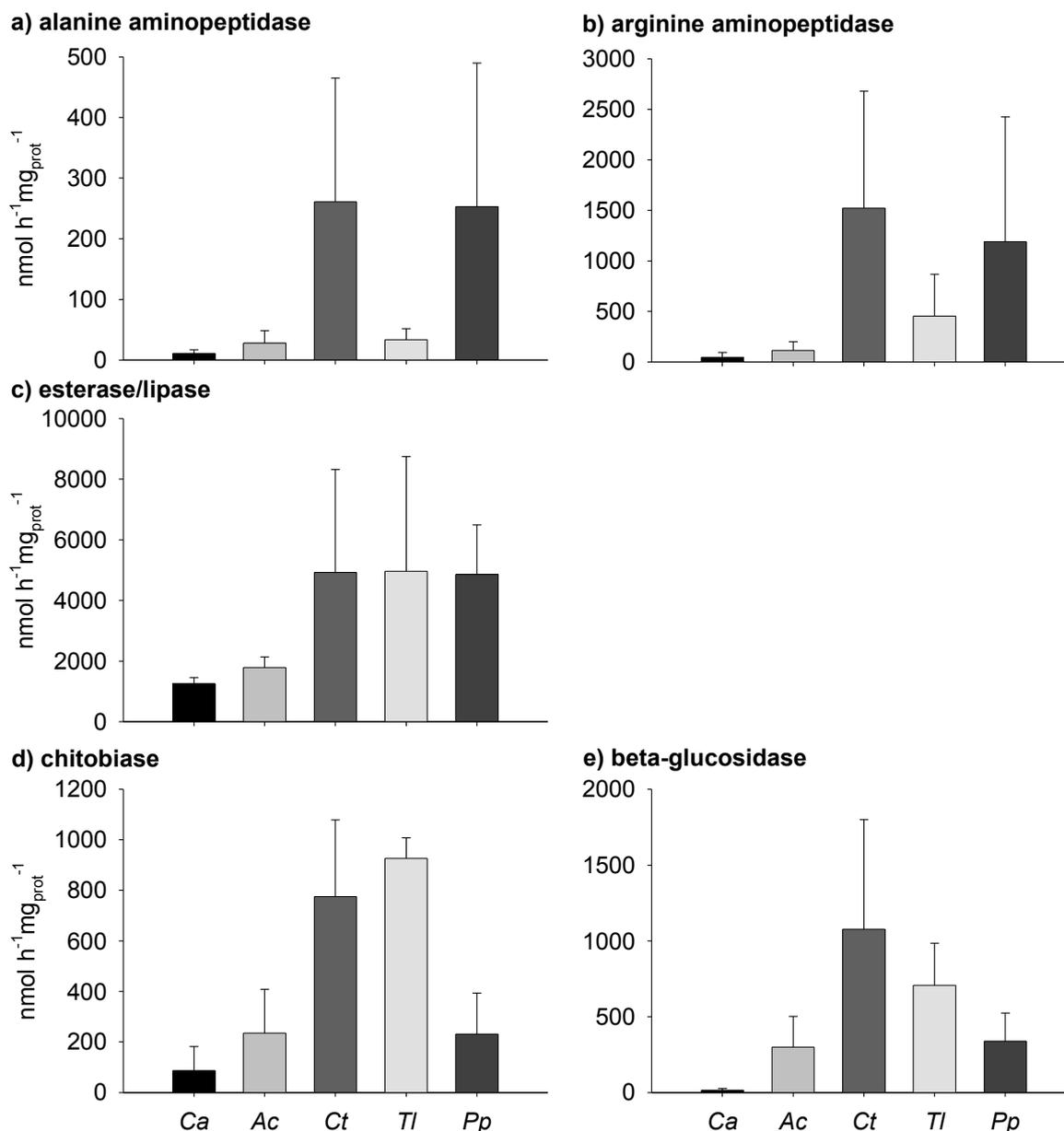


Fig. 2.3: Specific activities of enzymes ($n = 4-6$; means \pm SD). For abbreviations refer to Table 2.1.

Chapter 3: Sublethal physiological effects of food limitation in copepods

Most copepods from the North Sea do not accumulate significant amounts of lipids and, thus, are vulnerable to food limitation. The effects of short term starvation on digestive capabilities were studied in individuals of *Acartia clausi* and *Acartia tonsa*. In both species hunger caused a decrease in chitinase activity and an increase of alanine aminopeptidase activity. The water soluble protein content decreased. Activities of arginine aminopeptidase, beta glucosidase and esterase/lipase, however, remained unaffected. Similarly, the patterns of endopeptidase isozymes did not differ significantly between fed and starved individuals. Apparently, the expression of enzymes which perform extra-cellular digestion of food compounds, e.g. chitinase, is rapidly reduced while the expression of enzymes which potentially catalyse intracellular catabolic reactions remains unaffected. Proteases appeared to be an important group of enzymes in *Acartia clausi* and in *Acartia tonsa* showing several isozymes which were not affected by short term starvation. Comparison with other species (*Centropages typicus*, *Corycaeus anglicus*, *Paracalanus parvus*, *Temora longicornis*) showed that each species express a distinct species-specific pattern of proteases. The temporary utilization of endogenous compounds, particularly proteins, enables the copepods to survive short periods of food limitation.

3.1. Introduction

Copepods show fundamentally different life history traits in response to regional environmental conditions. Polar regions are characterised by a distinct seasonality in primary production. During the spring phytoplankton bloom food availability is sufficient while it is limiting during other seasons, particularly winter. Therefore, many polar and sub-polar copepod species spend the winter period diapausing or they metabolize stored lipids which they accumulated during the productive seasons. Their amount of storage lipids, e.g. wax esters may exceed 70% of the body dry mass (Lee et al. 2006). In contrast to *Calanus finmarchicus* and *Pseudocalanus elongatus*, the neritic copepods *Acartia clausi*, *Centropages hamatus* and *Temora longicornis* do not accumulate wax esters as storage products (Kattner et al. 1981). Only during the spring and late summer phytoplankton blooms the pool of storage lipids (triacylglycerides) and the amount of polyunsaturated fatty acids increase.

Chapter 3: Physiological effects of food limitation

The patterns of fatty acids change with seasons, implying that these copepods utilize different food sources throughout the year. An opportunistic nutritional strategy is supported by high activities of carbohydrases, esterases/lipases, and proteases which allow for rapid hydrolysis of alimentary lipids and proteins (chapter 2).

Fluctuations in food availability also appear in the otherwise highly productive coastal waters of the southern North Sea due to the prevalence of low quality food species. In winter when phytoplankton growth is low but nutrients are sufficient, high proportions of detritus may reduce food quality (Mayzaud et al. 1998). Accordingly, species which are physiologically not prepared to sustain periods of food limitation may severely suffer from starvation. *Acartia clausi*, *Centropages hamatus*, *C. typicus*, *Paracalanus parvus*, and *Temora longicornis* belong to the most frequent species of calanoid copepods dominating the zooplankton community in the southern North Sea (Krause 1995; Greve et al. 2004). *A. tonsa* is a brackish water species only abundant in estuaries around the North Sea and in the Baltic Sea. *Pseudocalanus elongates* is as abundant as the other small copepods. Similar to *Calanus helgolandicus* and *C. finmarchicus* which, however occur only in minor numbers in the southern North Sea, *P. elongates* is known to store wax esters. *Corycaeus anglicus* is a carnivorous poecilostomatoid species that is mainly abundant from summer throughout winter.

The sublethal physiological effects of starvation in copepods have not yet been sufficiently described. *Acartia clausi*, *A. tonsa*, *Centropages hamatus* but also *Calanus pacificus* exhibit a hunger response after starvation with increased respiration and feeding rates (Hassett and Landry 1988; Tiselius 1998; Thor 2003). Harris et al. (1986) proposed a compensatory mechanism for balancing between optimal foraging and assimilation as well as the costs of enzyme synthesis. Hassett and Landry (1990) agree with their model that low food concentrations may increase the digestive abilities of copepods to counterbalance threatening food limitation while enzyme activities may decrease at very high food concentrations to reduce the effort. However, Båmstedt (1988) pointed out that physiological responses to the environment can vary considerably even within a population.

Accordingly, it appears to be crucial that we demonstrate biochemical and physiological reactions on an individual basis. Studies on individual copepods, however, are rare due to the lack of appropriate and sensitive micro-analytical methods. The present work was aimed at studying the effects of short term starvation on the digestive potential of copepods. The specific activities of a set of important

Chapter 3: Physiological effects of food limitation

digestive enzymes were measured in individuals and the expression of proteinase isoforms was determined by zymograms (Guérin and Kerambrun 1982, García-Carreño et al. 1993).

3.2. Material and Methods

Laboratory experiments

A. clausi and *A. tonsa* cultures were maintained in filtered natural sea water (1 μm membrane filters) at $15 \pm 1^\circ\text{C}$ in a 16:8 hours light:dark cycle (culturing conditions). They were fed ad libitum with *Rhodomonas* sp. ($\sim 500 \mu\text{g C L}^{-1}$) grown in semi-continuous batch cultures in f/2-medium without silicate (Guillard and Ryther 1962; Guillard 1975).

Survival under different food regimes was determined in artificial seawater at a salinity of 30 (hw professional Meersalz, Wiegand GmbH, Krefeld, Germany). Ten to thirteen *A. clausi* specimens were incubated in each of nine 1 L-bottles for 4 days under culturing conditions. Water and food were exchanged every 24 h, copepods were counted, and dead copepods were removed from the bottles. Control groups were fed with the flagellate *Rhodomonas* sp. ($500 \mu\text{g C L}^{-1}$). For biochemical analyses 30 individuals of *A. tonsa* and *A. clausi* females were kept without food for 2 days. Control groups of 30 individuals of each species were fed ad libitum with *Rhodomonas* sp.

Digestive enzyme activities

Activities of chitobiase, beta-glucosidase, esterase/lipase, arginine- and alanine-peptidase were measured with fluorogenic substrates in McIlvaine buffer (McIlvaine 1921) at pH 5 (carbohydrases) and pH 7 (esterase/lipase and aminopeptidases). All enzyme activities were measured in the same extracts of each of 6 individuals per species and treatment (chapter 3). Enzyme activities were normalised to the average protein contents of 9-10 individuals of each species and treatment and were expressed as specific activities ($\text{nmol h}^{-1} \text{mg}_{\text{prot}}^{-1}$).

Water soluble protein content of individuals

Average water soluble protein contents of individual copepods were determined with a modified micro BCA-protein-assay (chapter 2) from Pierce Ltd. (no. 23231, 23232, 23234) (Smith et al. 1985).

Protease zymograms

Zooplankton was collected in autumn 2003 off Helgoland (German Bight, North Sea) with plankton nets of 280 and 150 μm mesh size. Females and males of the species *Acartia clausi*, *Centropages typicus*, *Corycaeus anglicus*, *Paracalanus parvus* and *Temora longicornis* were sorted out immediately after capture and maintained for 2 days at $15 \pm 1^\circ\text{C}$ and 16:8 hours light:dark cycle, and fed with a flagellate mixture of *Rhodomonas* sp., *Isochrysis* sp. and *Oxyrrhis marina* (Klein Breteler 1980). The flagellates were grown in semi-continuous batch cultures on f/2-medium without silicate (Guillard and Ryther 1962; Guillard 1975). Individuals of *A. tonsa* were obtained from cultures at the marine station that were kept under the same culturing conditions as *T. longicornis*. Specimens selected for biochemical analyses were briefly rinsed with deionised water, blotted dry and immediately deep frozen at -80°C . Individual copepods or pools of 10 animals were homogenised in 15 or 100 μL sample buffer (0.12 mol L^{-1} Tris/HCl buffer, pH 6.8) containing 30% glycerine and 4% sodium dodecyl sulphate (SDS) and traces of bromophenol blue. Samples were centrifuged (15000 g , 4°C , 10 min) and 5-10 μL of the supernatants were applied to mini-gels ($8 \times 10 \times 0.075 \text{ cm}$) for discontinuous SDS-PAGE (Laemmli 1970; Schagger and von Jagow 1987). The composition of the stacking gel was 4%T, 2.6%C (0.5 mol L^{-1} Tris/HCl buffer, pH 6.8), and the composition of the separation gel was 12.3%T, 2.6%C (1.5 mol L^{-1} Tris/HCl buffer, pH 8.8). Two gels with the same samples were run in parallel in a vertical electrophoresis unit (Hofer SE 250, 300 V, 15 mA per gel, 0°C)

Following the native SDS-PAGE gels were incubated in an icecold 3% casein solution (0.05 mol L^{-1} Tris/HCl, pH 8.0) for 30 min. Casein was allowed to penetrate into the gels for 30 min while being cooled in an ice water bath. Thereafter, temperature was raised to $\sim 25^\circ\text{C}$ and active proteases were allowed to digest casein in the gel for another 90 min (García-Carreño et al. 1993). Gels were rinsed thoroughly with deionised water and stained overnight (0.1% coomassie brilliant blue R-250 in 40% methanol and 10% acetic acid solution). The next morning gels were de-stained in a 40% methanol and 10% acetic acid solution.

Clear zones represented active endopeptidases due to casein digestion in an otherwise blue casein containing gel. Molecular weight markers (Sigma Low Range Marker (6.5-66 kDa) and a 10 kDa-step marker, Biomol) were applied to each gel.

Chapter 3: Physiological effects of food limitation

Gels were photographed (BioRad ChemiDoc™XRS camera system) and analysed using QuantityOne analysis software (BioRad).

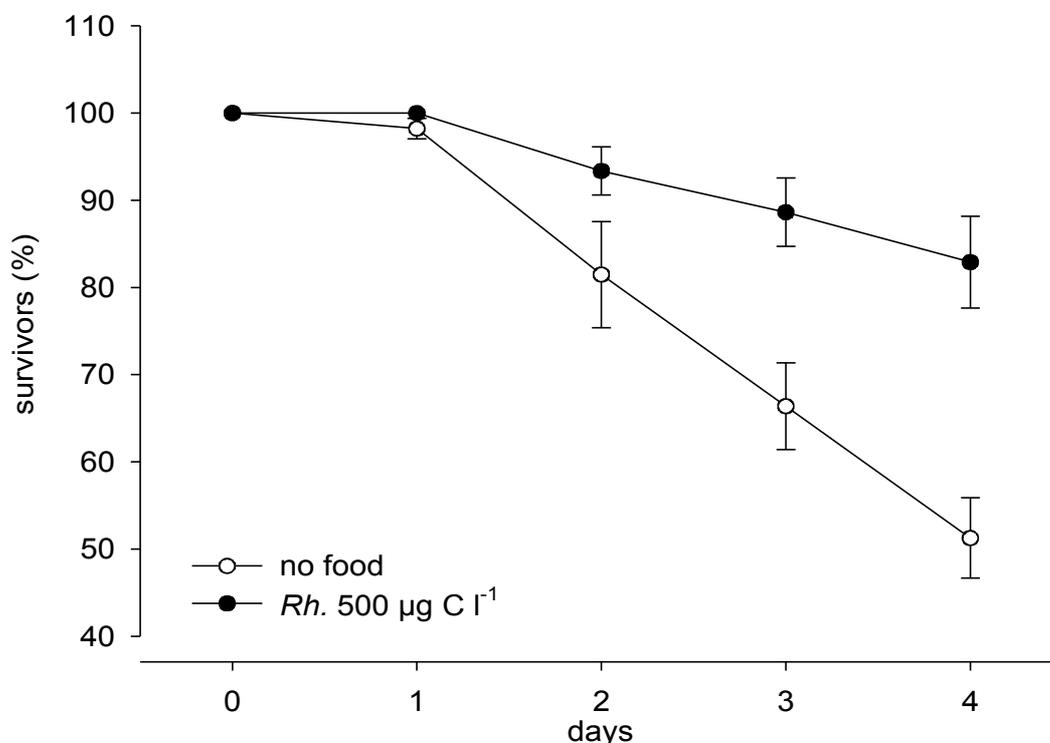


Fig. 3.1: *Acartia clausi*: percent survivors without food or fed with *Rhodomonas sp.* ($500 \mu\text{g C l}^{-1}$), (means \pm SE, N=9-10).

Statistics

Statistical analysis of data sets was carried out with the Statistica 6.1 software package (StatSoft, Inc., Tulsa, OK, USA). Homogeneously distributed data were tested for significant differences with an ANOVA and Least Significance Difference test or Student-t-test. Data that were not homogeneously distributed were tested for significant differences with the non-parametric Wald-Wolfowitz-Test and corrected for small sample sizes.

3.3. Results

Mortality and survival after starvation

After 24 hours, copepods died only in the treatments without food. From the second day on mortality occurred in both treatments (Fig. 3.1). For copepods without food the average daily mortality rate amounted to $19 \pm 2\%$ (means \pm SE) as compared to the $6 \pm 2\%$ rate in the *Rhodomonas sp.* control. Mortality rates and survival were significantly different between treatments on all four days (LSD-Test, $p \leq 0.05$).

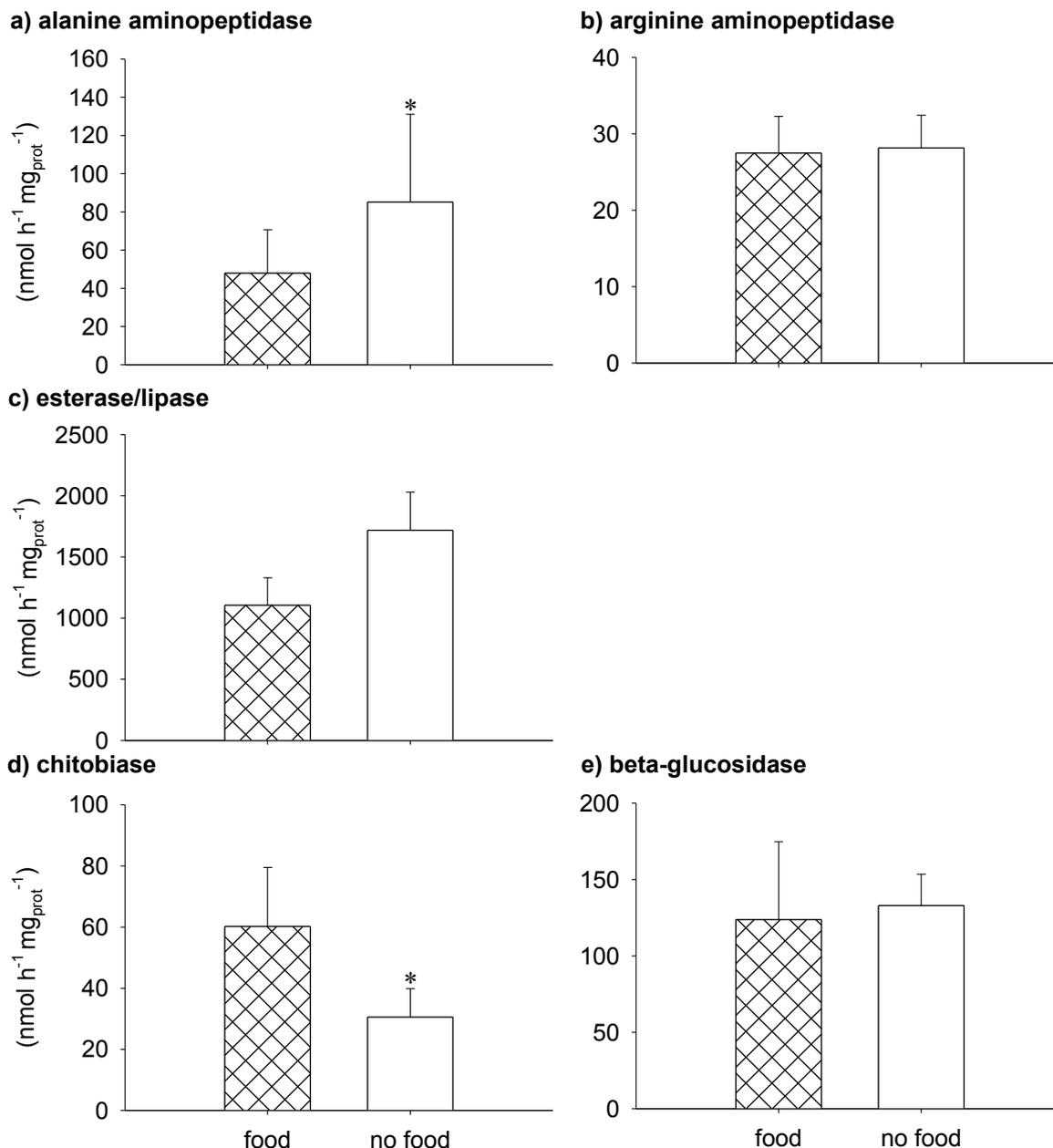


Fig. 3.2: *Acartia clausi*: specific digestive enzyme activities of fed and non-fed individuals; ($N=6 \pm SE$); *: treatments significantly different.

Hunger experiments – digestive enzyme activities and protein contents

Alanine aminopeptidase activity was significantly higher in starving than in fed *A. clausi* individuals ($p=0.034$) (Fig. 3.2a). No significant differences appeared in arginine aminopeptidase (Fig. 3.2b), esterase/lipase (Fig. 3.2c), and beta-glucosidase activity (Fig. 3.2e). Chitobiase activity was significantly lower in unfed than in fed individuals ($p=0.034$, Fig. 3.2d). In starving *A. tonsa* individuals only chitobiase activity was significantly lower than in fed individuals ($p=0.024$, Fig. 3.3d). A decrease of alanine aminopeptidase activity was caused by the no food treatment with only 89% probability ($p=0.11$, Fig. 3.3a). Activities of arginine

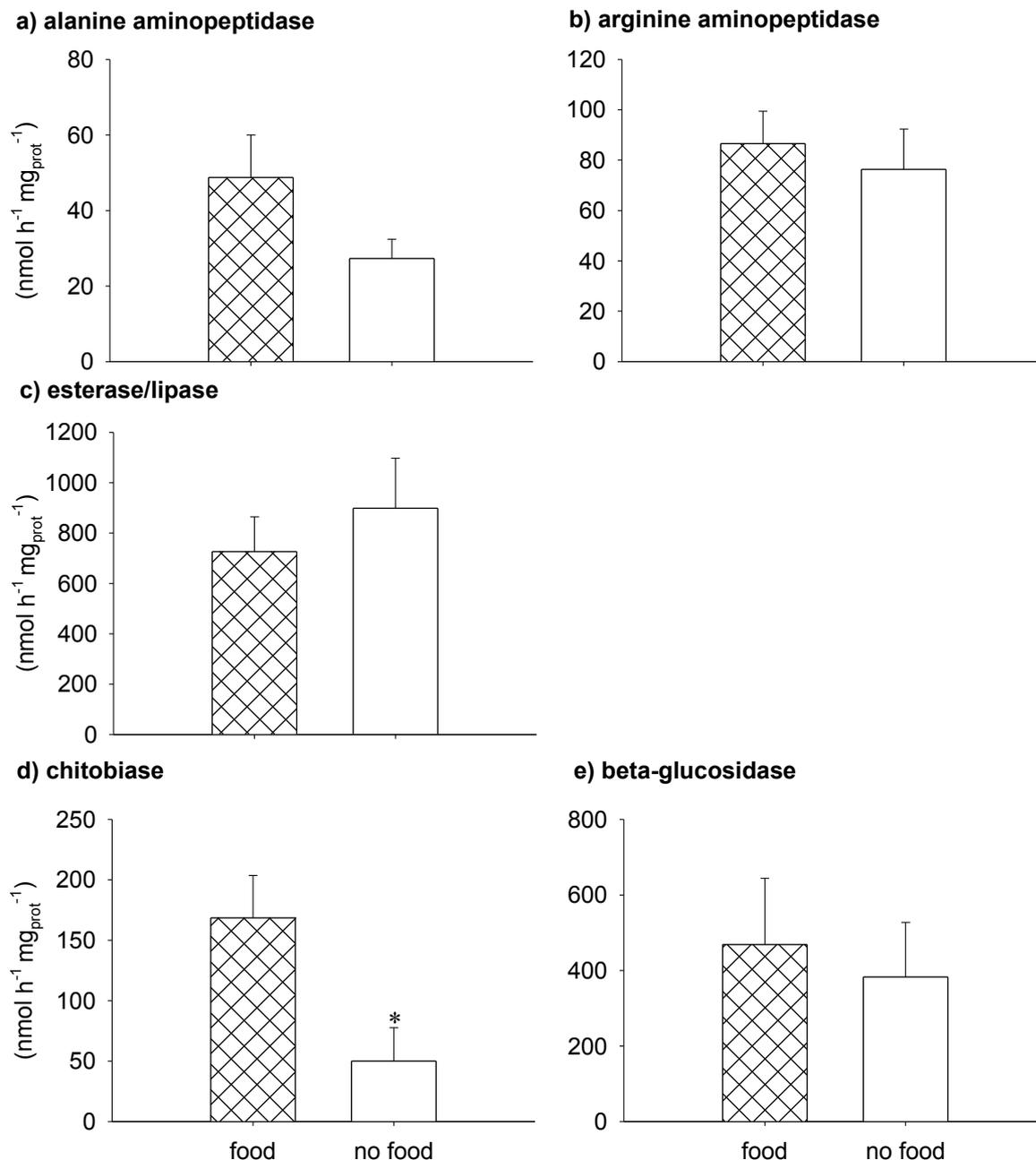


Fig. 3.3: *Acartia tonsa*: specific digestive enzyme activities of fed and non-fed individuals ($N=6 \pm SE$); *: treatments significantly different.

aminopeptidase (Fig. 3.3b), esterase/lipase (Fig. 3.3c) and beta-glucosidase (Fig. 3.3.e) did not differ significantly between treatments. Water soluble protein contents declined in starving *A. tonsa* ($p < 0.001$) while no significant difference appeared between fed and unfed *A. clausi* (Fig. 3.4).

Hunger experiments – protease zymograms

Protease zymograms of *A. tonsa* individuals did not show differences between hunger and control treatments (Fig. 3.5). However, individual variability was common, particularly in the expression of the 54 kDa band and to a lesser extent the

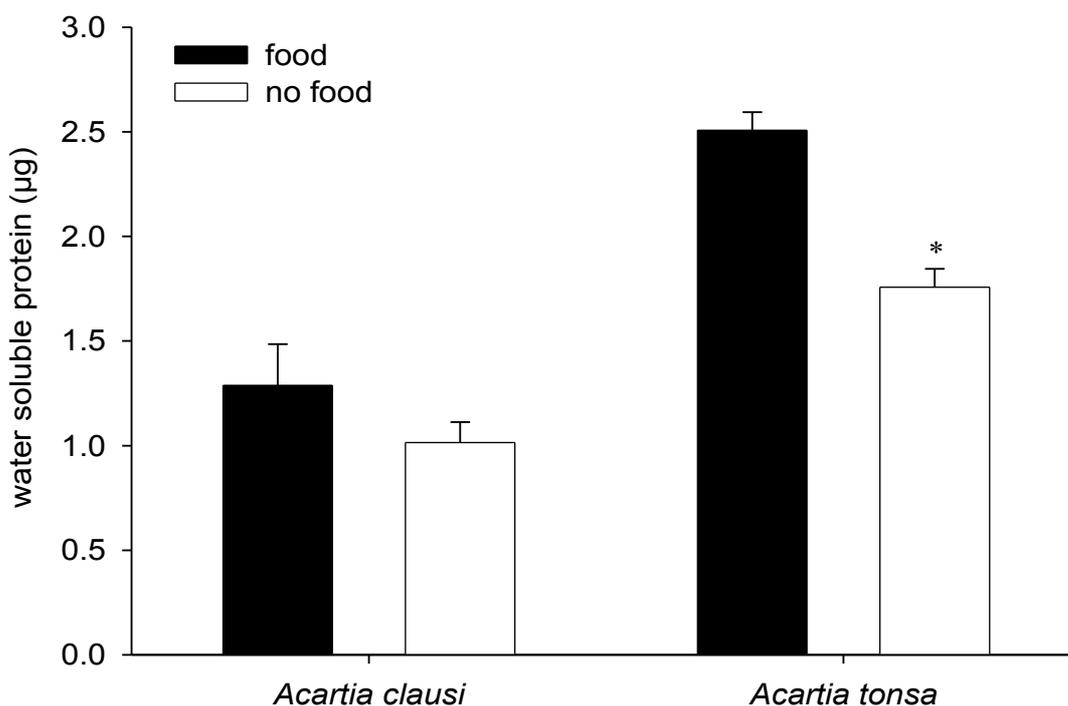


Fig. 3.4: Water soluble protein content of fed and non-fed *Acartia clausi* and *A. tonsa* specimens (means \pm SE, N=9-10); *: treatments significantly different.

expression of the 17.6 and 19.3 kDa enzyme bands. Activity bands in the two *A. clausi* gels were too weak to be photographed and visualized with QuantityOne Software. However, after trans-illumination on a light box visible details of the gels were identified and recorded manually. The 65.6 kDa band only occurred in 3 of 6 individuals in the control group. The 46.4 kDa band was present in all animals but appeared generally weaker in the hunger group. The 23.5 kDa band was present in all individuals, however, it was weaker in one of the starved individuals. The 20.1 kDa band was only visible in one individual of each group. The 15.5 kDa band was only weakly visible in 2 control and 3 hunger group individuals.

Species comparison – protease zymograms

The protease zymograms of 6 common copepod species revealed individual inter-specific patterns (Fig. 3.6a) with distinct activity bands (Fig. 3.6b). In most species several activity bands were present. The highest numbers of casein digesting proteases appeared in *A. clausi* (8 bands) and *T. longicornis* (7 bands). In *C. typicus* and *T. longicornis* some active proteins had not migrated into the gel (light band on top of the lanes, Fig. 3.6a). *A. tonsa* and *T. longicornis* zymograms of females and males showed the same pattern (Fig. 3.7). No sex-specific differences were present.

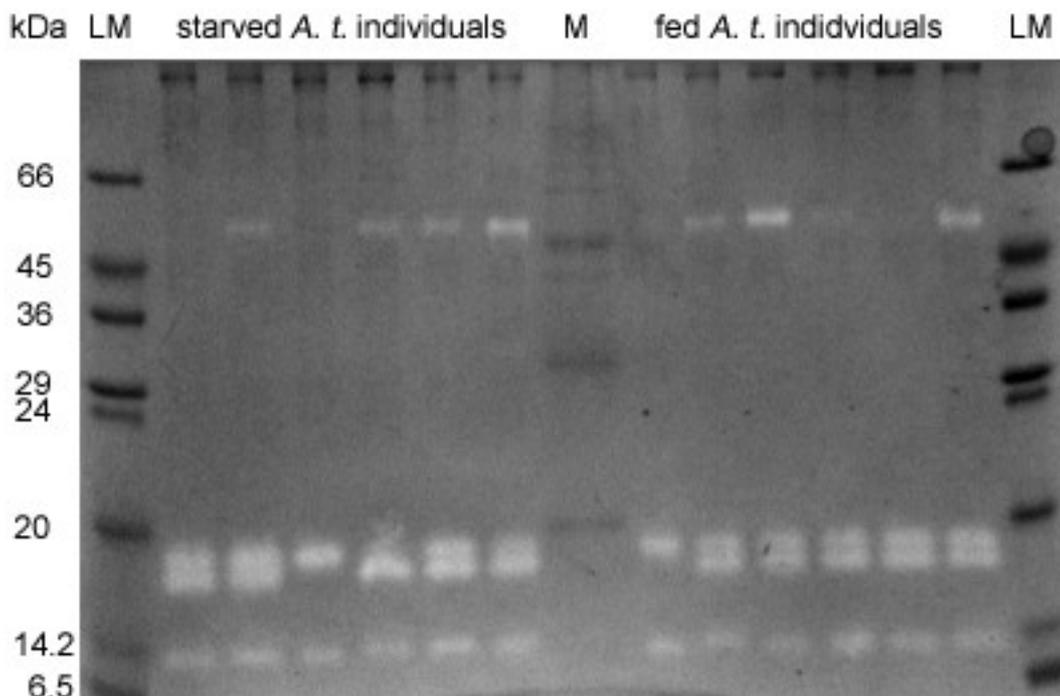


Fig. 3.5: Protease activity bands in 6 starved and 6 fed individuals of *Acartia tonsa* (*A. t.*); LM – Sigma low range marker, M – 10 kDa step marker starting with 20 kDa; left column: molecular weights of LM in kDa.

3.4. Discussion

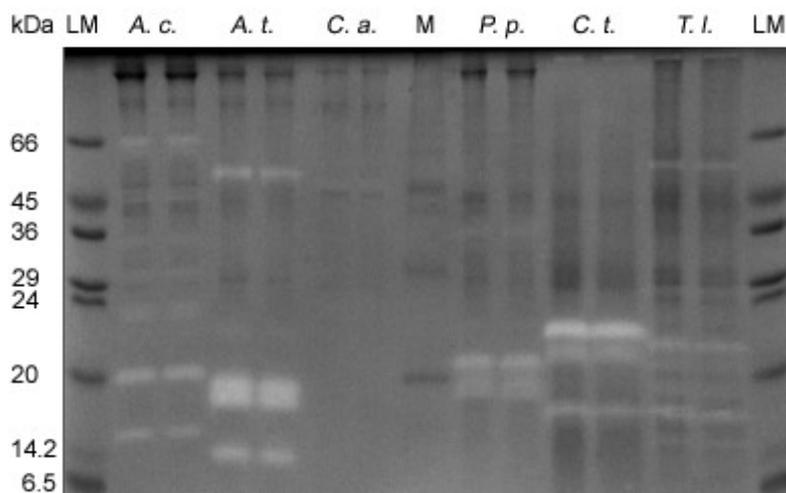
The reactions of copepods to their food environment strongly depend on their feeding history (Harris et al. 1986; Roche-Mayzaud et al. 1991; Mayzaud et al. 1992). When copepods encountered a period of rich food supply, the synthesis of enzymes would be enhanced and the abundance of enzyme synthesising B- and F-cells of the copepod midgut (Arnaud et al. 1980) would mask the present digestive processes and thus enzyme activities (Head et al. 1984). In order to eliminate past feeding effects, we fed the copepods for two days with the same plankton diet before the starvation period started.

Acartia species are more susceptible to starvation than lipid storing copepods such as *Calanus* species (Dagg 1977; Hassett and Landry 1990) or *Pseudocalanus elongatus* (Koski and Klein Breteler 2003). In our study, *Acartia clausi* already showed significantly impaired survival after two days. Therefore, an experimental period of two days was considered appropriate to study sublethal physiological effects due to short term starvation. Both *Acartia* species, *A. clausi* and *A. tonsa*, showed complex reactions in digestive enzyme activities. No significant variation appeared in the activities of arginine-aminopeptidase, esterase/lipase and beta-glucosidase while chitinase activity decreased in both species after two days. Different results between species were obtained for alanine-aminopeptidase of which

Chapter 3: Physiological effects of food limitation

activity increased in *A. clausi* but remained unchanged in *A. tonsa*. Apparently, both species do not react in the same way to short term starvation. A detailed interpretation, however, has to include the time scales of physiological reactions and the function of the analysed enzymes.

a)



b)

A.c.	A.t.	C.a.	P.p.	C.t.	T.l.
65.6					
	54.0				54.5
50.4		48.9			
46.4					
35.4			35.2		
27.8					27.6
23.5			22.5	22.3	21.8
20.1			20.5	21.1	20.6
	19.3		18.8		19.0
	17.6			17.0	17.1
15.5					15.0
	13.4				

Fig. 3.6: a) Protease activity bands of species (pools of 10 individuals): LM – Sigma low range marker, A. c. - *Acartia clausi*, A. t. - *Acartia tonsa*, C. a. - *Corycaeus anglicus*, M – 10 kDa step marker starting with 20 kDa, P. p. - *Paracalanus parvus*, C. t. – *Centropages typicus*, T. l. - *Temora longicornis*; left column: molecular weights of LM in kDa. b) Table with molecular weights of copepod proteases in kDa.

Time scales of reactions

Reactions of copepods to starvation and resulting changes in their enzyme activities have been studied in several species. Most distinct enzyme activity decreases appeared at the long term scale in different omnivorous *Calanus* species (Hirche 1996; Han et al. 2002), carnivorous cyclopoid species (Krylov et al. 1996) and harpacticoid copepods (Lonsdale et al. 1998) which undergo diapause during non-supportive environmental conditions. In order to save metabolic energy these species reduce the synthesis of metabolic superficial enzymes and increase the utilization of endogenous storage products such as lipids and proteins. The reduction of digestive enzyme activities coincides with reduced gut epithelia and, therefore, with depleted enzyme stores (Hallberg and Hirche 1980).

Reactions of copepod digestive enzymes to short-term starvation were less distinct and the available studies do not provide coherent results. No changes in enzyme activities were reported in *Calanus pacificus* and *Acartia tonsa* upon short-term starvation (Hassett and Landry 1988; Hassett and Blades-Eckelbarger 1995) nor did digestive enzyme activities follow diurnal feeding rhythms in *Temora longicornis* and *Centropages hamatus* (Head et al. 1984), or in *Pseudocalanus elongates* and

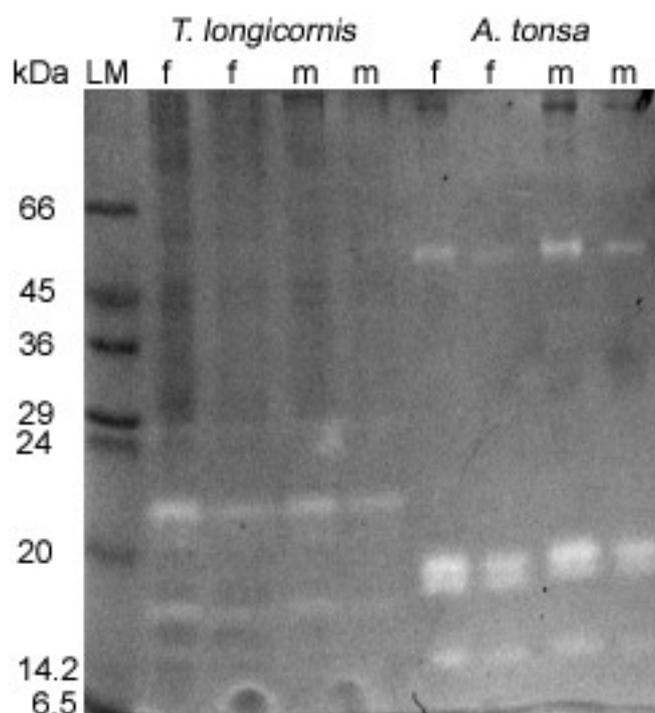


Fig. 3.7: Protease activity bands in *Temora longicornis* and *Acartia tonsa*; LM – Sigma low range marker, f – females, m – males; left column: molecular weights of LM in kDa.

Calanus helgolandicus (Baars and Oosterhuis 1984). In other studies with *Calanus finmarchicus* (Tande and Slagstad 1982) or *Anomalocera patersoni* (Kerambrun and Champalbert 1993), diurnal digestive enzyme patterns were apparent. A lack of enzyme activity variation may reflect a physiological insignificance to react to starvation, that is, these species may not face severe food limitation in their environment but are instead adapted to a patchy nutritional environment. Under low food concentrations, copepods increase their feeding range and therefore ingestion rate (Paffenhöfer and Lewis 1990) and they are able to stay within a food patch (Tiselius 1992).

Stable values or increases in enzyme activity during the first few days of starvation may indicate catabolic activity that mobilises body proteins and lipids to compensate for the food limitation. Another response to overcome food-shortage can be to intensify digestion by increasing substrate affinity of the enzyme (Mayzaud et al. 1998) or by prolonging the gut retention time as observed with diatom diet (Tirelli and Mayzaud 2005). These processes of adaptation to changing food environments depend as well on the considered zooplankton species as on food species and quality. *Acartia clausi*, *A. tonsa* and *Centropages hamatus* (Tiselius 1998) as well as *Calanus pacificus* (Hassett and Landry 1988) exhibit a hunger response after short-

Chapter 3: Physiological effects of food limitation

term starvation. Their ingestion rates increase beyond levels that are observed in the same food concentrations before starvation. The respiration rate is elevated in *A. tonsa* that are recovering from short-term starvation periods reflecting increased activity and metabolism (Thor 2003). Stable or elevated enzyme activity levels in the beginning of food limitation are a perfect match to a feeding behaviour that compensates for the experienced nutritional shortcomings during the search for a new food patch. Species-specific acclimation times to reduced food levels can be explained by different foraging strategies, habitat ranges and vertical migration patterns that avoid or are adapted to periodic food limitation (Tiselius 1998).

Instantaneous reduction of enzyme expression upon starvation was shown for trypsin in *Temora longicornis* (Kumlu 1997) and for 3 carbohydrases in *Calanus pacificus* (Hassett and Landry 1990). However, the dynamics in *Calanus pacificus* depended on the origin of the animals and their initial enzyme levels. Low initial enzyme levels were present in small specimens that partly comprised animals from the overwintering population; higher initial levels were measured in large animals of later spring generations. An enzyme activity decrease was only observed in the large animals. On a longer time scale all studies named above showed decreased enzyme activities in copepods upon starvation, decreases occurring later in lipid storing species. The drop in enzymatic expression was often followed by an increase in mortality. In these cases, the species apparently reduced synthesis of enzymes and saved metabolic energy to sustain longer starvation periods.

Digestive enzymes react faster to food quality changes than to changes in concentration (Mayzaud et al. 1998). Hence, the digestive system of copepods is a potential mediator for observed low quality food inhibition of growth and reproduction in copepods (Koski et al. 1998).

Role of enzymes

The interpretation of varying enzyme activities has to consider the detailed biochemical function of the studied enzyme. Enzymes which are released from gut cells to predominantly digest external food need to be distinguished from those enzymes which are also capable of hydrolysing internal storage products.

Chitinolytic enzymes are involved in digestion and in moulting. They express specific isoforms in the digestive tract and in the integument (Peters et al. 1999). Since adult copepods do not grow and moult, the presence of moulting chitobiases may be neglected. Accordingly, the observed decrease in chitobiase activity is most likely a

Chapter 3: Physiological effects of food limitation

reaction of the digestive enzyme to the lack of suitable substrates. Similar reactions were observed in euphausiids. Starvation caused a reduction of chitinolytic activity in the stomachs and in the midgut glands of Antarctic krill (Saborowski and Buchholz 1999). Chitinase pools are apparently quickly depleted and not needed for catabolism upon starvation.

Beta-glucosidase and lipase/esterase are enzymes that could participate in catabolism of body proteins and lipids. Beta-glucosidase hydrolyses terminal beta-D-glucose mainly from the mainly beta-linked structural polysaccharides and is also involved in metabolic processes. Lipases/esterases are degrading lipids. Copepods that do not store lipids put their energy uptake into reproduction as long as their basic metabolic needs are met. Triacylglycerols are directly transferred into egg production in copepods (Gatten et al. 1980).

Exopeptidases, such as alanine-aminopeptidase, may be important in the mobilisation of internal body stores. In starving zooplankton, internal proteins and lipids are degraded (Mayzaud 1976). The mode and rate of lipid and protein catabolism varies significantly between species and their ability to accumulate storage products. Elevated catabolism of endogenous proteins upon starvation was observed in *A. clausi* (Mayzaud 1976) and *T. longicornis* (Helland et al. 2003). Both species are not capable of storing significant lipid reserves and thus catabolise internal proteins upon starvation. Accordingly, activities of protein-hydrolysing enzymes have to remain high to supply metabolites. In particular, alanine represents an important metabolite which can enter and supply metabolic pathways in many positions.

Basic metabolism is maintained on reduced levels upon starvation (Thor 2003). However, egg production ceases more rapidly in non-lipid storing copepods than in lipid storing copepods (Dagg 1977). Quantitative and qualitative food limitation affects egg production even in lipid-storing copepods and the negative effect can prevail (Rey-Rassat et al. 2002b; Niehoff 2004). Such a negative effect is probably caused by high costs of gonad maturation (Niehoff 2000; Rey-Rassat et al. 2002a).

Zymograms

Proteins are a very important group of nutrients, thus proteolytic enzymes are essential in the digestive tract of most organisms. In crustaceans, endopeptidases appeared in various isoforms. The analysis of endopeptidases by zymography is a suitable tool to reveal differences between species, sexes, or treatments (Einsele

Chapter 3: Physiological effects of food limitation

1988, Schwenzen and Bulnheim 1991, Kerambrun and Champalbert 1975). The protease isozyme patterns of the two *Acartia* species were similar in fed and starved animals. These results are in agreement with the enzyme activity measurement, showing that the two copepod species do not visibly alter enzyme patterns. Apparently, the genetic variation of proteases was more pronounced than possible nutritional effects. Such an internal determination of isotrypsin patterns independent of potential external nutritional stimuli was described for whiteleg shrimp (*Litopennaeus vannamei*) (Sainz et al. 2005).

Species specificity

Inter-specific patterns of metabolic and digestive enzymes in copepods have been described (Riviere 1983) and used for the biochemical identification of species in the genera *Megacyclops* (Einsle 1988) and *Tisbe* (Schwenzen and Bulnheim 1991). The expression of different isoforms of digestive enzymes was identified as a factor that controls food preference in amphipods (Guarna and Borowsky 1993; Sotka 2003). A feedback mechanism between digestive system and food ingestion, mediated by chemoreception is suggested for copepods (Mayzaud et al. 1998). The endopeptidase zymograms of the copepods analysed for this study showed high species-specificity. Almost no overlap of protease bands was detected between species. Even closely related species like *A. clausi* and *A. tonsa* showed characteristic individual isozyme patterns.

Sexual differences

Similarities in the protease patterns between sexes as observed in *A. tonsa* and *T. longicornis* hint to a similar nutrition of males and females. This observation is in contrast to sexual dimorphic esterase zymograms in *Anomalocera patersoni* (Kerambrun and Champalbert 1975). Esterases participate in lipid degradation and, therefore, in the transfer of nutritional lipids into egg production (Kattner and Krause 1989).

Conclusion

In the south-eastern North Sea copepod species that do not store significant amounts of lipids are abundant. Their digestive physiology and feeding behaviour is optimally adapted to an environment where patchiness may appear but extended periods of starvation are rarely encountered. They are able to survive short starvation periods of only a few days. However, starvation has implications for population

Chapter 3: Physiological effects of food limitation

dynamics because it affects egg production. The energy that can be released from internal body nutrients is only sufficient for basic metabolism for a few days. The synthesis of enzymes that do not participate in catabolism decreases immediately. Some enzyme activities remain high during short-term starvation, probably so that the copepods can be prepared for compensatory feeding (hunger response) when the next food patch is foraged and/or to aid in metabolising body proteins and lipids to maintain basic metabolism.

Chapter 4: Influence of food quality and quantity on egg production of *Acartia clausi* and *Temora longicornis* during a spring phytoplankton bloom

Egg production of *Acartia clausi* and *Temora longicornis* was monitored off Helgoland (54°11N, 7°54E, North Sea, German Bight) from mid February until the end of May 2004. It was accompanied by a high-frequency sampling of abiotic and biotic environmental parameters, and the determination of size-fractionated grazing. The food quality parameters, seston C:N ratio, and particulate organic phosphorus (POP) content of total particulate phosphorus (TPP) explained more of the variance in carbon-specific and temperature normalized egg production than food quantity parameters. The quality of size classes changed seasonally, and the copepods preferred different size classes in different periods of spring. Similarities and differences in food gathering strategies of the two co-occurring copepod species are discussed.

4.1. Introduction

Copepods play a central role in marine food webs. Their population dynamics influence the constitution and development of primary producer communities, directly by grazing (Sommer 1988; Cyr and Curtis 1999), or indirectly through competition with other grazers, or by grazing on other herbivores (Kiørboe et al. 1996; Gasparini et al. 2000). Furthermore, their number and nutritional quality influence the reproduction and survival of secondary consumers such as fish (St. John et al. 2001; Malzahn 2006), or they act as transmitters of toxins such as paralytic shell fish poisoning (PSP) (Turner et al. 2000). As a consequence, it is of paramount importance to understand the factors that drive reproductive success in copepods. Many factors have been identified that influence reproductive success of copepods: temperature, salinity, and turbulence are the most important abiotic factors, whereas the nutritional environment, food quantity and quality are the main biotic drivers of reproductive success. However, it remains unclear, how these different factors interact.

Female size strongly effects egg production of *A. clausi* and *T. longicornis* (Halsband and Hirche 2001). Temperature and food concentration influence copepod size (Klein Breteler and Gonzales 1988) and gonad maturity, and therefore affect egg production (Niehoff 2003). Turbulence influences depth preference and prey encounter rate, and therefore grazing performance of copepods (Kiørboe and Saiz 1995; Visser et al.

Chapter 4: Food quality and egg production – spring bloom

2001). The evaluation of food quality effects is more complex. Nutrient limitation (John and Flynn 2000) and age (Estep et al. 1990) influence the biochemical composition of prey species. Biochemical deficient food can hamper reproductive success: highly-unsaturated fatty acids (Jónasdóttir et al. 1995), essential amino acids (Guisande et al. 2002; Arendt et al. 2005), and sterols (Klein Breteler et al. 1999; Hassett 2004) are important quality determinants. Potentially toxic food species decrease the effective food concentration, or when grazed upon, affect the reproductive success of copepods. Examples are: aldehyde-producing diatoms such as *Skeletonema costatum* and *Thalassiosira rotula* (Ivanora et al. 2004), dimethylsulfoniopropionate (DMSP) producing prymnesiophyte *Phaeocystis globosa* (Hansen 1995; Koski et al. 2005), and paralytic shell fish poisoning (PSP) producing *Alexandrium* sp. (Dutz 1998; Teegarden 1999).

Many copepods are known to feed omnivorously on phytoplankton, microzooplankton and on detritus (Kleppel 1993). However, copepods do not feed indiscriminately on all potential food particles. To increase feeding efficiency, they select for large size (Hansen et al. 1994) and high quality (Cowles et al. 1988; DeMott 1988; Koski and Klein Breteler 2003) of food particles. Grazing on cells that are of different nutritional quality but belong to the same food species can vary stronger than grazing on different species (Long and Hay 2006). Further, ingestion rates of copepods depend on their feeding history (Donaghay and Small 1979; Donaghay 1988).

In freshwater ecosystems the role of macronutrient ratios (C:N:P) has been researched extensively, as reviewed by Frost et al. (2005). Macronutrient ratios influence ecosystems, that is, autotrophs change their stoichiometry and concomitantly their biochemical composition depending on nutrient availability, while herbivores and higher level carnivores stay homeostatic (Sterner and Elser 2002). Therefore, nitrogen and/or phosphorus depletion lead to a potential imbalance between the homeostatic consumer and its food. This imbalance causes costs in the consumer that affect ontogenetic development, growth, and reproduction (Sterner and Schulz 1998; Boersma 2000). However, grazers can adjust their feeding behaviour and physiology to cope with nutritional imbalances: selective feeding (DeMott 1989; Acharya et al. 2004), compensatory ingestion (Plath and Boersma 2001), and adjusted digestion (Darchambeau 2005) can decrease negative effects of imbalanced food. Therefore, the implementation of stoichiometric food quality into models may greatly enhance model predictability (Mitra 2006).

Chapter 4: Food quality and egg production – spring bloom

We studied the influence of changing bulk seston macronutrient ratios on the reproduction of two dominant North Sea copepods, *Acartia clausi* and *Temora longicornis*. Concomitantly, size selective grazing on the natural seston particle assembly was measured for both species. The potential nutrient uptake of copepods was calculated for different particle size classes at three phases of spring 2004 (before phytoplankton increase, during increase, and at maximum).

4.2. Material and Methods

Sampling – animals and surface seawater

The copepods, *Acartia clausi* and *Temora longicornis*, were sampled twice weekly throughout a period from mid February until the end of May 2004 off Helgoland (54°11.3'N, 7°54.0'E, North Sea, German Bight). Adult females were selected and transferred to experimental vessels containing natural sea water at in situ temperature, at the same time other females were shortly rinsed with deionised water, blotted dry and frozen at -80 °C for biochemical and physiological analyses.

Surface seawater samples were taken simultaneously with zooplankton samples, pre-filtered (300 µm), and stored cold in opaque 10 L plastic containers until filtration or use in experiments within 4 hours. 250 mL were stored in brown bottles, fixed with Lugol's solution, and subsequently used to count particles. For each seston parameter (total particulate carbon (TPC), nitrogen (TPN), phosphorus (TPP), and particulate organic (POP) and inorganic phosphorus (PIP)) 3 to 6 parallels of 300-1200 mL were filtered onto pre-combusted (6 h, 420 °C) glass fibre filters (Whatman, GF/C, 25 mm diameter). Smaller animals were picked from the filters under a dissection microscope at 50x magnification after filtration. Filters were stored individually in reaction tubes at -80 °C.

Additional samples were taken three times during spring to investigate size-fractionated division of nutrients, grazing on the major size classes, and the enzymatic composition of the two copepod species under investigation. This was done within an early period of low food concentration (February and March = period a), a period when phytoplankton biomass was building-up (April = period b), and a period of highest phytoplankton biomass (May = period c). Four different size classes were created (< 10 µm, 10-40 µm, 40-100 µm, and 100-300 µm). The <10 µm fraction dominated at all times. Therefore, a subtractive sampling strategy would have masked results of less abundant size classes. Sea water samples were

Chapter 4: Food quality and egg production – spring bloom

fractionated by gently pouring the water through a set of sieves. The fractions $> 10 \mu\text{m}$ were resuspended in 450-500 mL filtered sea water (Whatman GF/C), gently mixed, and even volumes distributed between filters; the $< 10 \mu\text{m}$ fraction was filtered directly. Ten litre volumes per filter were necessary to yield enough material in the two larger size fractions, while 500-700 mL were sufficient for the $<10 \mu\text{m}$ fraction. The summed up fraction results gave total seston values that were comparable to values measured within 2 days earlier or later.

Abiotic environmental data

Sea surface temperature was determined during sampling. Wind and weather data for Helgoland were collected from Deutscher Wetterdienst (www.dwd.de), and lunar and tidal information from Bundesamt für Seeschifffahrt und Hydrographie (www.bsh.de) to check the data for biases caused by wind and tides.

Phytoplankton determination

Phytoplankton was counted by the Utermöhl method in 25 and 50 mL samples (Utermöhl 1931), and phytoplankton biomass and organic phytoplankton carbon were calculated from cell sizes (PhytoC) (Hillebrand et al. 1999). The percentage of phytoplankton carbon on total particulate carbon was determined (PhytoC percentage).

Dry mass, C, N and P determination

Total particulate carbon (TPC) and nitrogen (TPN) contents of freeze dried and heat dried (60°C , 12 h) seston filters and 5 to 50 copepods were measured with a CHN analyser (Fisons Instruments EA 1108). Acetanilid (Thermo Quest, 338 36700) served as standard. Molar C:N ratios were calculated. C and N were analysed in the same samples.

We differentiated between particulate organic phosphorus (POP) and particulate inorganic phosphorus (PIP) of the seston filters after (Aspila et al. 1976). Total particulate phosphorus (TPP) was determined by combusting the filters at 520°C for two hours, extraction in 6 mL 1N HCl for 18 hours and measuring dissolved inorganic phosphorus (DIP) in the neutralized extract after Grasshoff et al. (1983). PIP was determined by extracting the uncombusted filters for 18 hours in 6 mL 1N HCl and measuring DIP in the neutralized extract with an AutoAnalyser after Grasshoff et al. (1983). POP was calculated from the difference between average TPP and PIP. The percentage of POP on TPP was calculated (POP %).

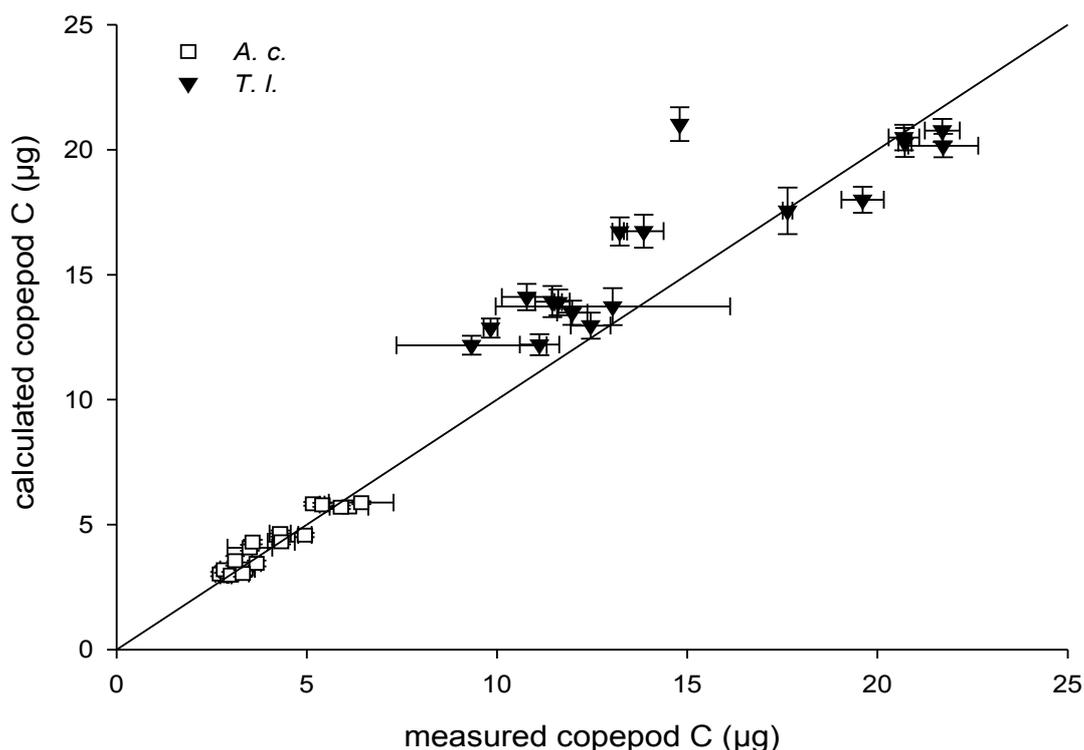


Fig. 4.1: Measured *A. clausi* and *T. longicornis* carbon values against calculated carbon (see methods), means \pm SE; line denoting 1:1 relationship.

Copepod egg production

Thirty females of each species were transferred into the wells of 6-well microplates. Each well contained 9-10 mL sieved seawater (55 μ m). Temperature and light conditions were adjusted to *in situ* conditions every day (WTB binder incubator). After 24 hours females were removed from the wells, and prosome lengths were determined under a dissection microscope using a video analysis system (analysis, Soft Imaging System). Individually based egg production of both species was correlated to temperature (*A. c.*: $r^2=0.39$, $p=0.002$; *T. l.*: $r^2=0.60$, $p=0.00003$) and animal carbon content (*A. c.*: $r^2=0.62$, $p=0.00001$; *T. l.*: $r^2=0.45$, $p=0.0006$). To detect correlations of egg production with nutritional factors, individual egg production was standardised to a temperature of 10°C with a $Q_{10}=3$ (Kjørboe and Sabatini 1995) and normalised to calculated carbon contents of the animals. Therefore, when egg production is mentioned in the following, temperature standardised (10 °C) carbon specific egg production is meant.

Carbon contents were calculated from the linear regression of measured carbon contents with average prosome lengths of all available Helgoland Roads data (*T. longicornis*: $\mu\text{g C}=0.0470*\text{length}-31.71$, $r=0.81$, $r^2=0.66$, $p<0.000001$, $N=118$ measured between 10/02/2004 and 24/05/2005; *A. clausi*:

Chapter 4: Food quality and egg production – spring bloom

$\mu\text{g C} = 0.01184 * \text{length} - 7.06$, $r = 0.93$, $r^2 = 0.87$, $p < 0.000001$, $N = 42$ measured between 10/02/04 and 23/07/04). Calculated carbon values correspond well with the fewer actually measured carbon values (Fig. 4.1). *Phaeocystis globosa* dominated the phytoplankton community in May (44 % of phytoplankton carbon). As this alga is a notoriously bad food for many copepods (Dutz et al. 2005), and we were mainly interested in more subtle effects of the food, we excluded the May data from the correlation analyses of biochemical nutritional factors with egg production. Indeed egg production during May (displayed by open symbols in Fig. 4.2) was low.

Copepod grazing selectivity

Seawater (300 μm pre-filtered) was gently siphoned into all experimental bottles. Initial prey field was determined in 3 sub-samples. Grazing experiments were conducted in three parallels in 1 L bottles with 7 *T. longicornis*, and 11 *A. clausi* females per bottle. Changes in the number of potential food organisms independent of copepod grazing were recorded in three controls without copepods. Experimental and control bottles were incubated for 24 hours under *in situ* temperature and light conditions (adjusted daily) and rolled overhead gently every hour during daytime; from 10 pm to 9 am bottles were not moved. Grazing experiments were started between 3 and 5 pm. Particle concentrations in size classes (3-5, 5-10, 10-20, 20-40 μm) were determined with a Casy particle counter (Schärfe System GmbH, Reutlingen, Germany).

Size class specific and carbon specific gross grazing (GFR_C) per day was calculated using the arithmetic mean of particles in control (N_{control}) and grazing bottles (N_{grazing}) after 24 hours available per copepod carbon (C_{ind}) for both copepod species because

control growth was negligible (Vanderploeg et al. 1984):
$$GFR_C = \frac{(N_{\text{control}} - N_{\text{grazing}})}{(24\text{h} * N_{\text{ind}} * C_{\text{ind}})}$$
 .

Negative ingestion values were set as zero. Particles larger than 40 μm were very rare and not considered. Medium food concentration available per μg copepod carbon was divided by the number of copepods in the grazing bottles (N_{ind}) and their average carbon content (C_{ind}).

Size selective grazing is displayed by plotting the percentage of the size class in the ingested food against the percentage of the same size class in the available food and

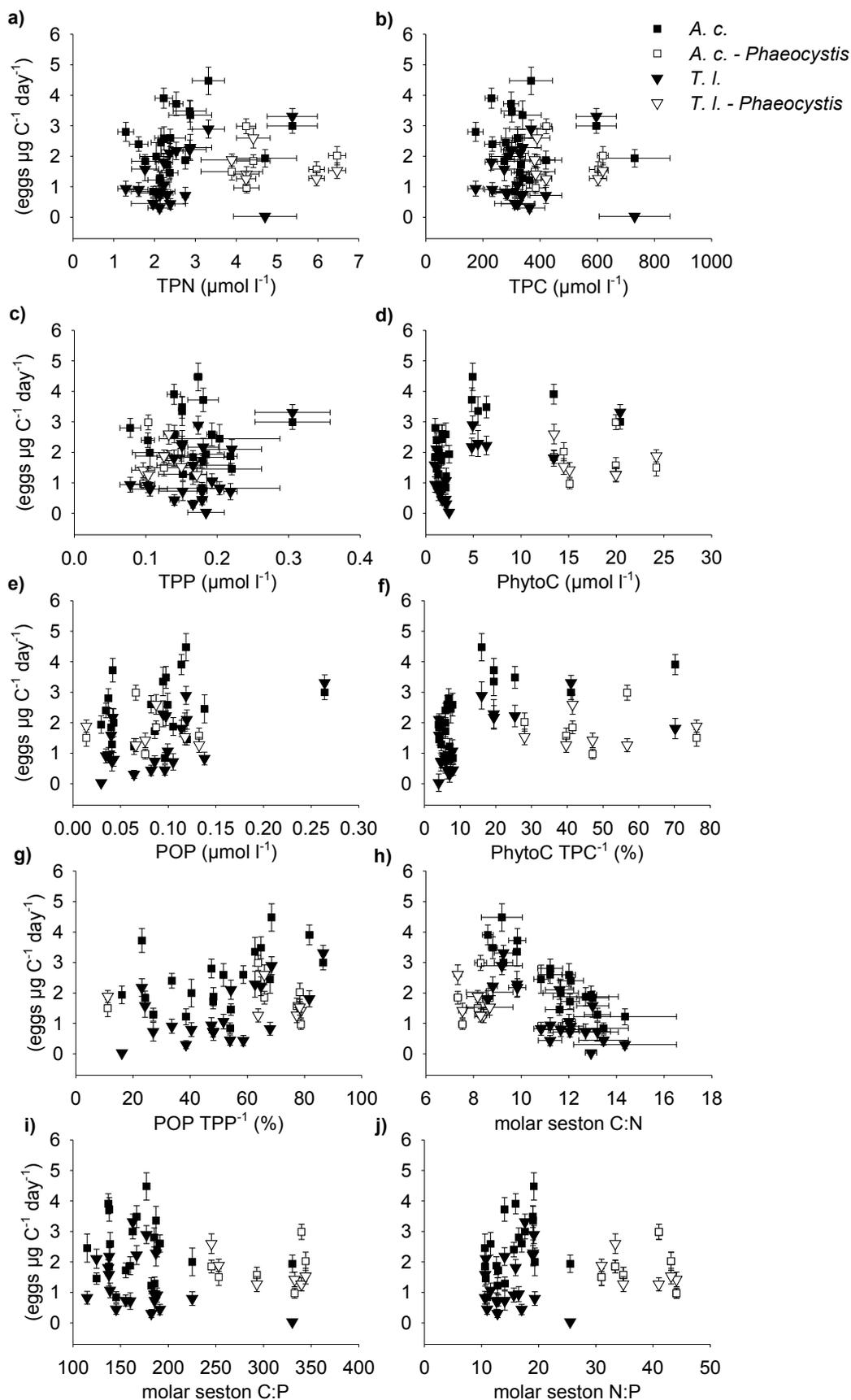


Fig. 4.2: Standardised egg production (means \pm SE) versus food quantity a) TPN, b) TPC, c) TPP, and quality d) PhytoC, e) POP, f) percentage of PhytoC on TPC, g) percentage of POP on TPP, h) molar seston C:N, i) molar C:P, j) molar N:P; open symbols represent data obtained in May when *Phaeocystis* sp. was dominant.

Chapter 4: Food quality and egg production – spring bloom

denoting non-selective grazing by a line with slope 1 (Fig. 4.6). Values above this line hint to positive selection while values below imply selection against the size class.

We assumed that the fractionated measurements of seston resemble approximately the values of the periods before and after the actual measurements. Therefore, the average mineral concentrations of particles in the size classes $< 10 \mu\text{m}$ and $10\text{-}40 \mu\text{m}$ were calculated for the 3 according periods with these approximated values. Specific ingestion of nutrients in these size classes was determined with the help of these estimated average values for both copepod species. Grazing on seston TPC was based on copepod carbon content. With help of the average measured spring C:N ratios of *A. clausi* (4.39 ± 0.06) (means \pm SE, N=22) and *T. longicornis* (4.78 ± 0.06) (means \pm SE, N=20) and a literature value of N:P = 20:1 (Walve and Larsson 1999; Van Nieuwerburgh et al. 2004) body contents of N and P were calculated from copepod C. With these ratios body mineral specific grazing was calculated for C, N, P and organic P. The proportion of ingested element on available element (both based on body element content) was calculated. From the elemental uptake the stoichiometric ratio uptake was calculated, based on the copepod ratios ($C:N_{\text{ing}}/C:N_{\text{body}}$).

Statistics

Statistical analyses were performed with the Statistica 6.1 software package (StatSoft Inc., Tulsa, OK, USA) and non-linear correlation analyses with the built in curve-fitting tool of SigmaPlot 8.0 (Systat Software Inc., Richmond, CA, USA). Homogeneously distributed data were tested for significant differences with an ANOVA and Least Significance Difference Test. Data that were not homogeneously distributed were tested for significant differences with the non-parametric Wald-Wolfowitz-Test and corrected for small sample sizes.

4.3. Results

Abiotic factors

The lowest temperature ($3.0 \text{ }^{\circ}\text{C}$) during the survey period was recorded in early March. Afterwards, temperature rose constantly up to $10.8 \text{ }^{\circ}\text{C}$ at the end of May. An average salinity of 32.2 ± 1.0 was recorded. Freshwater inflows in April and May coincided with the highest recorded phytoplankton biomasses. Moon phase or tides were not correlated to nutritional parameters or to egg production. But curiously *A. clausi* standardised egg production showed a weak correlation to the moon phase (

Chapter 4: Food quality and egg production – spring bloom

$r^2=0.19$, $p=0.05$, including May data) potentially indicating increased spawning activity around full moon.

Table 4.1: Food Quantity in size fractions ($\mu\text{mol l}^{-1}$) and fraction percentages on the total particulate material $< 300 \mu\text{m}$ ($N=3-8$; means \pm SD).

Fraction Date	TPC $\mu\text{mol L}^{-1}$	SD	%	TPN $\mu\text{mol L}^{-1}$	SD	%	TPP $\mu\text{mol L}^{-1}$	SD	%
<10 μm									
10/3/04	33.7	± 4.0	77.9	2.96	± 0.32	79.4	0.1621	± 0.0026	84.2
14/4/04	21.9	± 1.4	71.2	2.19	± 0.11	67.2	0.1220	± 0.0002	70.0
19/5/04	37.4	± 2.4	72.4	5.04	± 0.29	77.9	0.1074	± 0.0044	71.6
10-40 μm									
10/3/04	6.9	± 1.6	15.9	0.55	± 0.12	14.7	0.0210	± 0.0017	10.9
14/4/04	4.5	± 0.4	14.6	0.51	± 0.03	15.8	0.0173	± 0.0064	9.9
19/5/04	4.6	± 0.1	8.9	0.53	± 0.02	8.2	0.0083	± 0.0072	5.6
40-100 μm									
10/3/04	2.2	± 0.4	5.0	0.17	± 0.03	4.5	0.0089	± 0.0011	4.6
14/4/04	2.5	± 0.2	8.0	0.29	± 0.02	9.0	0.0175	± 0.0019	10.0
19/5/04	6.0	± 0.2	11.6	0.59	± 0.03	9.1	0.0253	± 0.0037	16.9
100-300 μm									
10/3/04	0.6	± 0.2	1.3	0.05	± 0.02	1.4	0.0006	± 0.0005	0.3
14/4/04	1.9	± 0.2	6.2	0.26	± 0.03	7.9	0.0176	± 0.0057	10.1
19/5/04	3.6	± 0.2	7.0	0.31	± 0.02	4.8	0.0088	± 0.0011	5.9

Prey community structure influencing egg production

Diatom numbers began to develop at the beginning of April and reached maximum densities by the end of April and in mid May. Standardised egg production of *T. longicornis* was linearly correlated to diatom concentrations (centrales: $r^2=0.40$, $p=0.007$; pennales: $r^2=0.35$, $p=0.01$). However, the correlations were of a saturation type exponential function similar as the function displayed by the PhytoC proportion on TPC concentration during spring as correlated with egg production (Fig. 4.3a and d). *A. clausi* standardised egg production was not correlated to diatom concentrations but copepod carbon content was (centrales: $r^2=0.24$, $p=0.03$; pennales: $r^2=0.24$, $p=0.05$). Ciliates peaked in mid February, in early March and in mid April. No significant correlations of ciliate concentrations with standardised egg production or copepod carbon content were found. During the whole period flagellates smaller than $15 \mu\text{m}$ were abundant, the smaller ones were more abundant in numbers than the larger ones. The diatom *Thalassionema nitzschoides* was dominant until the end of April. Besides this species the diatom *Paralia sulcata* played a major role in early spring until the end of March. In mid April other diatoms (*Chatonella sp.*, *Guinardia delicatula*, *Odontella aurita*, *Thalassiosira decipiens* and

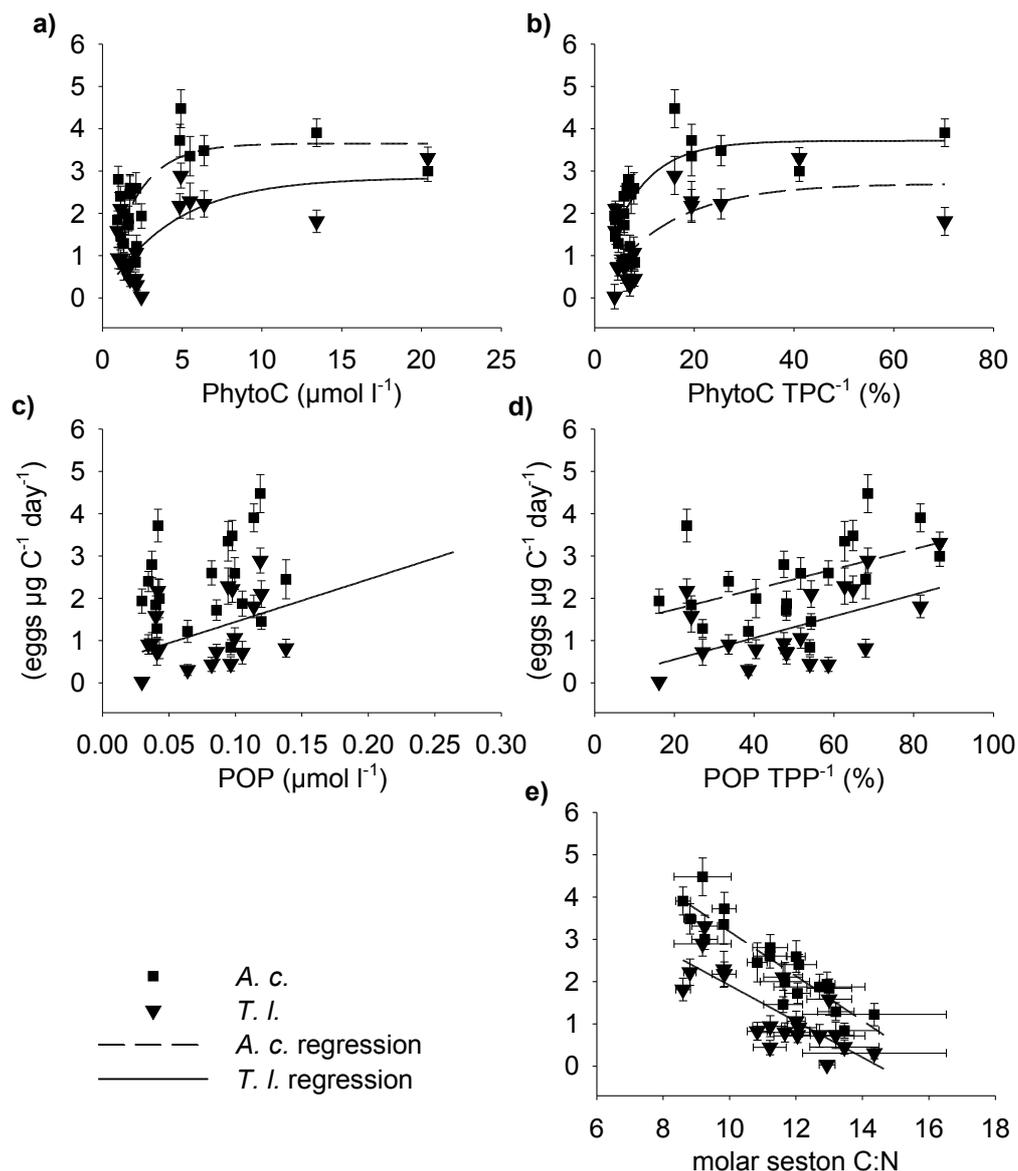


Fig. 4.3: Regression plots: significant correlations of egg production with nutritional parameters as in Table 4.1 (only May data); in a) + b) regression function $f(x) = a \cdot (1 - e^{-bx})$, otherwise linear regression.

T. nordenskjöldii) proliferated. During May, the haptophycean *Phaeocystis globosa* dominated with maximum numbers of 13-14 million cells L^{-1} from 10th to 21st May (with a dent of 2.8 million cells L^{-1} on the 13th May).

Stoichiometric food quantity and quality started to change considerably in the first week of April (Table 4.1, Table 4.2 and Fig. 4.4). The proportion of PhytoC on TPC (PhytoC %) increased from about 6 to 40%, the POP proportion of TPP (POP %) from about 40 to 80% (Fig. 4.4a). An increase in POP concentration and a decrease in PIP concentration interacted to lead to a higher POP proportion on TPP (Table 4.1).

Chapter 4: Food quality and egg production – spring bloom

Molar seston C:N improved from a poor quality 13:1 ratio to a higher quality 8:1 ratio (Fig. 4.4b). This was mainly caused by the over-proportional increase in TPN as compared to TPC. The ratios of seston C:P and seston N:P were lowest (= best quality) in early and late April but revealed an increasing imbalance between phosphorus and the other elements in May, exceeding any values determined before (Fig. 4.4c).

Table 4.2: Seston elemental contents during spring; data of size fractioned filters in italics.

Date	TPC ($\mu\text{mol L}^{-1}$)	TPN ($\mu\text{mol L}^{-1}$)	TPP ($\mu\text{mol L}^{-1}$)	POP ($\mu\text{mol L}^{-1}$)	PIP ($\mu\text{mol L}^{-1}$)
16/02/04	22.52 ± 1.66	2.01 ± 0.09	0.111 ± 0.007	0.047	0.0646 ± 0.0071
19/02/04	33.70	2.55 ± 0.21	0.182 ± 0.006	0.069	0.1138 ± 0.0033
23/02/04	30.25 ± 4.46	2.12 ± 0.28	0.166 ± 0.002	0.064	0.1020 ± 0.0135
26/02/04	25.99 ± 5.88	1.96 ± 0.54	0.179 ± 0.007	0.096	0.0824 ± 0.0189
01/03/04	28.15 ± 1.76	2.13 ± 0.08	0.152 ± 0.033	0.041	0.1105 ± 0.0026
04/03/04	35.01 ± 4.63	2.75 ± 0.08	0.219 ± 0.009	0.105	0.1134 ± 0.0021
08/03/04	23.83 ± 5.22	2.04 ± 0.41	0.106 ± 0.025	0.043	0.0631 ± 0.0084
10/03/04	43.34 ± 6.21	3.73 ± 0.49	0.193 ± 0.006	0.082	0.1106 ± 0.0078
11/03/04	26.76 ± 1.54	2.23 ± 0.14	0.193 ± 0.007	0.100	0.0931 ± 0.0051
15/03/04	19.39 ± 2.20	1.61 ± 0.23	0.103 ± 0.009	0.035	0.0683 ± 0.0310
18/03/04	14.52 ± 2.25	1.29 ± 0.19	0.078 ± 0.015	0.037	0.0412 ± 0.0029
22/03/04	60.88 ± 10.3	4.70 ± 0.77	0.184 ± 0.026	0.030	0.1546 ± 0.0601
25/03/04	27.81 ± 0.88	2.31 ± 0.06	0.179 ± 0.007	0.086	0.0931 ± 0.0026
29/03/04	26.79 ± 4.81	2.38 ± 0.38	0.140 ± 0.006	0.082	0.0580 ± 0.0009
01/04/04	22.88 ± 3.97	1.77 ± 0.33	0.166 ± 0.051	0.040	0.1259 ± 0.0007
05/04/04	27.48 ± 2.47	2.37 ± 0.20	0.220 ± 0.042	0.120	0.1008 ± 0.0226
08/04/04	23.33 ± 2.93	2.15 ± 0.24	0.203 ± 0.084	0.138	0.0652 ± 0.0027
13/04/04	28.25 ± 5.44	2.87 ± 0.52	0.151 ± 0.004	0.095	0.0563 ± 0.0063
14/04/04	30.71 ± 2.16	3.26 ± 0.18	0.174 ± 0.014	0.113	0.0615 ± 0.0009
15/04/04	30.69 ± 6.26	3.32 ± 0.40	0.173 ± 0.006	0.119	0.0546 ± 0.0009
19/04/04	25.14 ± 3.23	2.86 ± 0.41	0.151 ± 0.004	0.098	0.0531 ± 0.0001
22/04/04	24.89 ± 2.17	2.53 ± 0.16	0.180 ± 0.021	0.042	0.1386 ± 0.0099
26/04/04	19.12 ± 1.82	2.22 ± 0.22	0.139 ± 0.009	0.114	0.0255 ± 0.0115
29/04/04	49.72 ± 5.85	5.37 ± 0.62	0.305 ± 0.053	0.264	0.0411 ± 0.0024
03/05/04	32.47 ± 3.68	4.42 ± 0.41	0.132 ± 0.007	0.087	0.0450 ± 0.0155
06/05/04	32.07 ± 2.78	4.25 ± 0.31	0.096 ± 0.016	0.076	0.0205 ± 0.0010
10/05/04	50.25 ± 2.51	5.97 ± 0.20	0.171 ± 0.014	0.132	0.0389 ± 0.0177
13/05/04	31.75 ± 5.97	3.89 ± 0.76	0.125 ± 0.040	0.014	0.1115 ± 0.0103
17/05/04	35.15 ± 1.58	4.24 ± 0.23	0.103 ± 0.003	0.066	0.0375 ± 0.0189
19/05/04	51.60 ± 2.87	6.47 ± 0.35	0.150 ± 0.016	0.1175	0.0366 ± 0.0017
24/05/04	50.94 ± 2.95	5.83 ± 0.31	0.223 ± 0.015	0.149	0.0740 ± 0.0034
27/05/04	27.07 ± 3.72	3.27 ± 0.43	0.126 ± 0.044	0.082	0.0439 ± 0.0278

Food quantity (Table 4.1) and quality (Fig. 4.5) varied in the size classes. The < 10 μm fraction comprised 67 to 84% of the total quantity independent of measured parameter and without great seasonal variability. All parameters in the 10-40 μm size

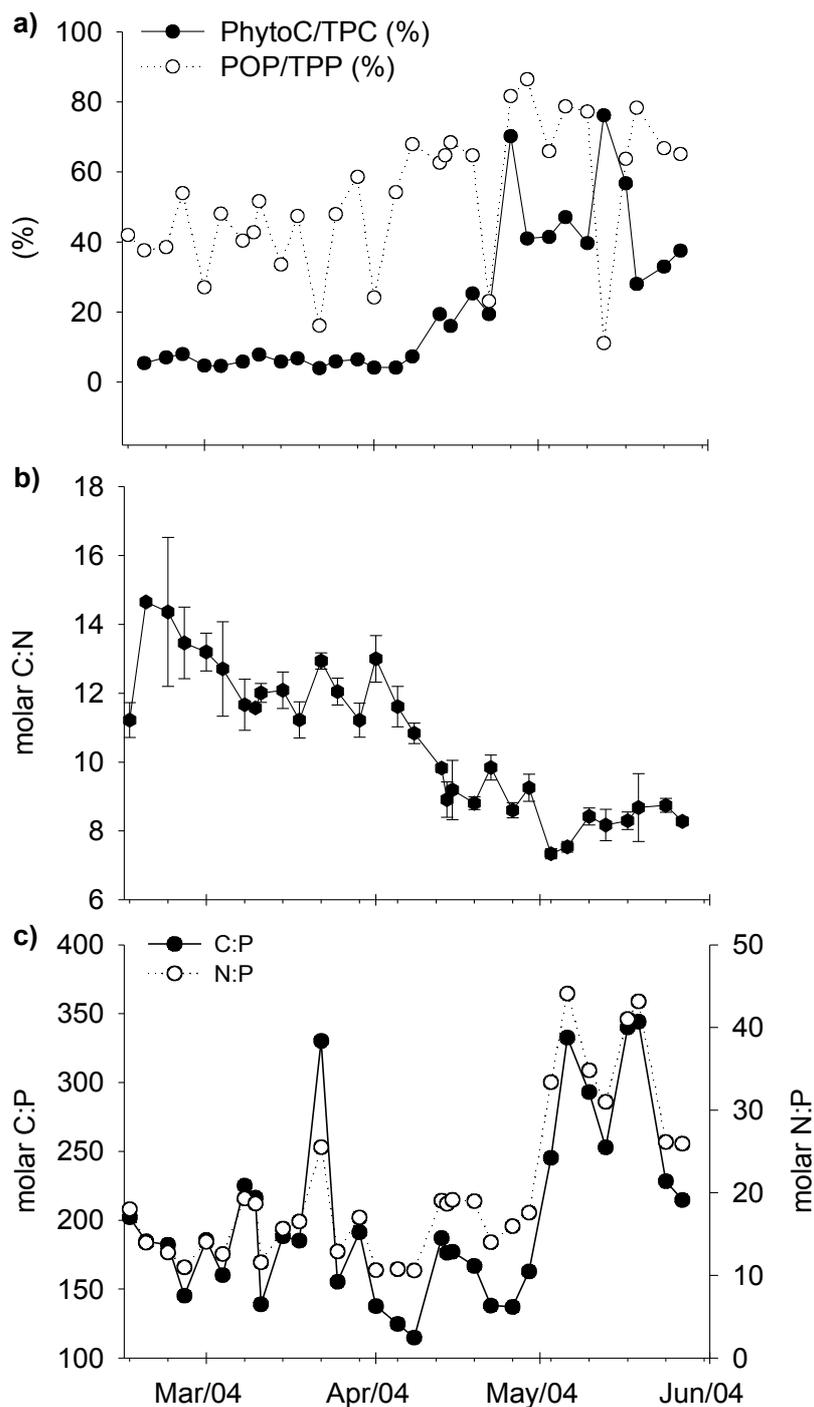


Fig. 4.4: Development of quality parameters during spring: a) percentage of POP TPP⁻¹ and of PhytoC TPC⁻¹ (calculated from means), b) particulate molar C:N (N=3-6; mean ± SD), c) particulate molar C:P and N:P (calculated from means); note different scales.

class decreased between March and May. Oppositely, in the 40-100 µm fraction all parameters increased within this period. TPC and TPN about doubled, TPP even quadrupled. The strongest seasonal changes were observed in the largest fraction (100-300 µm). For TPN and TPP highest percentages were reached in April, for TPC a further increase was recorded in May. However, the absolute percentages of the

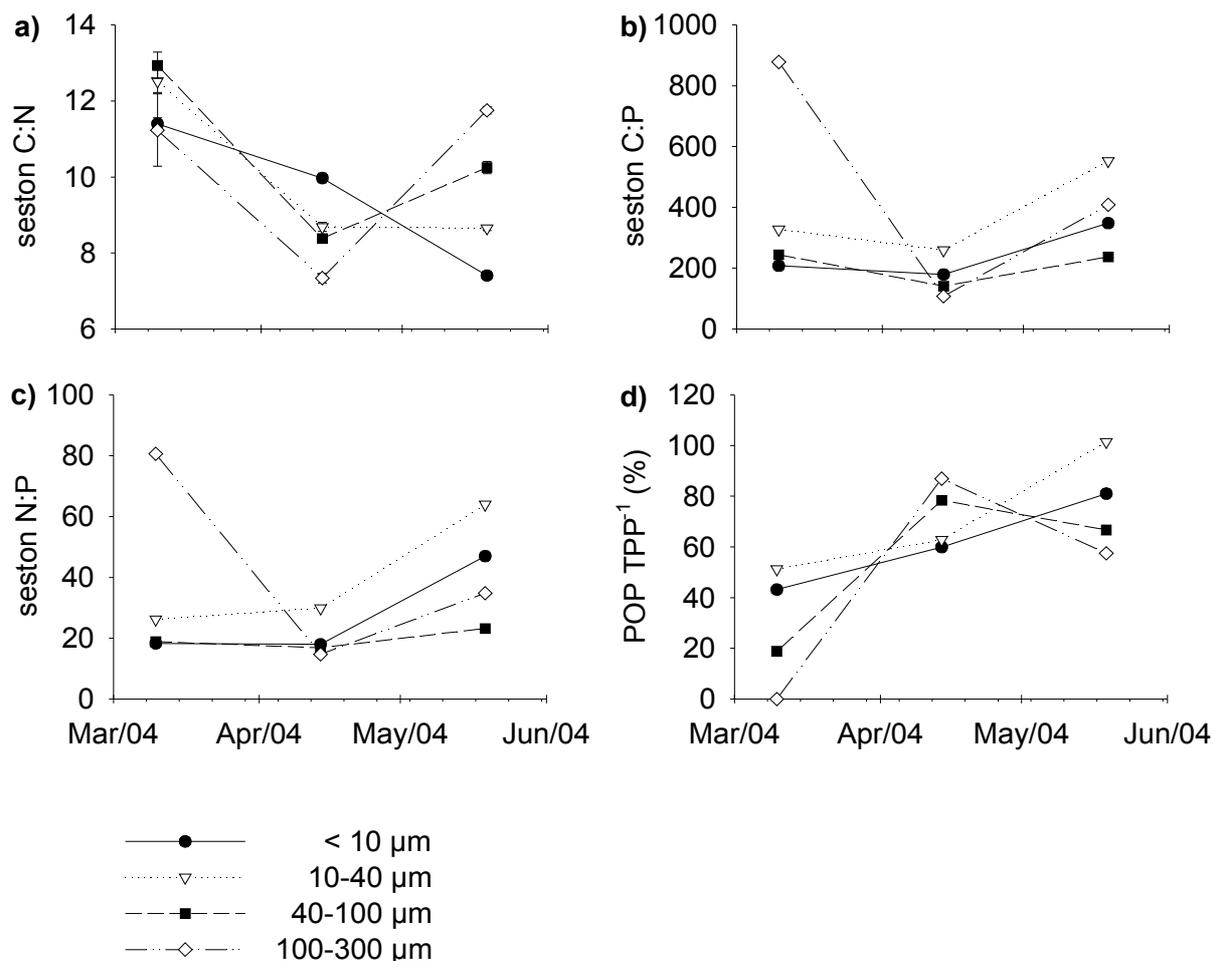


Fig. 4.5: Quality in size fractions (< 10 μm, 10-40 μm, 40-100 μm, 100-300 μm) a) molar seston C:N (N=3-6; means ± SE), b) molar seston C:P, c) molar seston N:P, d) percentage of POP TPP⁻¹ (calculated from averages).

100-300 μm size class stayed relatively low with maximum values of 7.0 (TPC), 7.9 (TPN) and 10.1 % (TPP).

Quality differences measured as C:N ratios in size classes between spring periods were obvious (Fig. 4.5a). In March C:N ratios were overall low. The 100-300 μm size class reached the lowest C:N ratio in April (7.3 ± 0.1), while the < 10 μm fraction reached the highest C:N ratio (10.0 ± 0.1) (means ± SE). In May it was vice versa (7.41 ± 0.04 and 11.8 ± 0.1). The seston C:P (Fig. 4.5b) and N:P (Fig. 4.5c) ratios did not show the same variability between fractions and seasons. A general rise was noted, with highest ratios in the 10-40 μm and lowest in the 40-100 μm size class. An exception was the very high ratio in the largest fraction in March due to an extremely low TPP content. The proportion of POP on TPP increased during the spring period starting from values around and below 50% in March (Fig. 4.5d). POP was not measurable in the 100-300 μm fraction in March, and only 19 % in the 40-100 μm fraction. In March and May, the smaller fractions contained a greater proportion of

Chapter 4: Food quality and egg production – spring bloom

POP than the larger fractions. In April it was the other way around. PIP was not measurable in the 10-40 µm size class in May, which resulted in a 100% POP proportion on TPP.

Table 4.3: Correlation of carbon specific egg production standardised to a temperature of 10°C, of percent reproducing females, and of calculated carbon content with nutritional parameters; May data excluded; significant correlations ($p \leq 0.05$) in bold.

Parameter	r(X,Y)	r ²	t	p	r(X,Y)	r ²	t	p
	<i>Acartia clausi</i>				<i>Temora longicornis</i>			
Carbon specific egg production standardised to 10°C								
TPC	-0.14	0.02	-0.56	0.580781	0.02	0.00	0.07	0.945469
PhytoC	0.50	0.25	2.21	0.042831	0.66	0.44	3.41	0.003867
TPN	0.21	0.04	0.83	0.421636	0.39	0.15	1.63	0.122909
TPP	-0.07	0.00	-0.27	0.789441	0.44	0.19	1.90	0.076749
POP	0.28	0.08	1.15	0.269077	0.63	0.39	3.12	0.007034
C:N	-0.89	0.79	-7.61	0.000002	-0.76	0.57	-4.50	0.000425
C:P	-0.10	0.01	-0.41	0.689021	-0.36	0.13	-1.66	0.114437
N:P	0.42	0.18	1.96	0.065114	0.12	0.01	0.50	0.621040
PhytoC %	0.61	0.37	3.00	0.009021	0.53	0.28	2.43	0.028062
POP %	0.52	0.27	2.38	0.031246	0.56	0.31	2.61	0.019732
Temp. °C	0.70	0.49	3.81	0.001698	0.69	0.48	3.73	0.001992
Copepod C	0.77	0.59	4.69	0.000289	0.64	0.41	3.23	0.005663
Percentage of reproducing females								
TPC	-0.13	0.02	-0.50	0.627514	-0.11	0.01	-0.43	0.674418
PhytoC	0.23	0.05	0.92	0.374194	0.62	0.38	3.04	0.008205
TPN	0.09	0.01	0.36	0.725493	0.27	0.07	1.07	0.301168
TPP	-0.11	0.01	-0.42	0.680536	0.42	0.18	1.80	0.092664
POP	0.14	0.02	0.53	0.604503	0.62	0.38	3.06	0.007971
C:N	-0.54	0.29	-2.48	0.025707	-0.77	0.59	-4.67	0.000304
C:P	-0.07	0.01	-0.29	0.776572	-0.55	0.30	-2.53	0.023269
N:P	0.27	0.07	1.09	0.293919	-0.07	0.01	-0.29	0.777772
PhytoC %	0.29	0.08	1.18	0.256473	0.55	0.30	2.53	0.022967
POP %	0.31	0.09	1.24	0.233044	0.58	0.33	2.73	0.015586
Temp. °C	0.23	0.05	0.91	0.379519	0.72	0.51	3.97	0.001234
Copepod C	0.51	0.26	2.54	0.020465	0.69	0.48	4.09	0.000685
Copepod carbon								
TPC	0.04	0.00	0.17	0.867938	-0.09	0.01	-0.34	0.738834
PhytoC	0.63	0.40	3.15	0.006626	0.45	0.21	1.98	0.066804
TPN	0.41	0.16	1.72	0.106404	0.24	0.06	0.98	0.343293
TPP	0.37	0.14	1.54	0.143669	0.31	0.10	1.25	0.228729
POP	0.57	0.32	2.69	0.016668	0.50	0.25	2.24	0.040887
C:N	-0.89	0.80	-7.76	0.000001	-0.83	0.69	-5.77	0.000037
C:P	-0.32	0.10	-1.42	0.172721	-0.35	0.12	-1.57	0.134448
N:P	0.20	0.04	0.85	0.408585	0.12	0.01	0.49	0.627277
PhytoC %	0.66	0.43	3.39	0.004053	0.48	0.23	2.11	0.051710
POP %	0.63	0.40	3.15	0.006580	0.56	0.32	2.64	0.018546
Temp. °C	0.84	0.71	6.12	0.000020	0.72	0.52	4.05	0.001043

Stoichiometric factors influencing egg production

Egg production was normalised to copepod carbon content (as a parameter of size) and standardised to a temperature of 10°C as described in the methods. Temperature was correlated to TPN, seston C:N, PhytoC, PhytoC % on TPC, PIP, POP % on TPP, thecate and atehcate dinoflagellates and central diatoms ($p < 0.05$). Therefore, temperature co-varied with some of the potential food quantity and quality parameters. Standardised egg production was correlated to the proportion of reproducing females in both species, no matter if May data were included or excluded. In *T. longicornis* the correlation was strong ($r^2 = 0.74$, $p < 0.000001$), in *A. clausi* it was less pronounced ($r^2 = 0.31$, $p = 0.003$); May data are included for this correlation analysis.

Food quantity and quality parameters correlated with standardised egg production, with the proportion of spawning females, and with copepod carbon (Table 4.3, Fig. 4.2 and 4.3). Food quantity given as TPC, TPN or TPP concentrations was neither correlated with egg production (Fig. 4.2a-c), nor with the proportion of egg producers, nor with carbon content of the two copepod species (Table 4.3). The POP concentration was correlated with *T. longicornis* egg production (Fig. 4.2e, 4.3c), with the proportion of spawning females, and with carbon content in both species. The correlation sank below a significance level of 5% in *T. longicornis*, when one very high POP concentration data point was removed from the data set. However, the correlation of POP with *A. clausi* carbon content remained significant. The proportion of POP on TPP (POP %) correlated with egg production of both species (Fig. 4.3d) and with copepod carbon content, in *T. longicornis*, also with the percentage of reproducing females (Table 4.3). The PhytoC concentration (Fig. 4.3a) and the percentage of PhytoC on TPC (Fig. 4.3b) were correlated with egg production and carbon content of both species, in *T. longicornis* also with the proportion of reproducing females (Table 4.3, Fig. 4.3a, b). However, almost maximum egg production was reached below 5 $\mu\text{mol L}^{-1}$ PhytoC and less than 10% PhytoC on TPC. Apparently, a linear correlation is misleading here. A curve fit with an exponential rise to maximum ($f(x) = a * (1 - e^{(-b*x)})$) of PhytoC concentration with *A. clausi* egg production gave a highly significant correlation with the coefficients $a = 3.6$ and $b = 0.5$ ($r^2 = 0.47$, $p < 0.0008$), and PhytoC % on TPC with egg production $a = 3.7$ and $b = 0.13$ ($r^2 = 0.62$, $p < 0.0001$). The function coefficients for the correlation of *T. longicornis* egg production with PhytoC concentration were $a = 2.7$ and $b = 0.23$

Chapter 4: Food quality and egg production – spring bloom

($r^2=0.49$, $p=0.0006$), and $a=2.7$ and $b=0.07$ ($r^2=0.54$, $p=0.0002$) for the correlation with PhytoC % on TPC. The seston C:N ratio was negatively correlated to egg production, carbon content and the proportion of reproducing females of both species with high correlation coefficients (Table 4.3 and Fig 4.3e). No significant correlations of egg production and carbon content with seston C:P and N:P ratios were detectable. However, the seston C:P ratio correlated with the proportion of reproducing females of the species *T. longicornis* ($r^2=0.02$, $p=0.02$).

Including May data for the correlation analysis, the same but weaker correlations (except that of seston C:P with the percentage of reproducing females) were found in *T. longicornis*. Additionally, seston TPN content became correlated to copepod carbon content, however, slightly below the significance level of 95% ($r^2=0.17$, $p=0.06$). For *A. clausi* all correlations with standardised egg production and proportion of reproducing females were lost by including May data. However, all correlations with copepod carbon were contained; they were only slightly reduced in significance. And comparably to the situation in *T. longicornis*, an additional correlation of copepod carbon with seston TPN concentration was noted ($r^2=0.21$, $p=0.03$).

Grazing behaviour

Copepods selected for larger cells. The two copepod species fed most differently on the smallest size class. *A. clausi* fed more randomly or selected small cells, *T. longicornis* fed randomly or selected against the size class. *A. clausi* ingested $68 \pm 4\%$ of the smallest size class, and was above expected values ($58 \pm 2\%$), in *T. longicornis* with $46 \pm 5\%$ ingestion it was below expected values ($60 \pm 2\%$) (Fig. 4.6a). Average availability of the 5-10 μm size class was 33 and $34 \pm 1\%$ for *A. clausi* and *T. longicornis*, while they fed more than expected with $43 \pm 5\%$ and $52 \pm 4\%$. No strong selection against the 5-10 μm size class occurred (Fig 4.5b). The 10-20 μm size class was available with $7 \pm 1\%$ for *A. clausi* and $6 \pm 1\%$ for *T. longicornis*, while *A. clausi* fed $18 \pm 5\%$ and *T. longicornis* $23 \pm 5\%$ (Fig. 4.6c). The largest size class of 20-40 μm made up only $0.9 \pm 0.1\%$ for *A. clausi* and $0.7 \pm 0.1\%$ for *T. longicornis*; it was preyed upon with very high rates of $3 \pm 1\%$ and $8 \pm 4\%$ (Fig. 4.6d).

Ingestion rates of all size classes were significantly correlated between the two species during the whole survey period ($r^2=0.49$, $t=8.31$, $p<0.00001$,

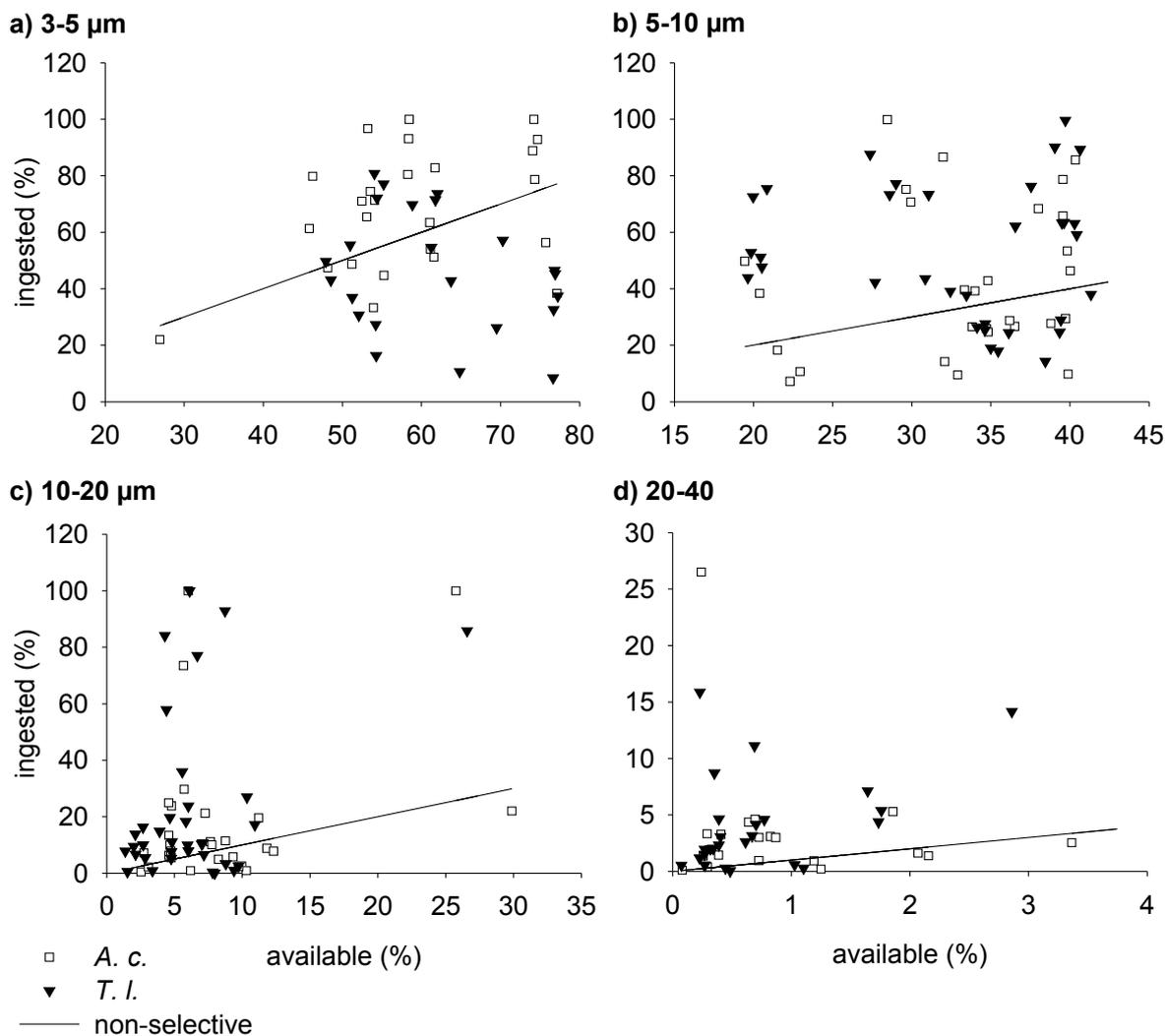


Fig. 4.6: Carbon specific grazing on a) 3-5 µm size class b), 5-10 µm size class c), 10-20 µm size class, d) 20-40 µm size class, ingested particles and available particle concentration, excluding zero ingestion data.

$N=75$). The *A. clausi* ingestion of the 10-20 µm size class was inversely correlated with standardised egg production of the species ($r^2=0.52$, $p=0.04$, $N=8$), and the *T. longicornis* ingestion of the 5-10 µm size class was inversely correlated with standardised egg production of *T. longicornis* ($r^2=0.45$, $p=0.03$, $N=10$). Both species showed a common tendency to ingest more food with rising medium food concentrations up to a saturation level (Fig. 4.7a-d).

Data were adjusted to an Ivlev-type exponential model with rise to a maximum $f(x)=a*(1-e^{(-b*x)})$ to test for significance. Ingestion rates depended significantly on food concentration only in the 5-10 µm size class (*A. c.*: $r^2=0.15$, $p=0.04$; *T. l.*: $r^2=0.14$, $p=0.03$). Saturation was reached with 10'000-20'000 ingested particles µg C⁻¹ d⁻¹(Fig. 4.7b). No saturation effect was detectable in the smallest size class (Fig. 4.7a). In the 10-20 µm fraction a maximum of about 5000 particles per µg

Chapter 4: Food quality and egg production – spring bloom

$C^{-1} d^{-1}$ occurred (Fig. 4.7c). About 600 ingested particles per $\mu g C^{-1} d^{-1}$ was the maximum in the largest size class (Fig. 4.7d). The percentage of the grazed particles

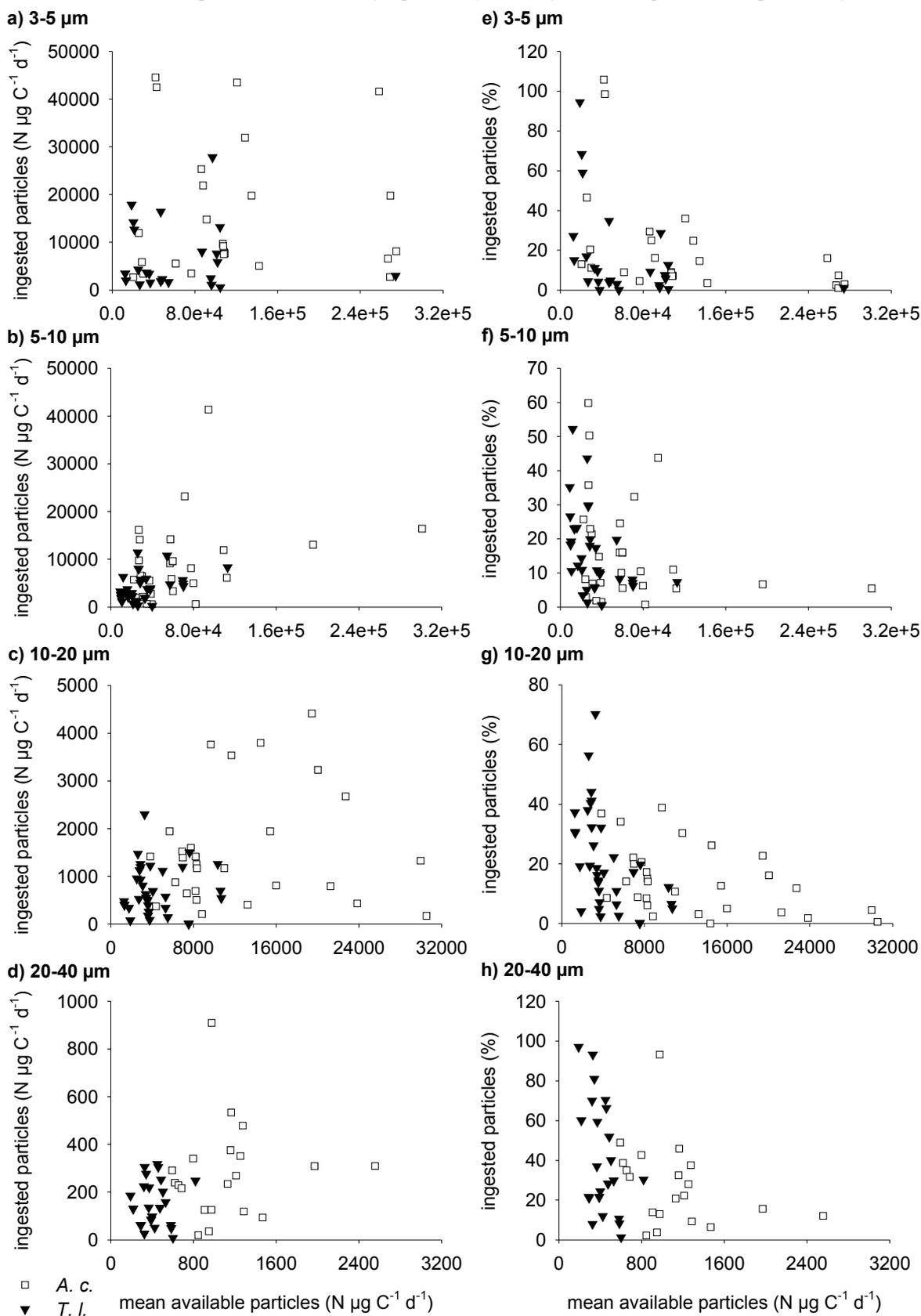


Fig. 4.7: Carbon specific particle ingestion (a-d) and percent of ingested on available particles (e-h) per μg copepod carbon; neighbouring graphs display results for the same size class (same x-axis).

Chapter 4: Food quality and egg production – spring bloom

on the available particles of a given size class was calculated, and is displayed in the neighbouring graphs (Fig. 4.7e-h). At low food concentrations the percentages of grazed particles on available particles varied over a wide range, which means that food levels were limiting.

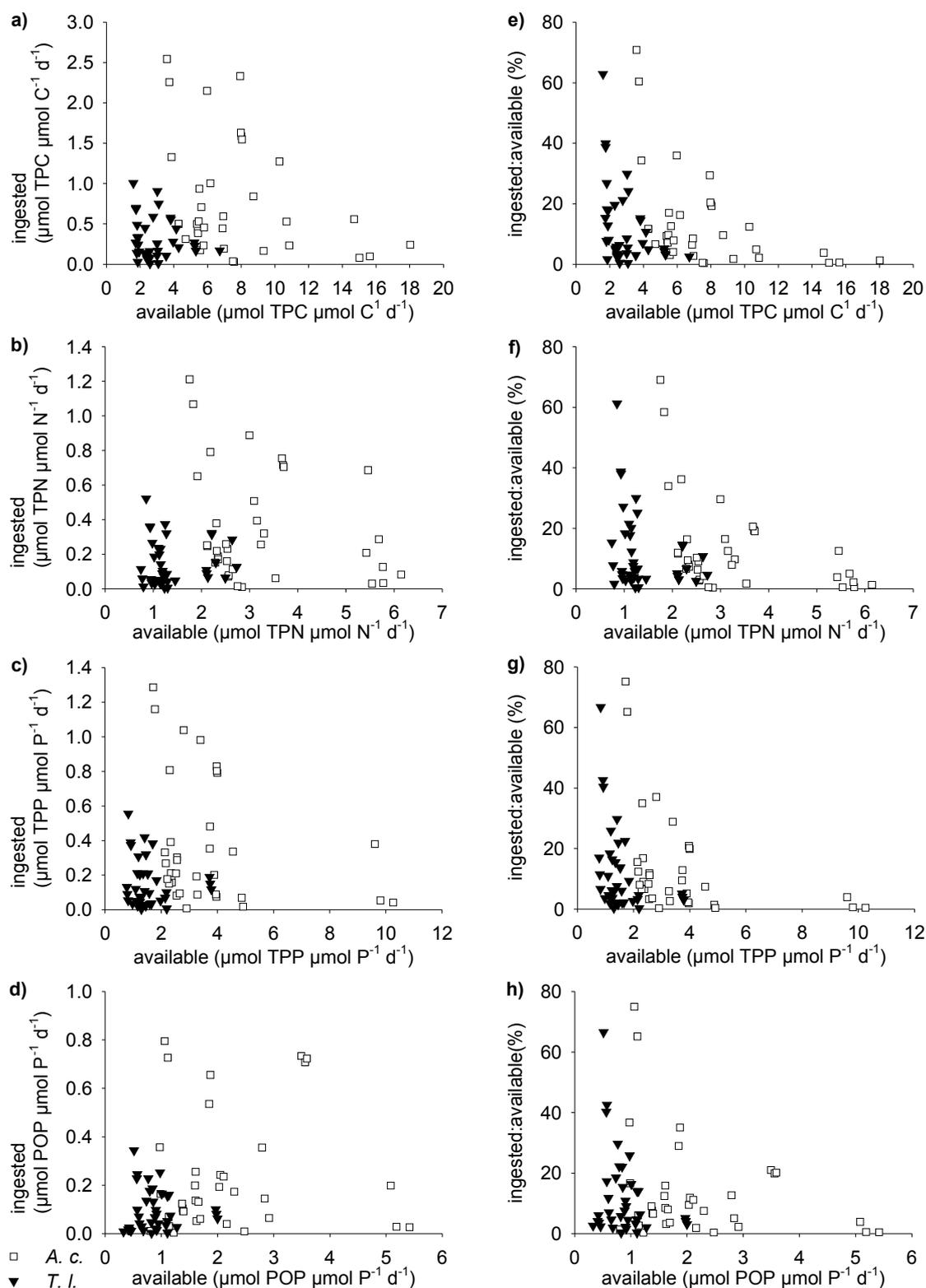


Fig. 4.8: Body nutrient specific grazing on seston nutrients: a-d) ingestion rates, e-h) % ingested of available nutrients per body nutrients.

Chapter 4: Food quality and egg production – spring bloom

Body specific nutrient ingestion on particles < 40 μm did not follow a saturation type feeding behaviour (Fig. 4.8). Maximum nutrient specific feeding occurred already at low available nutrient concentrations. Carbon uptake of *A. clausi* could well exceed 1:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$) and reached maximum rates of 2.5 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$) (Fig. 4.8a). Nutrient uptake of N, P and organic P rarely exceeded 1:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$) in *A. clausi* (Table 4.4, Fig. 4.8b-d). *T. longicornis* ingested generally lower nutrient ratios (seston:body) than *A. clausi*. Body specific C uptake never exceeded 1:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$) (Fig. 4.8a), specific N and P uptake was not higher than 0.6:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$) (Fig. 4.8b,c), and the uptake of organic P per body P did not exceed 0.4:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$) (Fig. 4.8d). Rapid satisfaction of nutrient demands was apparent with increasing nutrient availability. Saturation occurred more rapidly in *T. longicornis* than in *A. clausi* (Fig. 4.8e-h). However, the data range of available nutrients per body nutrients was smaller for *T. longicornis* due to the larger body mass of the species.

Table 4.4: Percentage of nutrient uptake in size classes < 10 μm and 10-40 μm , and average body nutrient specific nutrient uptake < 40 μm (N=35-38; means \pm SE).

ingested seston:body nutrient per day	<i>Acartia clausi</i>				<i>Temora longicornis</i>			
	< 10 (%)	SE	10-40 (%)	SE	< 10 (%)	SE	10-40 (%)	SE
TPC C ⁻¹ d ⁻¹	74 \pm	5	26 \pm	5	61 \pm	5	39 \pm	5
TPN N ⁻¹ d ⁻¹	73 \pm	5	27 \pm	5	61 \pm	5	39 \pm	5
TPP P ⁻¹ d ⁻¹	78 \pm	5	22 \pm	5	67 \pm	5	33 \pm	5
POP P ⁻¹ d ⁻¹	77 \pm	5	24 \pm	5	65 \pm	5	35 \pm	5
	< 40 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ d}^{-1}$)			SE	< 40 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ d}^{-1}$)			SE
TPC C ⁻¹ d ⁻¹	0.78 \pm			0.12	0.30 \pm			0.04
TPN N ⁻¹ d ⁻¹	0.35 \pm			0.05	0.15 \pm			0.02
TPP P ⁻¹ d ⁻¹	0.37 \pm			0.06	0.15 \pm			0.02
POP P ⁻¹ d ⁻¹	0.24 \pm			0.04	0.09 \pm			0.01

The < 10 μm size class was identified as the major source for nutrients (Fig. 4.9, Table 4.4). Maximum seston nutrient uptake by body nutrient content was reached in mid-April in both species irrespective of the type of nutrient (Fig. 4.9).

However high nutrient ingestion already occurred in the early spring phase, in March, when phytoplankton biomass had not yet begun to increase dramatically. The uptake of C:N and C:P revealed imbalances (Fig. 4.10a,b). However, the C:N imbalance decreased over time with increasing seston C:N quality (decreasing values). C:N was

Chapter 4: Food quality and egg production – spring bloom

taken up according to its seston ratio (Fig. 4.11a). Copepods took up food in an N:P ratio similar to their own N:P ratio most of the time, indicated by an uptake:body ratio

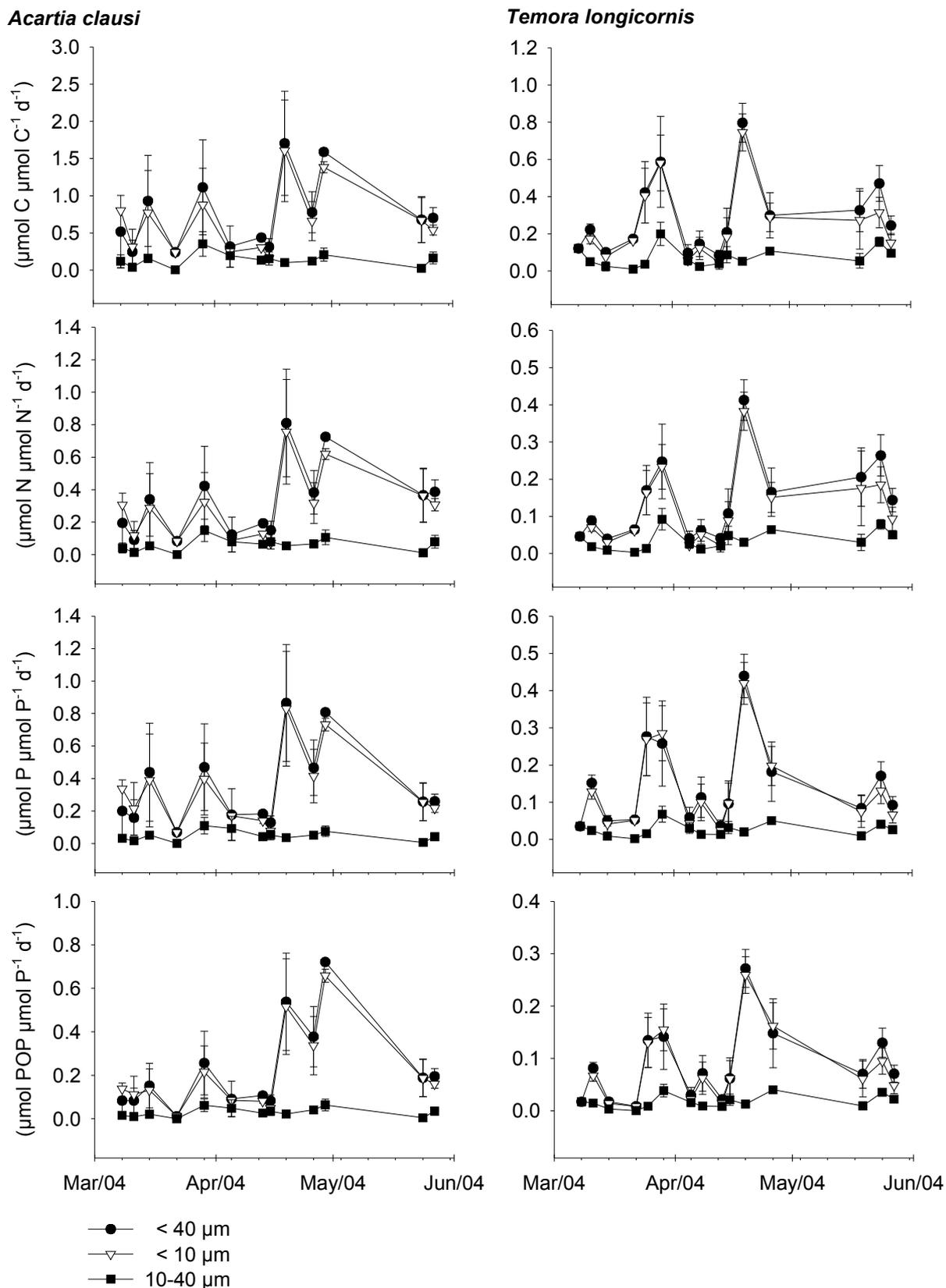


Fig. 4.9: Body specific nutrient uptake of seston nutrients (C, N, P, POP) in the size class < 10 μm and 10-40 μm and < 40 μm in *A. clausi* and *T. longicornis* during spring (no grazing measurements during the first half of May).

Chapter 4: Food quality and egg production – spring bloom

of 1:1 (Fig. 4.10c). The proportion of ingested organic P on TPP dropped at the end of March, and then rose to a high level (Fig. 4.10d) that reflected the development in the seston (Fig. 4.11d). Both species ingested C:N and POP:TPP according to the seston ratios, indicated by the line at a ratio of 1 (ingested ratio per copepod ratio divided by seston ratio) (Fig. 4.11a, d). Slightly increased body specific C:P and N:P ingestion occurred during April (Fig. 4.11b, c).

4.4. Discussion

Temperature is acknowledged as an important regulating factor of biological processes. In a study on dominant Helgoland species, including *A. clausi* and *T. longicornis*, temperature was described as a more important factor regulating egg production than food quantity (Halsband and Hirche 2001). Laboratory experiments show a clear dependence of egg production on temperature under saturating food concentrations (Halsband-Lenk et al. 2002; Castro-Longoria 2003; Holste and Peck 2006). However, in the field, food concentration and quality do not stay stable throughout longer periods, and potential temperature dependent laboratory egg production rates may not be reached (Hirst and Bunker 2003). Moreover, saturating food concentration increases with temperature (Bunker and Hirst 2004; Maps et al. 2005). In our field study, food quantity and quality were rising concomitantly with

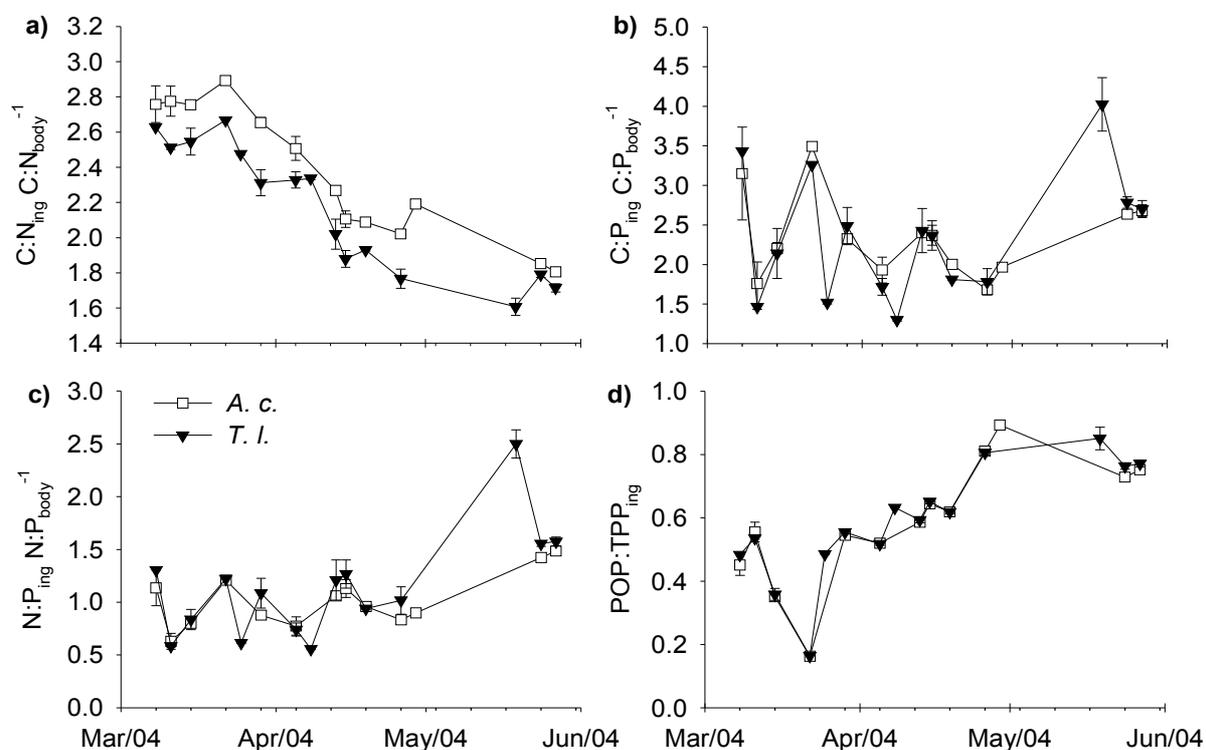


Fig. 4.10: Elemental ratios of ingested food versus body ratios over time: a) $C:N_{ing} C:N_{body}^{-1}$, b) $C:P_{ing} C:P_{body}^{-1}$, c) $N:P_{ing} N:P_{body}^{-1}$, d) $POP:TPP_{ing}$.

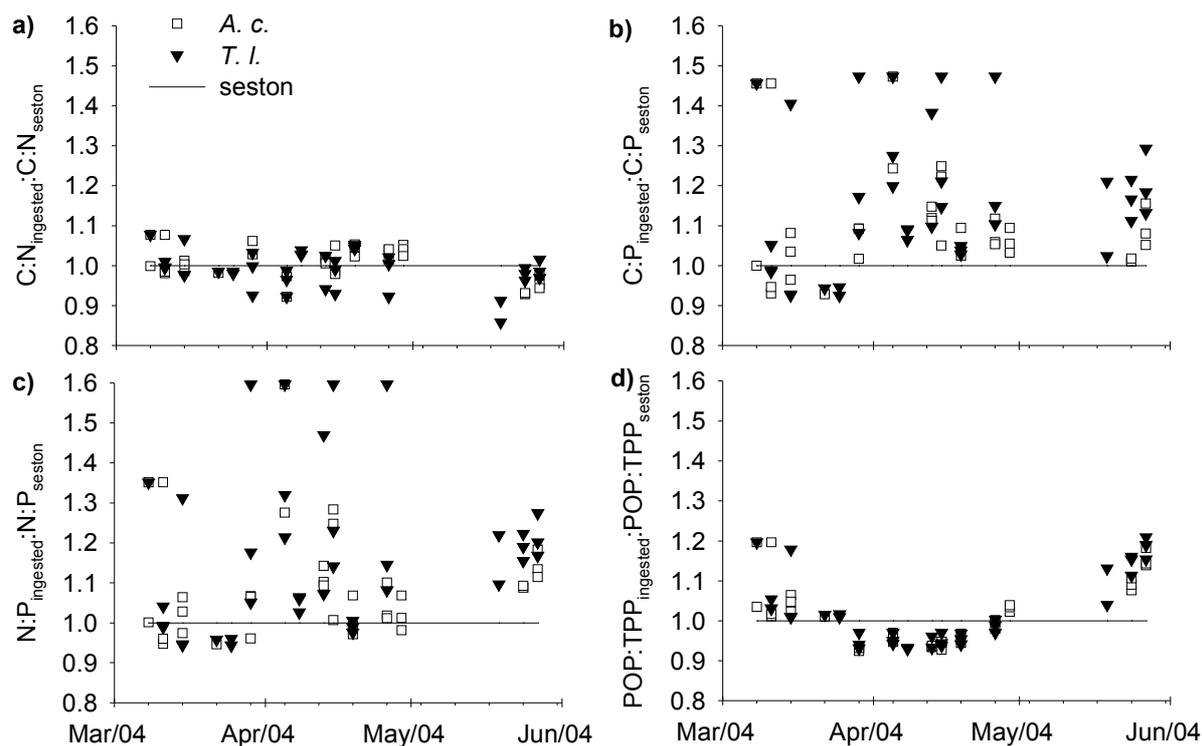


Fig. 4.11: Individual uptake (based on copepod C) of nutrient ratios in relation to seston ratios: a) $C:N_{ingested} : C:N_{seston}$, b) $C:P_{ingested} : C:P_{seston}$, c) $N:P_{ingested} : N:P_{seston}$, d) $POP:TPP_{ingested} : POP:TPP_{seston}$.

temperature. To evaluate nutritional factors besides a temperature effect we applied a general temperature factor ($Q_{10}=3$) to correct egg production for a standard temperature of 10 °C (Kjørboe and Sabatini 1995). Depending on species and study area the Q_{10} of egg production can however vary (Bunker and Hirst 2004). In our study, the standardised egg production of *A. clausi* and *T. longicornis* still showed a strong correlation to temperature (Table 4.3). This may be due to co-variance of nutritional factors with temperature. Or the theoretical Q_{10} of 3 may be too low for *A. clausi* and *T. longicornis* in our study area. In laboratory studies with *T. longicornis* and *Pseudocalanus elongatus* food concentration was positively but temperature negatively correlated with body size (Klein Breteler and Gonzalez 1988). And in the Helgoland study of Halsband and Hirche (2001) body size was increasing before temperature was rising. Correlations of food concentration with growth and fecundity of copepods, but not with temperature, were described for several copepod species in the Benguela upwelling region (Richardson and Verheye 1998).

Egg production is size dependent as long as no food limitation occurs. Larger animals have higher absolute energy demands to sustain their metabolism, so they are potentially more easily food limited than smaller individuals. Temperature can enhance a limitation. A general increase in grazing with size was noted in our data

Chapter 4: Food quality and egg production – spring bloom

sets supporting this view. Therefore, we standardised grazing and egg production to copepod carbon content. With such corrected egg production data sets we analysed the correlation of egg production with nutritional factors.

Seston quantity factors given as TPC, TPN, and TPP concentration were not correlated with egg production. This lack of correlation points to a qualitative rather than a quantitative food limitation of copepod reproduction. Phytoplankton carbon and proportion of TPC as well as concentrations of distinct phytoplankton taxa have a quantitative as well as a qualitative aspect. The very steep slope of the exponential relation between phytoplankton carbon concentration and copepod egg production denotes that copepods were obviously not limited by total phytoplankton carbon. The Ivlev-type correlations of diatom concentration with *T. longicornis* egg production and *A. clausi* body carbon showed a steep increase. Almost maximum egg production values were found at very low phytoplankton and diatom concentrations. Therefore, one can conclude that phytoplankton and the tested taxa do not greatly affect copepod reproduction. However, high phytoplankton concentrations probably support maximum egg production rates. Among stoichiometric ratios, the strong correlation with the seston C:N ratio supports the view that nitrogen is the most important limiting factor in this marine environment. However, our data also support a potentially limiting copepod demand for P to maximise egg production.

In the marine environment most of the P occurs in dissolved form as compared to particulate and organismic P (Hassett et al. 1997). The Helgoland seston N:P ratios pointed to no or moderate nutrient deficiency, while the C:P ratios depicted moderate to severe deficiencies, and the C:N ratios showed moderate deficiency. However, the ratios can vary between phytoplankton species (Klausmeier et al. 2004). Therefore, copepods should use strategies such as selective or compensatory feeding to circumvent nutrient limitations. They are able to differentiate not only the size but also the quality of a potential prey (Cowles et al. 1988; Donaghay 1988). High detritus loads, for example, decrease food quality and can prevent a saturation of the ingestion rate (Paffenhöfer and Van Sant 1985; Mayzaud et al. 1998). Ingestion on all size classes followed similar saturation type patterns. However, we did only measure size-specific and not food-species-specific feeding. The nutrient specific feeding revealed highest maximum ingestion rates at low available nutrient concentrations. Maximum uptake rates of N, P and organic P were 1:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$) and slightly above. In carbon terms, most values stayed around 1:1 (μmol

Chapter 4: Food quality and egg production – spring bloom

$\mu\text{mol}^{-1} \text{ day}^{-1}$) but uptake rates could rise up to 2.5:1 ($\mu\text{mol} \mu\text{mol}^{-1} \text{ day}^{-1}$). These patterns and the very high carbon ingestion rates point to a compensatory feeding to circumvent N and P limitation. At many times, however, feeding rates were so low at low food or nutrient concentrations that they were probably not sufficient to sustain a good fitness of the grazers. Copepods have been shown to maximise protein ingestion (Libourel-Houde and Roman 1987). Additionally to the accepted view that marine sites are widely N limited (Elser and Hassett 1994), evidence is accumulating that P can be limiting in marine environments as well, especially in open ocean areas (Downing et al. 1999; Guildford and Hecky 2000). The presented data support the view that both elements can be limiting in the ocean.

Responses to food quality are more obvious at higher than at lower food concentrations (Koski and Klein Breteler 2003) but are also measurable at low concentrations (Boersma and Kreutzer 2002).

Copepods cannot only adapt their grazing behaviour but also their digestive system to changes in food quantity and quality, so that they are able to compensate possible negative effects (Hassett and Landry 1990). Nevertheless, nutritional imbalances can be passed on from prey to copepod (Van Nieuwerburgh et al. 2004). The results of Van Nieuwerburgh et al. (2004) support the hypothesis that copepods do not regulate their body functions homeostatically but rheostatically, adapting to their ever changing environment (Villar-Argaiz et al. 2002). Such a slight adaptation can reduce the danger of nutrient limitation for copepods.

In this study, feeding on the largest cells was not measured because particles larger than 40 μm were rare. Copepods in the field may cruise a larger area than a 1 L bottle volume and thereby may be selecting differently in nature. Copepod grazing impact on herbivorous microzooplankton can lead to negative grazing results on smaller size fractions. Pico- and nanoplankton may benefit from the top-down control of copepods on microzooplankton herbivores. But an increase in the small fractions can also be due to the break-down of larger cells. The selection against the 3-5 μm fraction in *T. longicornis* (Fig. 4.5a) may reflect such a connection, especially since otherwise, *T. longicornis* seems to prefer larger particles stronger than *A. clausi*.

Our data hint to a diatom preference of *T. longicornis* over other prey taxa. *Phaeocystis* seems to affect egg production (Bautista et al. 1994; Devreker et al. 2005) despite stoichiometric quality (this study). However, growth phase and nutrient condition of the algae seem to play a role in toxin-production (Koski et al. 2005).

Chapter 4: Food quality and egg production – spring bloom

The number of spawning females greatly effected egg production in other species as well (Campbell and Head 2000). Food quantity and quality factors can act on gonad maturity, thereby, influencing the number of spawning females (Niehoff 2004). In *A. clausi* nutritional factors seemed to act more directly on the individual female egg production. Adult copepods do not grow or moult. Therefore, they can transfer most of the energy they take up, into egg production.

We have shown that the < 10 µm size class usually contains the largest proportion of C, N and P. Copepods did take up the main proportion of nutrients through grazing on this small seston fraction. From culturing it is known that several copepod species thrive when fed exclusively with the rather small *Rhodomonas* sp. (5-8 µm) and *Oxyrrhis marina* (13 µm) cells (Klein Breteler 1980). Especially, when larger food particles are scarce, animals have to rely on smaller food particles and inferior food to circumvent food limitation (Chervin 1978). Nevertheless, they usually strongly select for larger prey.

The results that we present here may be biased because we calculated with average nutrients in size classes, and did not know what proportion of nutrients the copepods really took up. This may be a rather rough estimate at times. Small flagellates and detritus mainly comprise the size class < 10 µm. Larger flagellates, small diatoms and small protozoans can be found in the size class between 10 and 20 µm, larger protozoans and largest flagellates between 20 and 40 µm. Large diatoms and diatom chains, and largest dinoflagellates belong to the size class > 40 µm. In the early spring phase, detritus formed a large part of the seston particles. Detritus is ingested by copepods but it is of inferior quality compared to living prey (Chervin 1978; Mayzaud et al. 1998).

Conclusion

Stoichiometric imbalances between seston and copepods interfered with copepod reproduction. The effect was probably transferred by the grazing behaviour of the copepods. Their ingestion of seston C:N reflected the environmental seston C:N ratio, the same was true for the POP:TPP proportion uptake. Both factors were correlated to egg production. The analyses of potential nutrient uptake showed that N and P are potentially limiting for copepods in the marine environment. Both species took up the largest part of their nutrient demand by grazing on the < 10 µm seston size fraction which contained the bulk of particle bound nutrients.

Chapter 5: Stable isotopes as predictors of copepod reproductive success

The stable isotope signatures of seston and copepods varied considerably in time and space, and also within seston size fractions. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiles of marine seston had a modulating effect on egg production patterns of dominant North Sea copepod species, *Acartia clausi* and *Temora longicornis*. The seston $\delta^{15}\text{N}$ signature was significantly negatively correlated with the molar *T. longicornis* C:N ratio. *A. clausi* showed the same trend. Over the year the seston $\delta^{13}\text{C}$ was positively correlated with seston C:N and negatively with egg production of both species.

A. clausi and *T. longicornis* fed on similar trophic levels ($\delta^{15}\text{N}$ signature) during a spring bloom but they utilized different carbon sources ($\delta^{13}\text{C}$ signature). These differences could be due to selective feeding and/or variable digestion between the two species. Copepod digestive enzyme activities showed distinct species-specific and seasonal patterns. Chitobiase activity indicated carbon assimilation while arginine aminopeptidase, esterase/lipase and beta-glucosidase activity were indicators of nitrogen assimilation.

5.1. Introduction

External and internal factors determine fecundity of copepods. External factors, i.e. hydrography, temperature, food quantity and quality, are the same for all co-occurring species in space and time, but different species may react in distinct ways to these factors as they differ in physiology and behaviour.

Stable isotope analyses have become a useful tool for food web studies. Animals with high turnover rates such as heterotroph protozoans and zooplankton reflect the signals of their food, slightly accumulating the heavy isotopes compared to their food. Animals or tissues of similar metabolic characteristic can be placed into trophic schemes with help of stable isotope analysis. Each trophic level is enriched in $\delta^{15}\text{N}$ and also in $\delta^{13}\text{C}$. A ^{15}N enrichment of 1.3 to 5.3 with an average of 3.4 ± 1.1 ‰ per trophic level has been determined across several food chains (Minagawa and Wada 1984). An enrichment of < 1 ‰ per trophic level is usually apparent in $\delta^{13}\text{C}$ food web signatures (Fry et al. 1984). Autotroph species vary considerably in their stable isotope signatures (Leboulanger et al. 1995). However, light regime, source of nutrients and nutritional stress can influence the stable isotope signature of primary producers as well as turnover rates and temperature (Leboulanger et al. 1995; Adams and Sterner 2000).

Chapter 5: Stable isotopes and reproductive success

The internal physiology of an animal affects its reactions to the environmental condition. Especially, digestive physiology holds a key position as it determines the way how an organism deals with its resources. For example, research on amphipods provides strong evidence that selective feeding behaviour is determined by the genetically determined digestive physiology (Guarna and Borowsky 1993; Sotka 2003). Moreover, the principal set of digestive enzymes such as iso-trypsins in *Litopenaeus vannamei* (Sainz et al. 2005) or proteases in copepods (chapter 3) does not change as a result of changes in quality or quantity of resources but rather shows a genetically determined variability. However, the activity of single enzymes can be adjusted to both food quantity and quality. Harris (1986) suggested that digestive enzymes play a regulating role to deal with varying food concentrations, with enhanced digestion at low food concentrations and reduced digestive activity when food is abundant. This compensatory mechanism is variable and depends on previous feeding experience of the copepods (Roche-Mayzaud et al. 1991; Mayzaud et al. 1992). In general, individuals that survived food shortage show a hunger response, overshooting feeding and respiration rates (Tiselius 1998; Thor 2003).

Copepods adapt their feeding behaviour to the concentration of the food. They are able to switch between non-selective and selective feeding modes depending on the particle distribution, size and quality they encounter (Price et al. 1983; Price and Paffenhöfer 1986). If food particles are scarce the animals tend to feed indiscriminately, if food particles are abundant they tend to select the particles with the best nutritional value for their needs (Donaghay 1988). They prefer actively growing phytoplankton (Cowles et al. 1988) and select against toxic cells (Teegarden 1999). Copepods are able to discern different food qualities by amino acids (Poulet and Ouellet 1982; Schütte 2006). Moreover, copepods can compensate for a lack in quality by compensatory feeding or prolonged gut evacuation times (Tirelli and Mayzaud 2005).

Nutritional quantity and quality of seston change over time and space. Seasonal changes in seston composition and concentration are pronounced, and hydrographic phenomena such as fronts or eddies can create steep gradients of food quantity and quality (Raabe et al. 1997). In the transitional zone of fronts, nutrients and particles accumulate. Thus, frontal systems, especially the transitional zone, are high productivity areas in the sea that structure plankton communities horizontally (Thibault et al. 1994; Nielsen and Munk 1998; Albaina and Irigoien 2004).

Chapter 5: Stable isotopes and reproductive success

This study was designed to reveal possible links between external factors and copepod physiology that all together explain variances in fecundity and differences between species. The different parameters were studied during a one-year cycle including 2 spring times. The variability in time at station Helgoland Roads (7°54.0'E, 54°11.3'N) was compared with spatial variability in the German Bight and adjacent southern North Sea zones during the GLOBEC-cruise HE211 of R/V Heincke in June 2004 (Fig. 5.1).

5.2. Material and Methods

Seasonal Study

We used a two-pronged approach for a seasonal study at Helgoland Roads. In spring 2004, we carried out an intensive sampling strategy (see also chapter 4), where we sampled seston and individual copepods twice weekly. During the rest of the year, we sampled seston and *Temora longicornis* females on a weekly basis. To assess the importance of spatial variation we sampled different water masses during a North Sea cruise (R/V Heincke, GLOBEC-cruise HE211, 18/06/2004-23/06/2004), determined nutritional characteristics of *Temora* to be able to compare the results of the time-series analysis with the spatial analysis.

Sampling – animals and surface seawater

The copepods *Acartia clausi* and *Temora longicornis* were sampled twice weekly during a period from mid February until the end of May 2004 off Helgoland (North Sea, German Bight). Adult females were selected and transferred to experimental vessels containing natural sea water at *in situ* temperature, or they were shortly rinsed with deionised water, blotted dry and frozen at -80 °C for biochemical and physiological analyses. From May 2004 to May 2005, we sampled only *Temora longicornis* individuals once a week.

Surface seawater samples were taken simultaneously with zooplankton samples, pre-filtered (300 µm), and stored cold in opaque 10 L plastic containers until filtration. For each seston parameter (total particulate carbon (TPC), nitrogen (TPN), phosphorus (TPP), particulate organic (POP) and inorganic phosphorus (PIP), and stable isotope ratios of $^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$), three parallels of 300-1200 mL were filtered onto pre-combusted (6 h, 420 °C) glass fibre filters (Whatman, GF/C, 25 mm diameter). After filtration visible animals > 100 µm were picked off manually under a

Chapter 5: Stable isotopes and reproductive success

dissection microscope at 50x magnification. Filters were stored individually in reaction tubes at -80 °C.

Copepod egg production

During spring and summer of 2004, we measured the *in situ* egg production rates of the two focal species of this study on a twice weekly basis, from September to December on a monthly basis. Thirty females each of the two species (*Acartia clausi* and *Temora longicornis*) were transferred into single wells of 6-well microplates. Each well contained 9-10 mL sieved seawater (55 µm). Temperature and light conditions were adjusted to *in situ* conditions daily (WTB binder incubator). After 24 hours females were removed from the wells, and eggs were counted.

Stable isotope determination

Stable isotope analyses give us insights into the nutritional sources plants and animals are using. The stable isotope pattern of a grazing organism reflects the stable isotope pattern of its food source. Especially, the signatures of ¹³C and ¹⁵N are widely used in ecology. The ratio between ¹³C and ¹²C gives an indication of the carbon source that was foraged. The enrichment of the stable nitrogen isotope ¹⁵N can be used as a trophic tracer because the stable isotope signatures of a consumer generally reflects the isotopic composition of their diets and enriches the heavier isotopes in a relatively dependable manner (Minagawa and Wada 1984). Stable isotopes of carbon and nitrogen were measured in 2 to 6 parallels with 5 to 17 heat dried (60°C, 12 h) adult female copepods per measurement (UC Davis-Stable Isotope Facility, CA, USA). Occasionally only one sample was measured due to a lack of material. Carbon and nitrogen stable isotope ratios were determined by continuous flow isotope ratio mass spectrometry. Stable isotope ratios are given using the δ notation expressed in units per mil as follows:

$$\delta (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad , \quad \text{and} \quad R = \frac{^{13}\text{C}}{^{12}\text{C}} \quad \text{or} \quad R = \frac{^{15}\text{N}}{^{14}\text{N}} \quad .$$

δ¹⁵N was calibrated versus air, δ¹³C versus Peedee belemnite carbonite.

Protein content and enzyme activities

Water soluble protein contents of individual females (N=5-10) were measured with the bicinchoninic acid assay (BCA, Pierce Ltd.) (chapter 2). Bovine serum albumin (BSA, 1 to 5 µg per well) was used as standard.

Chapter 5: Stable isotopes and reproductive success

Enzyme activities were measured in individual females (optimal pH, 25 °C) with fluorogenic substrates (chapter 1). Individual copepods were homogenized in 200 μL of ice cold citrate-phosphate buffer, pH 8 (McIlvaine, 1921). The extracts were used for the analysis of two protein degrading exopeptidases (arginine and alanine aminopeptidase), lipid hydrolysing esterase/lipase (all at pH 7), and two carbohydrases (chitobiase and beta-glucosidase) measured at pH 5. Standard curves were prepared with 4-beta-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC). Enzyme activities were calculated in relation to the average protein content of either species and were presented as specific activities ($\text{nmol h}^{-1} \text{mg}_{\text{prot}}^{-1}$).

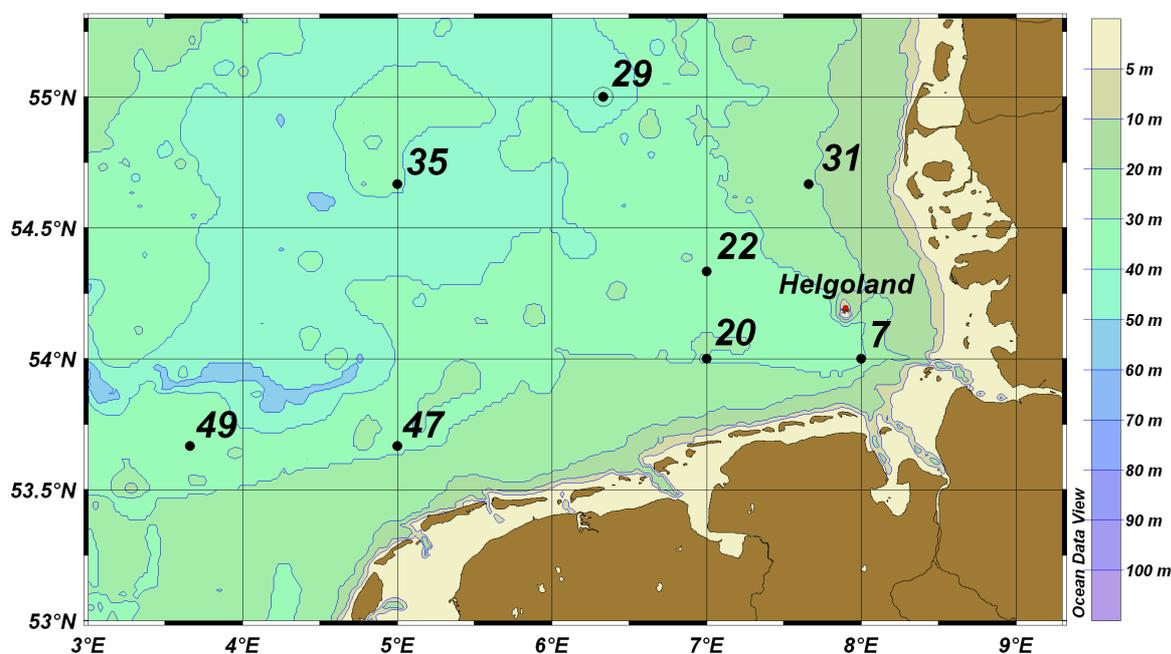


Fig. 5.1: Helgoland (red dot) and GLOBEC sampling stations (station numbers) in the North Sea (map created with Ocean Data View Version 3.1.0, Schlitzer 2006).

Spatial study

Seven water bodies in the German Bight (Fig. 5.1) were sampled. From every station, we took water samples from a depth of 5 m. One to two L of pre-filtered seawater (100 μm) were filtered through pre-combusted (6 h, 420 °C) Whatman GF/C filters (3 per parameter and station), and filters dried (60°C overnight) for C, N and P determination. At every one of the seven stations we collected adult females of *T. longicornis* by vertical hauls with a WP2 net. The animals were sorted on board and stored at -80 °C until further (isotope and biochemical) analysis.

Chapter 5: Stable isotopes and reproductive success

Table 5.1: Ranges of stable isotope values in seston and copepod samples (N=3-6, means \pm SD); total period: 16/02/2004-19/04/2005; spring 04: 16/02-27/05/2004; spring 05: 15/02-19/04/2005.

Parameter/ Period	Min (‰)	Min day	Max (‰)	Max day
Seston $\delta^{15}\text{N}$				
Total	4.9 \pm 0.1	26/04/2004	11.9 \pm 0.4	06/08/2004
Spring 04	4.9 \pm 0.1	26/04/2004	10.4 \pm 0.5	14/04/2004
Spring 05	7.5 \pm 1.6	01/03/2005	10.0 \pm 0.6	10/04/2005
<i>T. l.</i> $\delta^{15}\text{N}$				
total	8.5 \pm 0.7	19/02/2004	15.6 \pm 0.4	22/03/2005
Spring 04	8.5 \pm 0.7	19/02/2004	15.0 \pm 0.1	29/03/2004
Spring 05	12.7 \pm 0.7	08/03/2005	15.6 \pm 0.4	22/03/2005
<i>A. c.</i> $\delta^{15}\text{N}$				
Spring 04	7.7 \pm 0.0	29/04/2004	13.8 \pm 0.3	13/04/2004
Seston $\delta^{13}\text{C}$				
Total	-23.7 \pm 0.3	05/04/2004	-12.8 \pm 0.4	19/11/2004
Spring 04	-22.3 \pm 0.1	13/05/2004	-16.7 \pm 0.2	22/03/2004
Spring 05	-23.7 \pm 0.3	05/04/2005	-16.8 \pm 0.1	15/02/2005
<i>T. l.</i> $\delta^{13}\text{C}$				
Total	-21.1 \pm 0.3	01/07/2004	16.7 \pm 0.10	04/11/2004
Spring 04	-20.8 \pm 0.0	19/04/2004	-17.8 \pm 0.03	27/05/2004
Spring 05	-20.1 \pm 0.2	12/04/2005	-18.7 \pm 0.20	22/02/2004
<i>A. c.</i> $\delta^{13}\text{C}$				
Spring 04	-23.5 \pm 0.0	15/04/2004	-17.4 \pm 0.02	27/05/2004

C, N and P determination

Seston filters were analysed for total particulate carbon (TPC) and nitrogen (TPN) using a CHN analyser (Fisons Instruments EA 1108). Acetanilid (Thermo Quest, 338 36700) served as standard. Molar C:N ratios were calculated. Differentiation between particulate organic phosphorus (POP) and particulate inorganic phosphorus (PIP) was done after Aspila et al. (1976). Total particulate phosphorus (TPP) was determined by combusting the filter at 520°C for two hours, extraction in 6 mL 1N HCl for 18 hours and measuring dissolved inorganic phosphorus (DIP) in the neutralized extract after Grasshoff et al. (1983). PIP was determined by extracting the uncombusted filter for 18 hours in 6 mL 1N HCl and measuring DIP in the neutralized extract with an AutoAnalyser after Grasshoff et al. (1983). POP was calculated from the difference between average TPP and PIP.

5.3. Results

Seasonal Study

Stable isotope patterns of copepods and seston varied considerably during a year (Fig. 5.2), and between size fractions as measured in 3 phases of spring 2004 (Fig. 5.3). Stable $\delta^{15}\text{N}$ (Fig. 5.2a, Table 5.1) ranged from 4.9 \pm 0.07 ‰ to 11.9 \pm 0.4 ‰ in seston (means \pm SD, also the following values), from 8.5 \pm 0.7 to 15.6 \pm 0.4 in *T. longicornis*, and from 7.7 to 13.8 in *A. clausi* which was only measured during spring

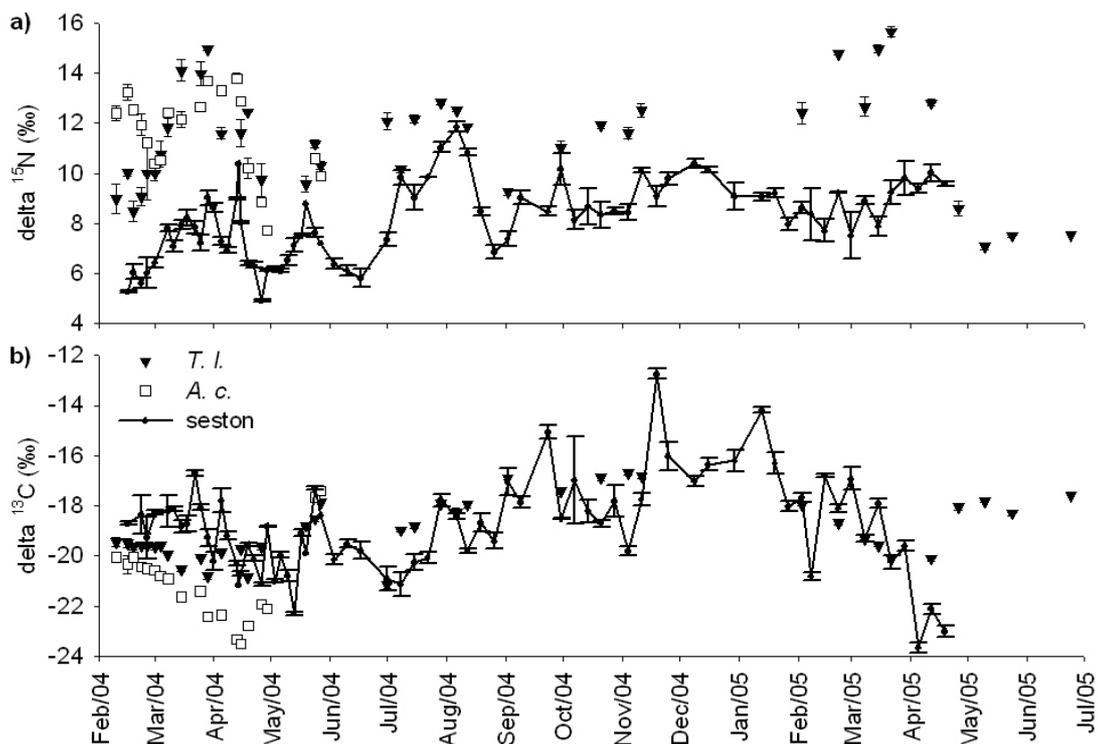


Fig. 5.2: Seston and copepod stable isotopes during a year including 2 spring times (N=3-6, means \pm SE), *Acartia clausi* was only sampled during spring 2004.

2004. Seston $\delta^{15}\text{N}$ increased during spring 2004, dropping steeply from mid-April to the lowest value end of April, then increasing again toward mid-May, dropping again. Maximum values occurred during July and August, another minimum followed at the end of August. From October throughout February the seston $\delta^{15}\text{N}$ signature stayed relatively stable at a medium level of 8.9 ± 0.8 (N=18; mean \pm SD). Values started to rise again in March 2005. The copepods more or less follow the seston pattern but on a higher level.

Seston $\delta^{13}\text{C}$ ranged from -23.7 ± 0.3 ‰ to -12.8 ± 0.4 ‰, in *T. longicornis* from -21.1 ± 0.3 ‰ to -16.7 ± 0.1 ‰, in *A. clausi* from -23.5 ± 0 ‰ to -17.41 ± 0.02 ‰ (Fig. 5.2b, Table 5.1). During both spring times the values decreased due to a shift in the seston composition. Lowest values occurred in late April and early May, and in June and July. From August until the end of February the seston $\delta^{13}\text{C}$ stayed relatively high, with highest values from mid-November till mid-January. The copepod $\delta^{13}\text{C}$ ratios were at similar levels as seston. The $\delta^{13}\text{C}$ signal of *A. clausi* was consistently lower than the signal of *Temora*. This is considered a first hint that the two species selected distinct food items out of the bulk seston.

The four size fractions of three spring phases showed some peculiarities. The phases were (as in chapter 3): March: early phase with low phytoplankton and high seston C:N; April: phytoplankton growth phase with decreasing seston C:N; May: late

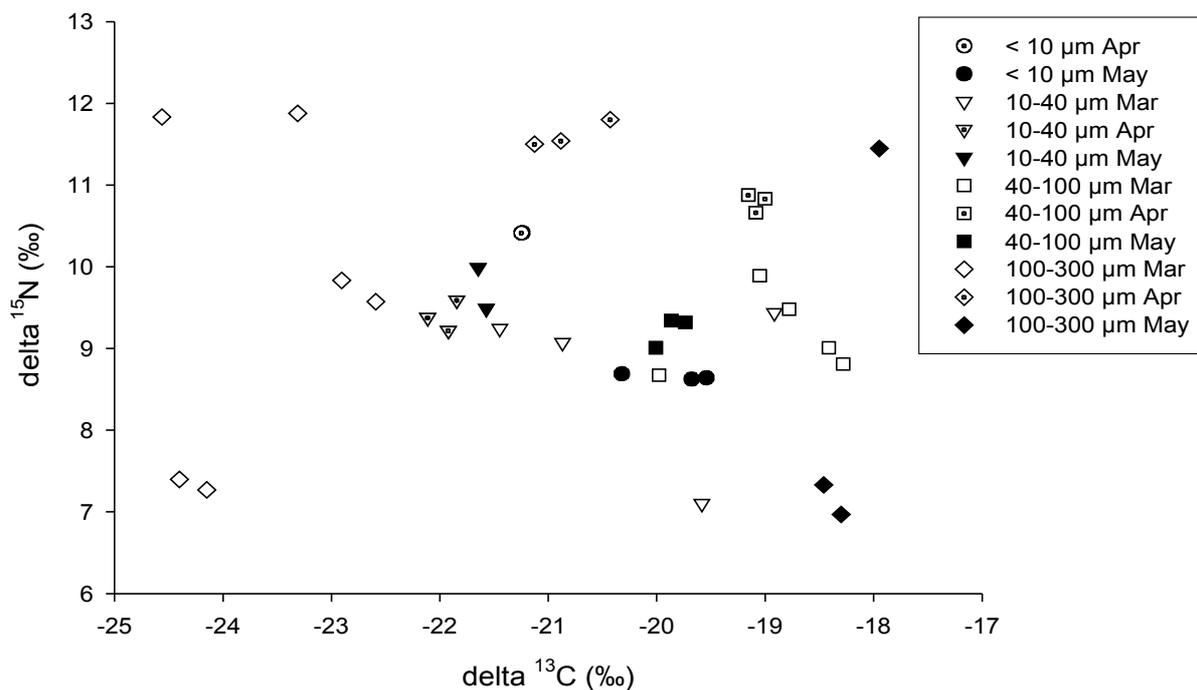


Fig. 5.3: Stable isotopes in size fractions during 3 spring phases of phytoplankton development.

phase with maximum phytoplankton carbon dominated by *Phaeocystis* and lowest C:N. In March (open symbols), inter-sample variation was much higher than in April and May (Fig. 5.3). Highest trophic levels as denoted by high $\delta^{15}\text{N}$ were reached by the largest size fraction (diamonds), but also four out of five of the lowest $\delta^{15}\text{N}$ values were found in the largest size fraction. Probably, as organisms in this fraction are scarcer coincidental catches of organisms played a more important role than in the other size classes. Moreover, large single cell diatoms and chain forming diatoms were probably an additional reason for reduced $\delta^{15}\text{N}$ levels. Some higher level heterotrophic organisms may have been overlooked when removing visible zooplankton from the filters. In March, the largest fraction samples were more ^{13}C depleted than all other samples. In April (dotted diamonds), the large size class displayed intermediate $\delta^{13}\text{C}$ ratios combined with highest $\delta^{15}\text{N}$. May samples (filled diamonds) were among those with highest $\delta^{13}\text{C}$ of all samples. The smallest size class was not measured in March. In April the replicates were grouped closely together and were laying near the 10-40 μm size class. In May the <10 μm fraction was grouped together with the 40-100 μm fraction (filled squares) at a $\delta^{15}\text{N}$ about 9 ‰ and a $\delta^{13}\text{C}$ of about -20 ‰. April samples of the latter size class (dotted squares) were on the highest trophic level. March samples of the 40-100 μm size class (open squares) had higher and May samples had lower $\delta^{13}\text{C}$ ratios compared to April

Chapter 5: Stable isotopes and reproductive success

samples. The 10-40 μm class (top-down triangles) was quite homogeneously grouped together throughout all spring phases ($\delta^{15}\text{N}$: 9 to 10; $\delta^{13}\text{C}$: -22.5 to -21) with the exception of 2 March samples (open symbols). Both had higher $\delta^{13}\text{C}$ ratios, and one of them was on a lower $\delta^{15}\text{N}$ level (7 ‰).

Copepod stable isotope patterns were correlated to the seston isotope patterns (Fig. 5.4). The correlation of *T. longicornis* $\delta^{15}\text{N}$ with seston was significant ($r^2=0.24$, $p=0.004$). The correlation of *T. longicornis* $\delta^{13}\text{C}$ with seston ($r^2=0.14$, $p=0.04$) was weaker. In *A. clausi*, the $\delta^{15}\text{N}$ correlation with seston was weaker ($r^2=0.27$, $p=0.03$) than that of $\delta^{13}\text{C}$ ($r^2=0.38$, $p=0.01$). However, a negative relationship between all *T. longicornis* $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ($N=113$, $r^2=0.08$, $p=0.002$) as compared to a weak positive relationship of the two stable isotope ratios in the seston ($N=189$, $r^2=0.02$, $p=0.03$) hint to a selective use of the total seston source.

A comparison of the stable isotopes of both species during spring revealed that *T. longicornis* (Fig. 5.5a) was feeding on a more ^{13}C enriched source than *A. clausi* (Fig.

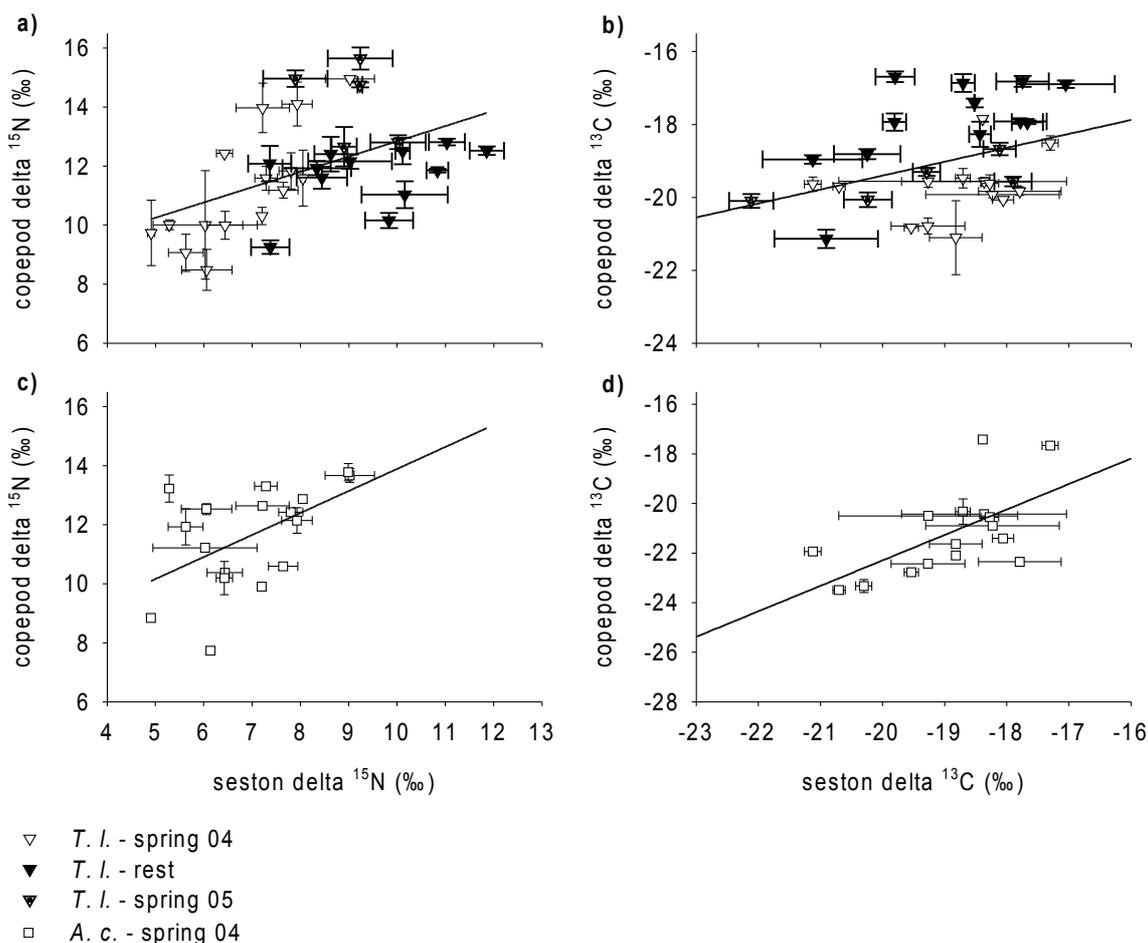


Fig. 5.4: Copepod stable isotopes versus seston stable isotopes with regression lines ($N=3-6$, means \pm SD); occasionally only one parallel.

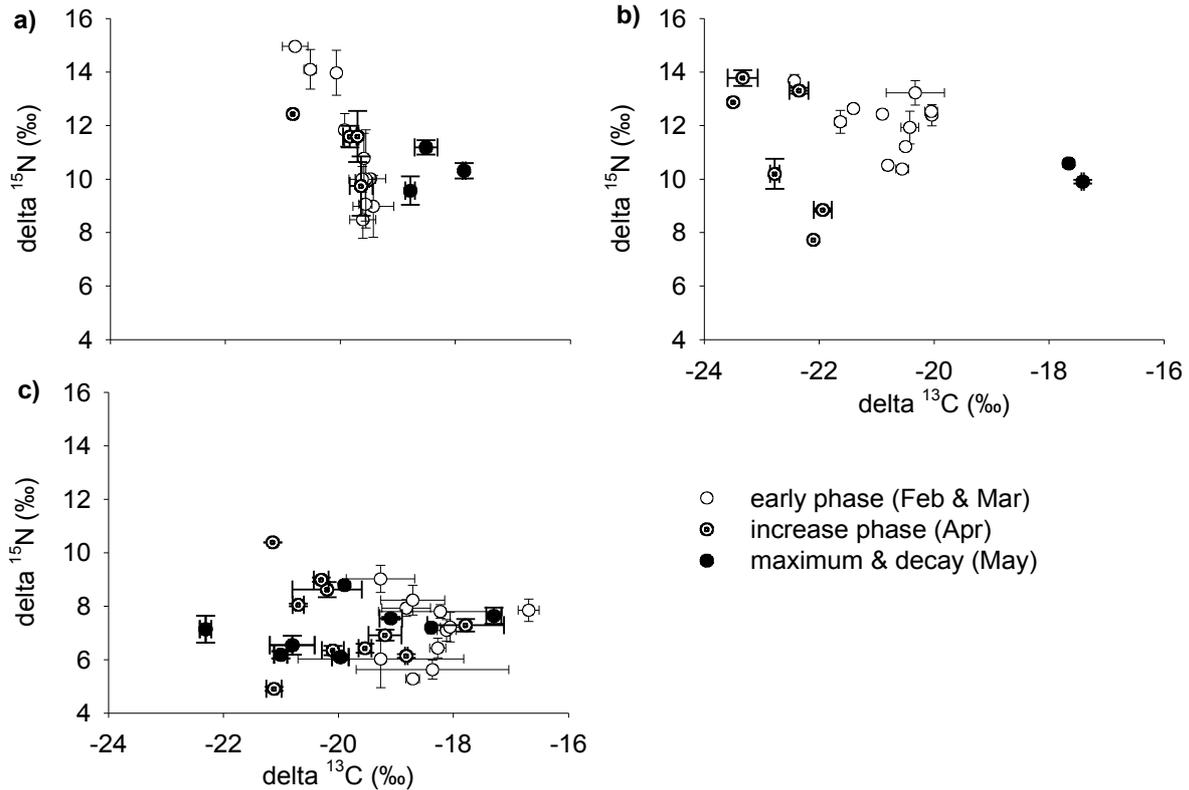


Fig. 5.5: Stable isotopes of a) *Temora longicornis*, b) *Acartia clausi* and c) seston during spring 2004, phytoplankton bloom phases signified by different symbols.

5.5b) except in May. In the early spring phase *T. longicornis* was grazing on highest $\delta^{15}\text{N}$ levels but switched to very low levels during March, this indicates the usage of another main food source. The span of $\delta^{13}\text{C}$ depicts that *A. clausi* was utilizing carbon sources of greater variability than *T. longicornis*. Seston $\delta^{15}\text{N}$ was generally lower than copepod $\delta^{15}\text{N}$ but the lowest $\delta^{13}\text{C}$ were still higher compared to copepod values (Fig. 5.5c). During the phytoplankton increase phase *A. clausi* acquired more

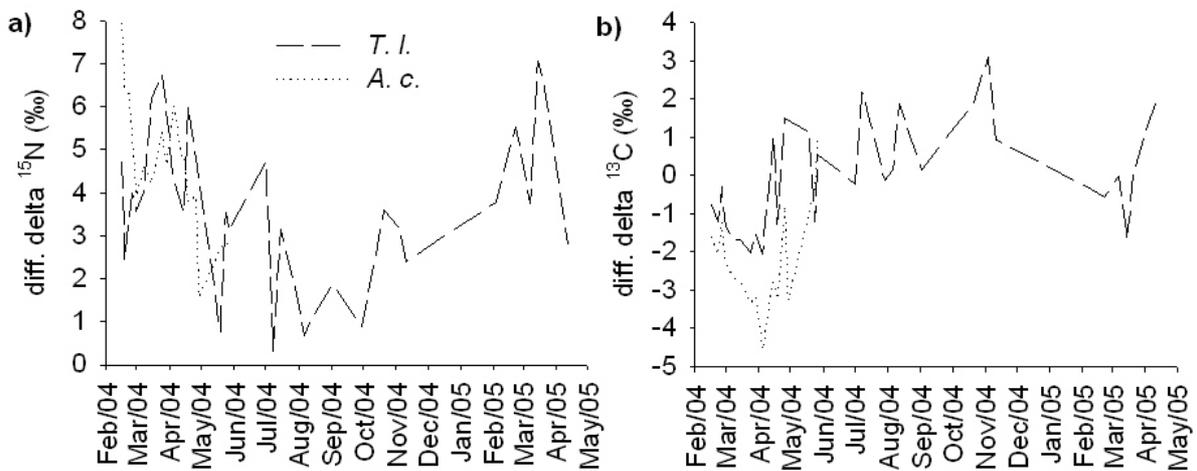


Fig. 5.6 Difference between *Temora longicornis*/*Acartia clausi* and seston a) $\delta^{15}\text{N}$ and b) $\delta^{13}\text{C}$; (N=3, means, no copepod results in December and January).

Chapter 5: Stable isotopes and reproductive success

^{13}C depleted carbon than at other times. The two copepods had a similar $\delta^{15}\text{N}$ signature distance to seston during spring that only deviated in early February, being higher in *A. clausi* (Fig. 5.6a). In March and April, the distances between copepods and seston appear to reach maxima denoting a relative increase of the phytoplankton proportion in the environment that decreases the seston $\delta^{15}\text{N}$ signal. During summer the heterotroph proportions of seston increased to maximum levels (Fig. 5.2a) probably reducing the distance of the copepods to the seston signal. *A. clausi* kept a constantly larger, more depleted distance to seston $\delta^{13}\text{C}$ as *T. longicornis* during early spring 2004. The negative distance between *T. longicornis* and seston $\delta^{13}\text{C}$ began to change from mid April on, the copepod becoming more enriched compared to seston; *A. clausi* followed the same trend in May. The distance of *T. longicornis* to seston $\delta^{13}\text{C}$ ranged between 0 and +2 throughout summer. During spring 2005 *T. longicornis* became depleted in comparison to seston similarly to spring 2004 but for a shorter period. This indicates highly selective feeding on a carbon source with a low $\delta^{13}\text{C}$ during spring when phytoplankton food was abundant.

Both stable isotope ratios showed significant relationships with copepod egg production and body biochemistry and physiology. Egg production was negatively correlated to seston $\delta^{13}\text{C}$ in *T. longicornis* ($r^2=0.17$, $p=0.02$) and *A. clausi*

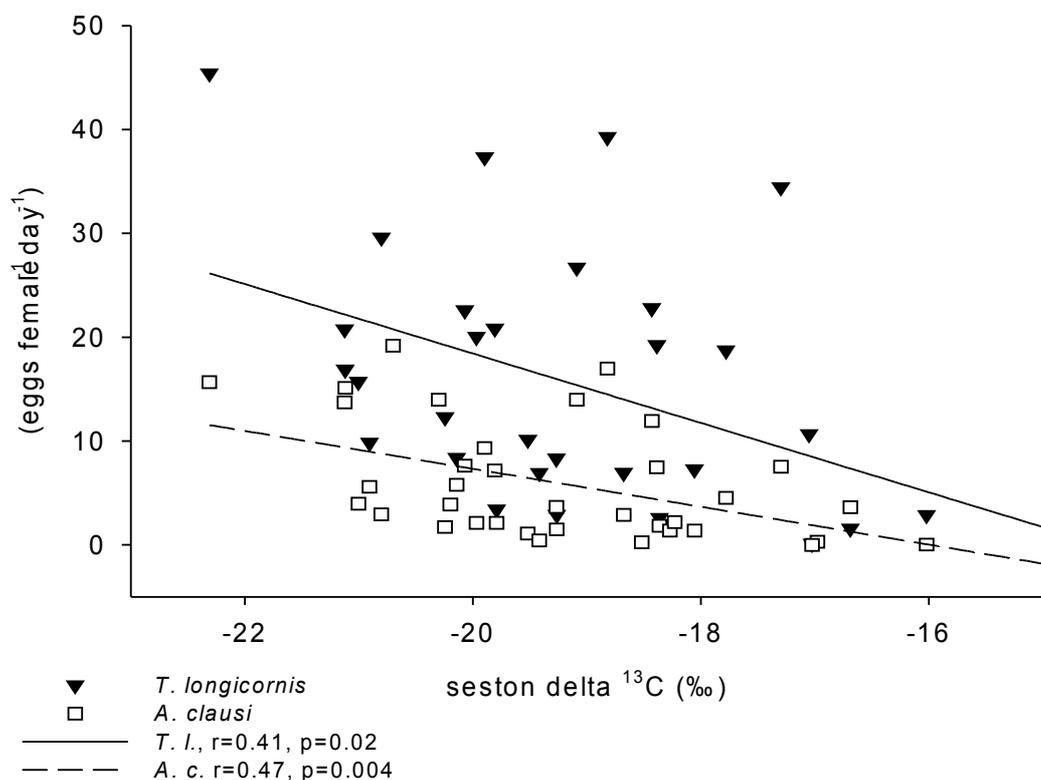


Fig. 5.7: Egg production of copepods is negatively correlated to seston $\delta^{13}\text{C}$ over the year.

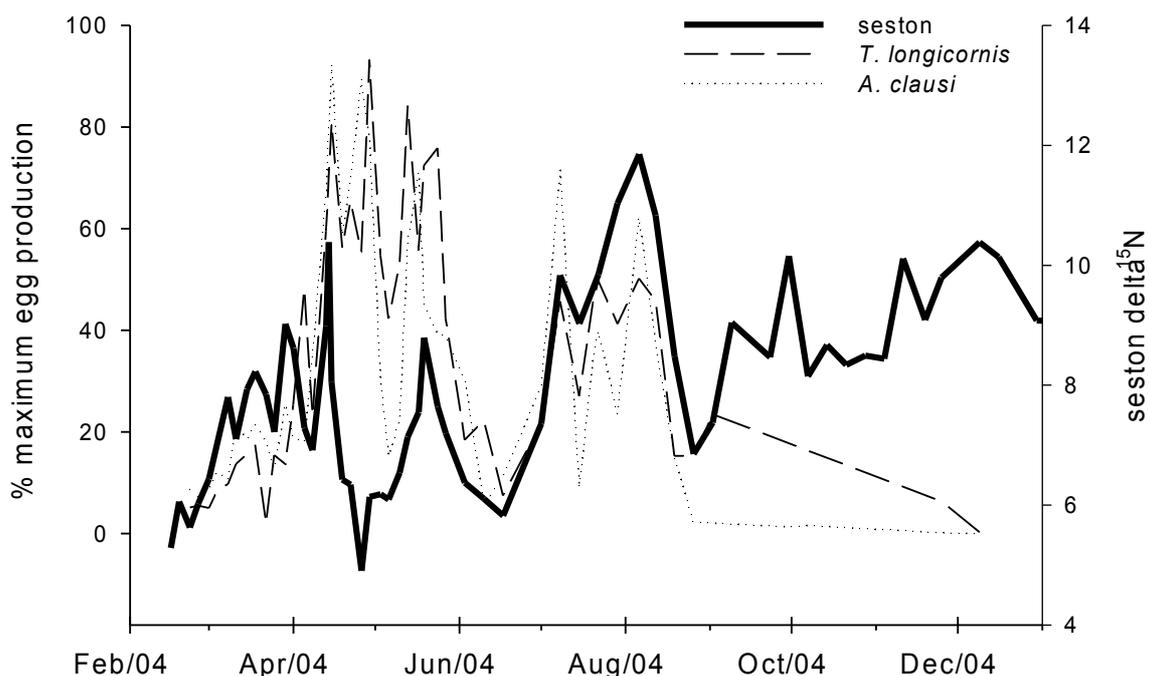


Fig. 5.8: Egg production pattern of *A. clausi* and *T. longicornis* during 2004 as compared with seston $\delta^{15}\text{N}$.

($r^2=0.22$, $p=0.004$) (Fig. 5.7). The seston C:N correlated with the seston $\delta^{13}\text{C}$ ratio, the correlation was highly significant ($r^2=0.62$, $p<0.00001$). And, seston C:N was strongly correlated to egg production (chapter 4). The seston $\delta^{15}\text{N}$ was not linearly correlated to egg production over the year in *A. clausi* and *T. longicornis*. However, comparing the graphs of relative *T. longicornis* and *A. clausi* egg production with the seston $\delta^{15}\text{N}$ signal a possible dynamically time-shifted (probably due to a temperature effect) modulating pattern is recognizable (Fig. 5.8). Prominent decreases in egg production were preceded by decreases in seston $\delta^{15}\text{N}$ during spring and directly accompanied by decreases in the seston signal during summer when highest temperatures prevailed.

In autumn the pattern is probably decoupled due to food limitation and decreasing temperatures. The seston $\delta^{15}\text{N}$ was negatively correlated with copepod C:N ($r^2=0.34$, $p=0.02$) (Fig. 5.9). Starving copepods are protein depleted and have therefore higher C:N ratios (chapter 3). Oppositely, a low C:N ratio indicates good copepod fitness and concomitantly high egg production ability. Copepods have high turnover rates; therefore, a seston signal can leave its traces within a day when temperature is high. At lower temperatures metabolic rates decrease and seston signals are integrated over longer time spans.

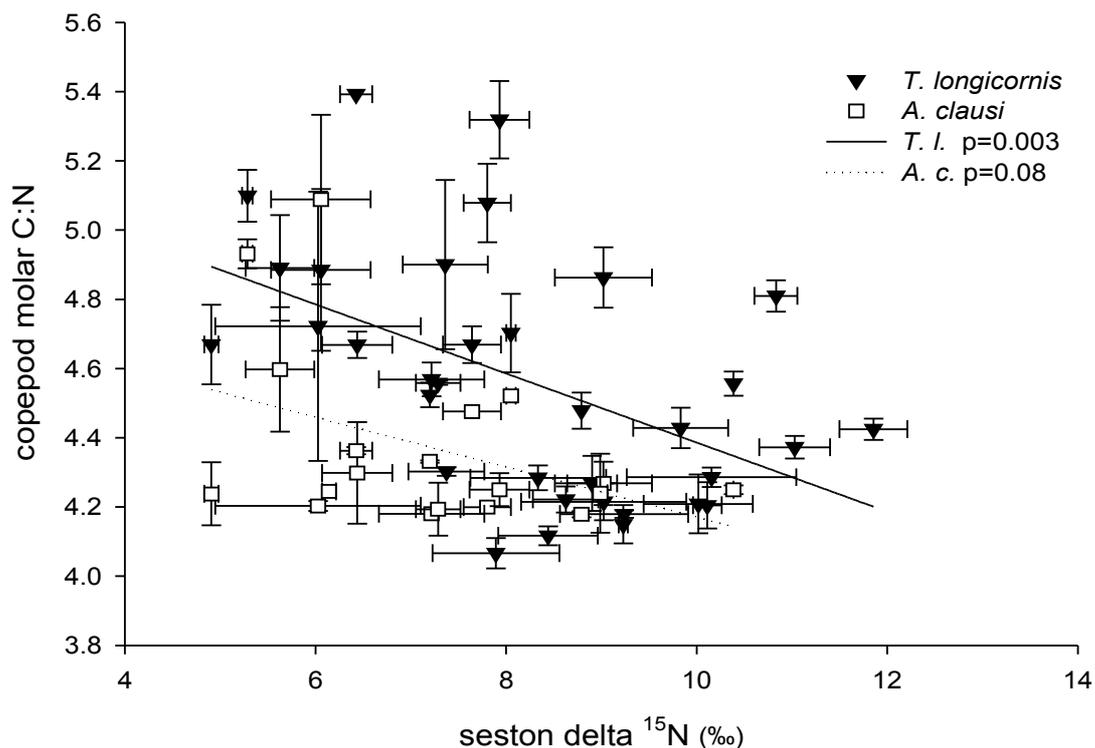


Fig. 5.9: Correlation of seston $\delta^{15}\text{N}$ with copepod C:N ($N=3$, means \pm SD), linear regressions for both species: *T. l.*-C:N = $5.4 - 0.1 * \text{seston } \delta^{15}\text{N}$ ($r=0.49$; $r^2=0.24$, $F=10.2$, $p=0.003$); *A. c.*-C:N = $4.9 - 0.07 * \text{seston } \delta^{15}\text{N}$ ($r=0.41$, $r^2=0.17$, $F=3.4$, $p=0.08$).

Protein and digestive enzyme activities

In both species water soluble protein contents increased throughout the season. In *A. clausi* all values were significantly different from each other ($p < 0.001$), in *T. longicornis* the protein content in February did not differ significantly from that in March, but all other values were significantly different from each other. Relative protein content about doubled in *A. clausi* while it stayed relatively stable in *T. longicornis* (Table 5.2). Activities of the same enzymes measured between February and May were significantly different between both species (Student-t-Test, $p < 0.05$) with the exception of alanine aminopeptidase. In *A. clausi* the seasonal patterns of arginine aminopeptidase (Fig. 5.10a), esterase/lipase (Fig. 5.10c) and chitobiase activity (Fig. 5.10d) were similar with lowest activities in March. However, seasonal patterns of arginine aminopeptidase (Fig. 5.10a) and esterase/lipase (Fig. 5.10c) were not significant.

Lowest alanine aminopeptidase activities in April were significantly different from the other months (LSD-Test, $p < 0.05$, Fig. 5.10b). Beta-Glucosidase activity was constantly rising, activities in May being significantly different from the other months (LSD-Test, $p < 0.05$). Enzyme activities of *T. longicornis* followed other seasonal

Chapter 5: Stable isotopes and reproductive success

patterns. However, seasonal activity patterns of arginine (Fig. 5.10a) and alanine aminopeptidase (Fig. 5.10b) and esterase/lipase (Fig. 5.10c) were not significant.

Table 5.2: Absolute and relative protein contents on dry mass (dm) of *A. clausi* and *T. longicornis* ($N=5-10$, means \pm SE).

Date	<i>Temora longicornis</i>			<i>Acartia clausi</i>		
	Prot (μ g)	SE	Prot dm ⁻¹ (%)	Prot (μ g)	SE	Prot dm ⁻¹ (%)
26/02/04	6.7	\pm 0.6	25.3	2.5	\pm 0.2	31.2
08/03/04	8.0	\pm 0.6	26.2	3.5	\pm 0.2	48.5
15/04/04	11.9	\pm 1.1	24.4	7.1	\pm 0.5	59.9
24/05/04	15.6	\pm 0.9	32.3	5.3	\pm 0.3	64.3
08/07/04	11.9	\pm 0.7	32.3			
20/07/04	7.9	\pm 0.2	23.7			
06/08/04	10.6	\pm 0.7	26.9			
02/09/04	7.2	\pm 0.5	29.3			
30/09/04	6.3	\pm 0.4	24.6			
21/10/04	13.1	\pm 0.8	29.9			
11/11/04	9.7	\pm 1.0	30.6			
03/02/05	8.2	\pm 1.1	41.0			
08/03/05	7.9	\pm 1.1	30.6			
12/04/05	12.7	\pm 0.6	26.9			
10/05/05	10.7	\pm 0.6	25.2			
24/06/05	9.9	\pm 0.6	26.4			

Table 5.3: Seston and/or copepod parameters with correlations to digestive enzymes, significant correlations < 0.05 in bold, correlations < 0.1 in bold + italics; TI – *Temora longicornis*, dm – dry mass, ala – alanine aminopeptidase, arg – arginine aminopeptidase, est – esterase/lipase, chito – chitinase, b-gluc – beta-glucosidase.

	r(X,Y)	r ²	t	p
seston $\delta^{15}\text{N}$				
ala	0.34	0.12	1.21	0.2516
arg	0.55	0.30	2.19	0.0510
est	0.58	0.33	2.35	0.0383
chito	-0.44	0.20	-1.64	0.1288
b-gluc	0.60	0.36	2.49	0.0301
TI C:N				
ala	-0.37	0.14	-1.31	0.2161
arg	-0.62	0.38	-2.61	0.0243
est	-0.59	0.34	-2.40	0.0353
chito	0.85	0.72	5.33	0.0002
b-gluc	-0.38	0.14	-1.34	0.2059
TI $\delta^{13}\text{C}$				
ala	0.45	0.20	1.67	0.1239
arg	0.48	0.23	1.82	0.0963
est	0.57	0.33	2.31	0.0410
chito	-0.65	0.42	-2.82	0.0168
b-gluc	0.29	0.09	1.02	0.3297

Chapter 5: Stable isotopes and reproductive success

Arginine showed a tendency to decrease from February to May. Chitobiase (Fig. 5.10d) and beta-glucosidase (Fig. 5.10e) activities followed the same pattern with highest activities in April and lowest values in February and May. Alanine aminopeptidase activity showed a tendency to the same activity pattern but seasonal differences within spring were not significant.

Comparing enzyme activities of *T. longicornis* with seston stoichiometric and stable isotope parameters revealed some significant correlations, results with a probability of $p < 0.06$ are also noted (Table 5.3). The only seston parameter that was significantly correlated to any enzyme activity was the seston $\delta^{15}\text{N}$. It was positively correlated with arginine aminopeptidase ($p = 0.05$), esterase/lipase and beta-glucosidase activities. This indicates a possible link of these enzymes to carnivorous feeding. Chitobiase activity showed a highly significant positive correlation with copepod C:N, and arginine aminopeptidase and esterase/lipase activities a negative

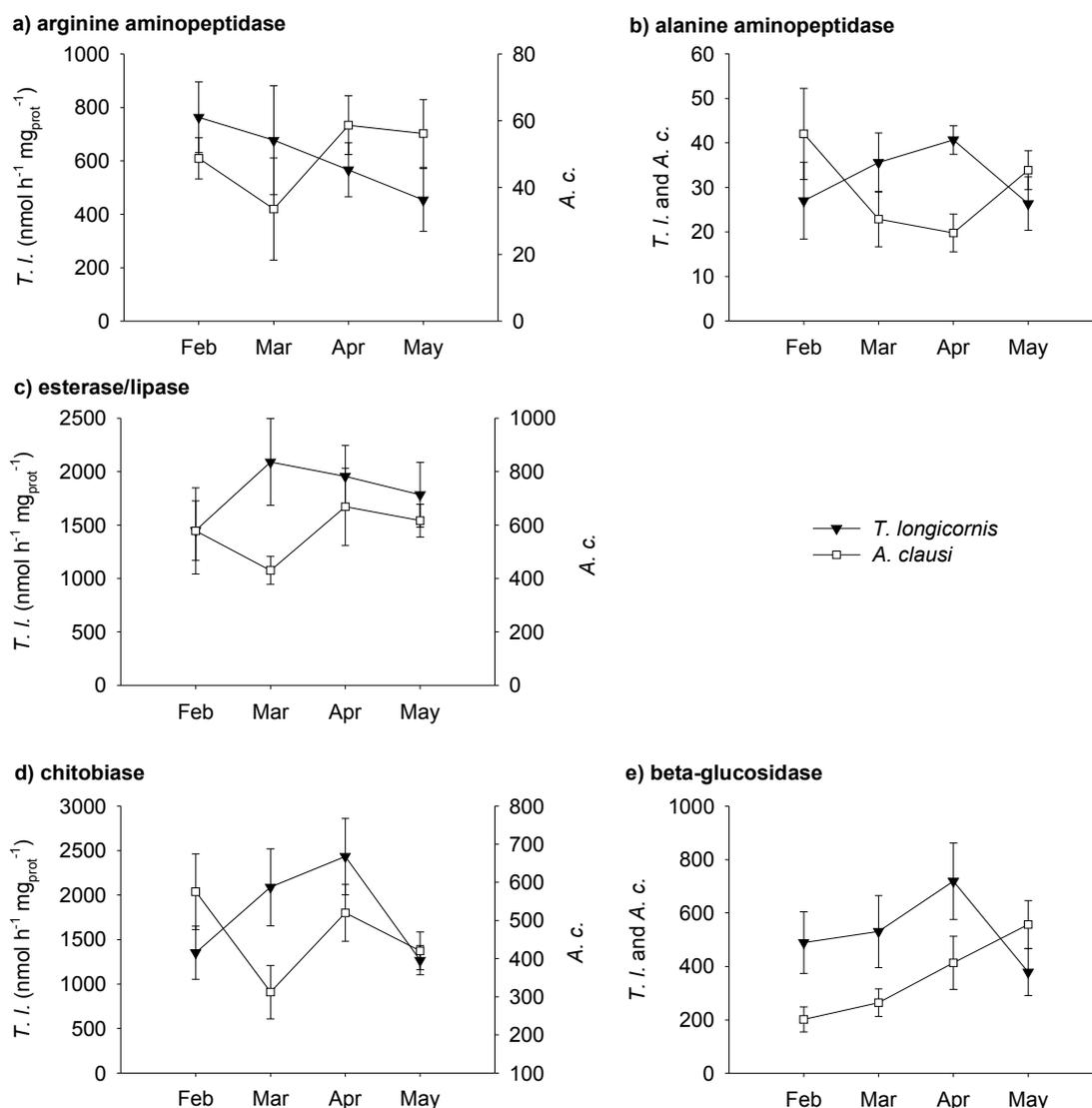


Fig. 5.10: Digestive enzymes during spring in *Acartia clausi* and *Temora longicornis* ($N=6$, means \pm SE).

Chapter 5: Stable isotopes and reproductive success

correlation to copepod C:N. Therefore, chitinase activity indicates carbon assimilation, while arginine aminopeptidase, esterase/lipase and beta-glucosidase indicate the assimilation of nitrogen.

The copepod $\delta^{13}\text{C}$ ratio was negatively correlated with chitinase activity and positively with arginine aminopeptidase and esterase/lipase activity. Hence, a low copepod $\delta^{13}\text{C}$ denotes high chitinase activity accompanied by high C assimilation. No direct correlations of egg production with enzyme activities could be observed (there were too few overlapping data points with enough variance).

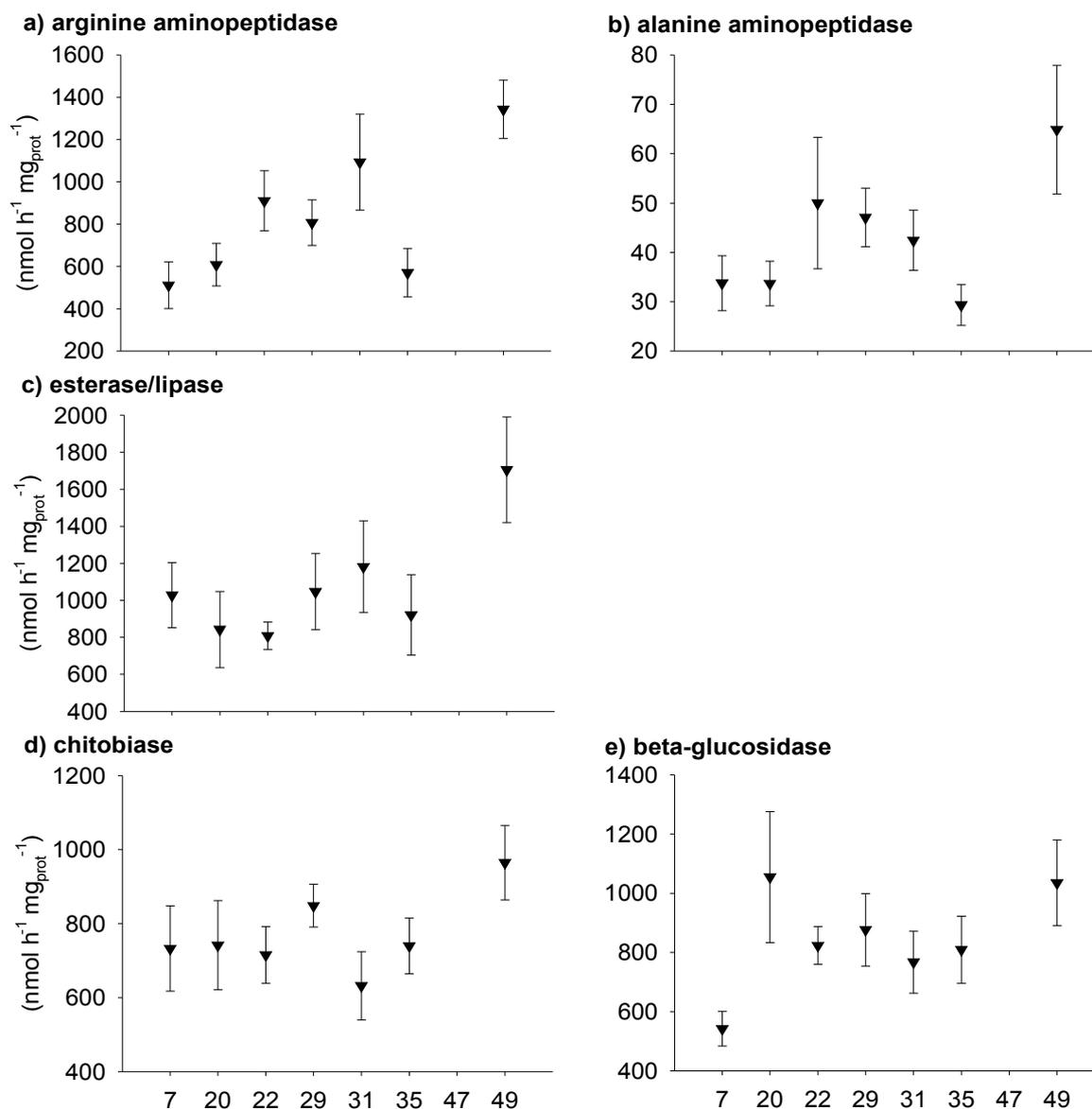


Fig. 5.11 *Temora longicornis*: specific enzyme activities in the German Bight, June 2004 ($N=6$; means \pm SE).

Spatial study

Measurements of *T. longicornis* enzymatic (Fig. 5.11), biochemical and stable isotope (Fig. 5.12) and also seston stoichiometric parameters (Fig. 5.13) at 7 stations (Fig. 5.1) distributed over the German Bight and adjacent areas of the southern North Sea revealed high spatial variability. The stations were sampled within 5 days in June 2004. The arginine aminopeptidase of *T. longicornis* was the enzyme that had the highest variability between stations (Fig. 5.11a). Enzyme activities at station 49 close to the Channel had highest over all enzyme activities of all stations. No correlations of enzyme activities with any seston or animal parameters were found within this spatial data set. The control of enzyme activity is very complex and may not be easily assigned to bulk food parameters because copepods can feed selectively and adapt

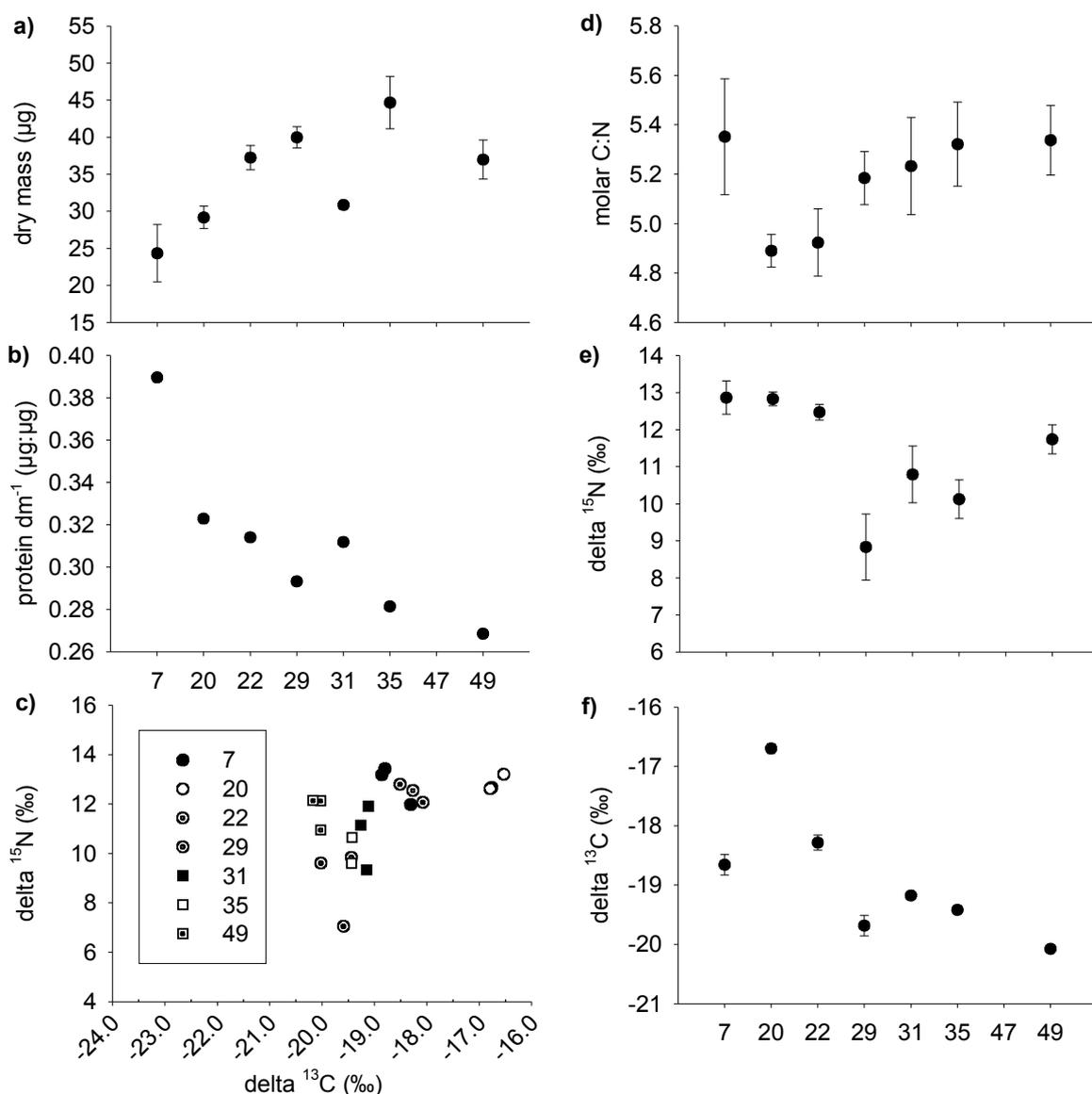


Fig. 5.12 Biochemical and stable isotope data of *T. longicornis* in the southern North Sea: a) dry mass, b) protein proportion of dry mass, c) copepod $\delta^{15}\text{N}$ versus $\delta^{13}\text{C}$., d) molar C:N, e) $\delta^{15}\text{N}$, f) $\delta^{13}\text{C}$ (means \pm SE).

Chapter 5: Stable isotopes and reproductive success

their enzyme activities to the environmental conditions they encounter to optimise energy transfer (chapters 2 and 3).

T. longicornis dry mass (Fig. 5.12a) and prosome length, width, N, C, and protein content varied significantly between stations (ANOVA, $p < 0.03$). The proportion of body protein on dry mass (Fig. 5.12b) was positively correlated with C, N and P content of the two small seston size fractions ($p < 0.03$). However, the copepod molar C:N (Fig. 5.12d) did not differ significantly between stations. The copepod stable isotope signatures were significantly different between stations (Fig. 5.12c, e, f). The C (Fig. 5.13a) and N (Fig. 5.13b) contents and molar C:N (Fig. 5.13e) of all seston size fractions varied significantly between stations (ANOVA, $p < 0.003$).

C and N contents of all seston size fractions except for the largest (30-100 μm) were negatively correlated with body mass and body C and N contents ($p < 0.04$). Copepod dry mass, C and N content were negatively correlated with seston P content of the 10-20 μm seston size class ($p < 0.05$). These correlations could be interpreted in a way, that the smaller seston particles do not support favourable energy transfer to copepods. Copepod C:N ratio was positively correlated with seston C:P (Fig. 5.13f) in the 30-100 μm size class ($p < 0.03$). The C:P ratio of the 30-100 μm size class was negatively correlated to both stable isotope ratios. So, indirectly one can say that the stoichiometric quality in terms of P proportion in this large seston class leads to a high quality and fitness of the copepod. The N:P ratio (Fig. 5.13g) of this seston size class was correlated with body $\delta^{13}\text{C}$ ($p < 0.05$).

5.4. Discussion

Internal factors such as selective feeding and selective digestion control how different species utilize their environment. The stable isotope patterns of grazers reflect the stable isotope signatures of their food. Therefore, differences in stable isotope patterns between co-occurring species hint to selective assimilation abilities, no matter whether controlled by selective feeding or by selective digestion.

Stable isotopes

We found an inverse relationship between seston $\delta^{15}\text{N}$ and copepod molar C:N. Low copepod molar C:N as well as a high protein:dry mass ratio are favourable for egg production (Checkley 1980a). The higher the seston $\delta^{15}\text{N}$ value, the higher is the proportion of heterotroph particles in the seston, or in other words the potential for carnivorous feeding for copepods. Starving and N limited zooplankton loose body

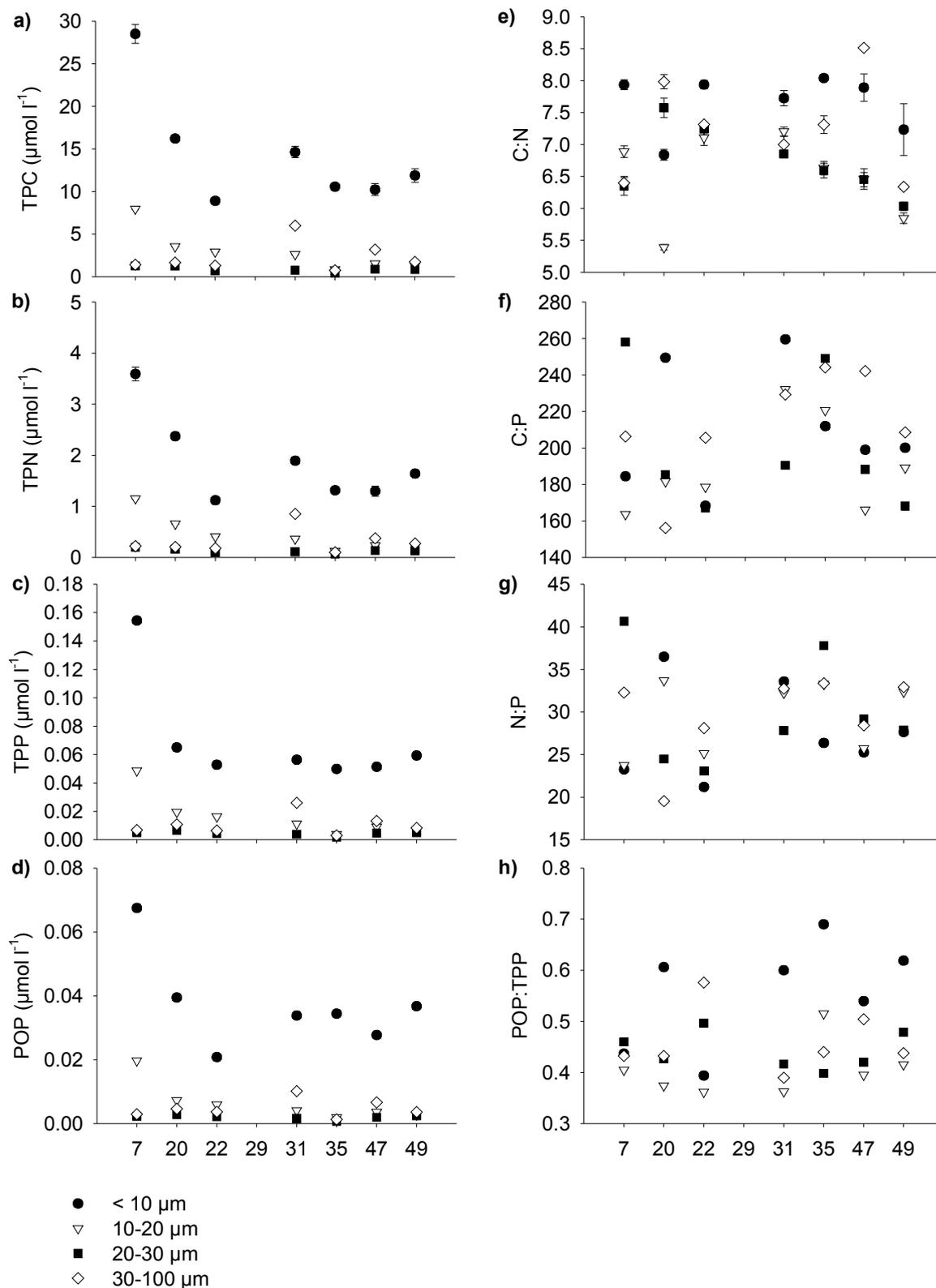


Fig. 5.13 Seston quantity and quality parameters at GLOBEC stations in the North Sea, June, 2004 (N=3; means \pm SE or average ratios).

protein (and N), hereby increasing their C:N (Adams and Sterner 2000; chapter 3). The same algae, grown under nutritional replete and depleted conditions, can cover a $\delta^{15}\text{N}$ range of 6 ‰ (Adams and Sterner 2000). Starving *Daphnia* showed

Chapter 5: Stable isotopes and reproductive success

enrichment of ^{15}N , probably due to the catabolism of body proteins, and the concomitant increased excretion of ^{14}N compared to ^{15}N (Adams and Sterner 2000). Accordingly, light ^{12}C is preferably excreted over heavy ^{13}C (Klein Breteler et al. 2002).

In spring, concomitantly with increasing phytoplankton biomass, copepod reproduction increased with decreasing $\delta^{13}\text{C}$. Both copepod species contained a lower proportion of heavy ^{13}C than the bulk seston, indicating highly selective feeding on a source with a relatively low proportion of ^{13}C compared to bulk seston. In spring 2005 the seston $\delta^{13}\text{C}$ dropped more dramatically than in 2004. But *T. longicornis* fed on a similar food source as in spring 2004 in respect to $\delta^{13}\text{C}$. In the summer months, in autumn, and in winter, copepods exhibited a $\delta^{13}\text{C}$ signature that was less depleted in heavy isotope than bulk seston. Therefore, selective feeding may be most prominent during spring and less so during other seasons.

An increase in seston $\delta^{13}\text{C}$ values can signify nutrient limitation. Under phosphorus limitation a marine diatom accumulated heavy ^{13}C with a similar rate as carbon enriched phytoplankton (Gervais and Riebesell 2001). The positive correlation of seston N:P (30-100 μm size class) with body $\delta^{13}\text{C}$ at the North Sea stations does support this finding. Larger phytoplankton was possibly enriched in ^{13}C due to phosphorus depletion, and this effect may be transferred to copepods. Otherwise, diatoms in the growth phase are enriched in ^{13}C ($\delta^{13}\text{C}$ ranging from -15 to -19) compared to most particulate matter that is rather ^{13}C depleted ($\delta^{13}\text{C}$ ranging from -21 to -25) (Fry and Wainright 1991). *T. longicornis* $\delta^{13}\text{C}$ was only below -20 ‰, when being on very high $\delta^{15}\text{N} > 12$ ‰ at the same time. However, marine microalgal $\delta^{13}\text{C}$ can vary greatly from -30.2 to -12.7 in different species and under varying growth conditions (Leboulanger et al. 1995).

In winter, high average wind speeds mix the water column of the shallow North Sea areas. The detritus proportion on seston increases. Detritus is usually depleted in $\delta^{13}\text{C}$ (Bouillon et al. 2000) and an inferior food source for copepods (Mayzaud et al. 1998). High seston C:N together with low seston $\delta^{13}\text{C}$ would be an indicator of high amounts of detritus and benthic material or of freshwater influence. Low salinity coastal water is influenced by river run-off, and therefore, contains high nutrient loads compared to offshore water and terrestrial material. Nitrogen input makes algae lighter and decreases $\delta^{15}\text{N}$, riverine phytoplankton is depleted of ^{13}C compared to

Chapter 5: Stable isotopes and reproductive success

oceanic phytoplankton and can have similar low values as terrestrial material of -28‰ (Peterson 1999).

Digestive enzymes

Both species adapt their digestive enzymes to the same food environment in a species-specific way. The strong increase in relative protein content during the spring bloom in *A. clausi* as compared to *T. longicornis* denotes a protein limitation in *A. clausi* in the beginning that is decreasing during the observed period. Either, *T. longicornis* was not protein limited or the ingested food did not support increased protein assimilation. The species-specific sets of digestive enzymes may enable the two species to utilise deficient particles in a way that circumvents imbalances (Anderson et al. 2004). The lower enzyme activities in March and April 2004 may denote an increased ingestion of easily digestible heterotrophic food or food with lower protein contents.

The specific set of digestive isozymes within some crustacean species is rather genetically determined than environmentally (Sainz et al. 2005; chapter 3). This genetic determination of the digestive system explains food choice in amphipods (Guarna and Borowsky 1993; Sotka 2003) and may also control food selection in copepods. Stable isotope analysis of copepods and seston hints to selective feeding on the available seston components. Enzyme activities could be related to seston and body stable isotopes. High chitinase activity was correlated to a high body C proportion and a rather depleted body $\delta^{13}\text{C}$. The seston $\delta^{13}\text{C}$ was inversely correlated to egg production in *T. longicornis* and other copepods. Further, as stated above, nitrogen limited growth decreases the phytoplankton $\delta^{15}\text{N}$ and leads to a high body C:N of cladocerans (Adams and Sterner 2000). Accordingly, we found a negative correlation between seston $\delta^{15}\text{N}$ and copepod C:N. The negative correlation of arginine aminopeptidase and esterase/lipase with copepod C:N and the positive correlation to seston $\delta^{15}\text{N}$ may hint to a compensatory mechanism on the digestive physiology level of *T. longicornis* to circumvent nitrogen imbalances. But more probably it indicates high utilization rates of heterotroph organisms in the seston. This view is supported by the correlation of increasing seston $\delta^{15}\text{N}$ with decreasing body C:N. Arginine aminopeptidase cleaves arginine and lysine from oligopeptides. Esterase/lipase hydrolyses carboxylic esters and thioesters. High enzyme activities usually denote the importance of the substrate in the nutrition of the animal. Nevertheless, high activities are also found when the substrate is not easily

Chapter 5: Stable isotopes and reproductive success

digestible, for example autotroph protein sources require higher protease levels than heterotrophic sources (Jones et al. 1997; chapter 1). And, at low substrate concentrations high enzyme activities may be needed to compensate for threatening food limitation or to prepare the digestive system for compensatory feeding when the next food patch is foraged (Harris et al. 1986). *T. longicornis* and *A. clausi* are adapted to a patchy food environment where they can encounter short-term food limitation but do not experience long term starvation.

No clear patterns of biochemical and physiological parameters were discernible between stations or water masses in the southern North Sea. The range of stable isotopes in *T. longicornis* was similar over all stations including the Helgoland station. The animals may use a similar total spectrum of food over all stations and in time. The C:N ratios did not differ much but tended to be lower at German Bight stations 20 and 22. The most significant variability between stations was seen within the range of arginine aminopeptidase activities, relative protein contents, and delta ^{13}C ratios. The highest arginine aminopeptidase activity occurred at the Channel station (49), the lowest at the most estuarine station (7). The most remote station 35 showed similar enzyme patterns as station 7 and 20 in the German Bight and the highest dry mass combined with low relative protein content. *T. longicornis* did rarely show $\delta^{13}\text{C}$ below -20 ‰ and if, then in combination with a high $\delta^{15}\text{N}$. Such a signature combination was seen at the Channel station 49. At this station individual dry mass was among the highest, the relative protein content was the lowest of all stations, combined with over-all highest enzyme activities, and higher arginine aminopeptidase than chitobiase activity. The enzyme activities and the low protein proportion do hint to food limitation. Highest $\delta^{15}\text{N}$ values were found at station 49, and at the German Bight stations except for the northernmost station (31). The highest relative protein content at station 7 closest to the Elbe and Weser estuaries was combined with the lowest individual dry mass of all stations and the lowest beta-glucosidase activity but a high ratio of chitobiase to arginine aminopeptidase activity. The enzyme activities were overall low. At the northern offshore station (29) the combination of the lowest $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were found implying a more herbivorous food source than at the other stations.

Conclusions

Seston stable isotopes, especially the $\delta^{13}\text{C}$ can be seen and implemented as a general modulator of egg production in copepods despite differences between

Chapter 5: Stable isotopes and reproductive success

copepod species. At certain times such as spring, the seston $\delta^{15}\text{N}$ pattern may be used to predict copepod egg production with a time shift of about 10-15 days. Further studies of seston-zooplankton- $\delta^{15}\text{N}$ relations are necessary to strengthen this statement. Digestive enzymes may indeed be used as additional tools to signify species-specific selective feeding. And, at least chitobiase and arginine aminopeptidase activities in adult copepods seem to depict the nutritional status of the animal and therefore reflect the reproductive ability of females. The combination of these different measurements is certainly a promising avenue in our quest to unravel the factors that determine reproductive success of copepods in the world's seas.

Chapter 6: General discussion: synthesis and outlook

6.1. Synthesis

This study shows that internal parameters of copepods greatly influence the energy transfer between prey and grazer. Grazing behaviour and digestive system of copepods interact in mediating the environmental signals of nutritional quality to the copepod. Food quality was identified as an important parameter influencing egg production rates in copepods, more important than food quantity.

Temperature has been suggested as the major controlling factor of reproductive output in North Sea copepod species (Halsband and Hirche 2001), with food concentrations only having minor effects (Koski and Kuosa 1999; Halsband-Lenk et al. 2004). The present study and several other field studies have, however, shown that egg production is often decoupled from temperature and food concentration and rather depends on other factors such as food quality in terms of C:N:P ratios (chapter 3), fatty acids (Jónasdóttir et al. 1995; Arendt et al. 2005), amino acids (Guisande et al. 2000; Guisande et al. 2002) or species composition with species that have different biochemical qualities (Kleppel and Burkart 1995; Schmidt et al. 1998).

However, a temperature effect on copepod metabolism generally interacts with temperature effects on nutritional factors, and hence the direct and indirect effects of temperature are difficult to evaluate separately. Nevertheless, the results of this thesis suggest that temperature and food concentration should be seen as absolute switches setting a baseline which is modulated by the food quality effects on copepod nutritional condition and egg production ability.

Acartia clausi and *Temora longicornis* ingested nitrogen and phosphorus in ratios that suited their body elemental composition (chapter 4). Highest body specific elemental uptake rates were found when the concentrations of these elements in the seston were low. This indicates elemental food limitation at certain times, with resulting compensatory feeding, as was reported earlier for *A. tonsa* (Prince et al. 2006) or freshwater cladocerans (Plath and Boersma 2001). In the grazing experiments it was observed that the C:N of the ingested food was similar to seston C:N and the relative ingestion of POP compared to TPP also followed the seston signal. However, the C:N of ingested particles in relation to copepod body C:N revealed imbalances. With decreasing seston C:N (rising quality) the ingested excess carbon decreased, in principal lowering the imbalance between ingested C:N and

Chapter 6: General discussion

body C:N. This reduction of the stoichiometric imbalance should therefore lead to a gain in energy that should be available for egg production.

The imbalance of ingested food with copepod body stoichiometry may be less pronounced in reality than is indicated by this study. First, copepod acquisition of minerals and imbalances between ingested and body stoichiometric ratios were calculated under the assumption of homeostasis in grazers. However, the measured body C:N of *A. clausi* and *T. longicornis* does vary over a range of 1 carbon atom per nitrogen (Fig. 6.1). High variability in body C:N was obvious in *T. longicornis* while *A. clausi* rather switched from a high level body C:N ratio during high seston C:N to a lower level with minor variability during times of better stoichiometric food quality. Of course, it has to be remembered that one would expect a difference between body carbon and ingested carbon, since carbon is oxidised in respiratory metabolism, and dissipates as CO₂. In fact, a gross growth efficiency for carbon of around 30% is normal, which necessitates a higher relative uptake for carbon than for nitrogen, and the high molar rates of 2.5:1 ingested carbon:body carbon may not be that imbalanced. Nevertheless, in the instances of these high values, a change in the body C:N of the copepods was observed. It remains to be seen whether this is an adaptation to low food quality (reducing the imbalance between food and body ratios)

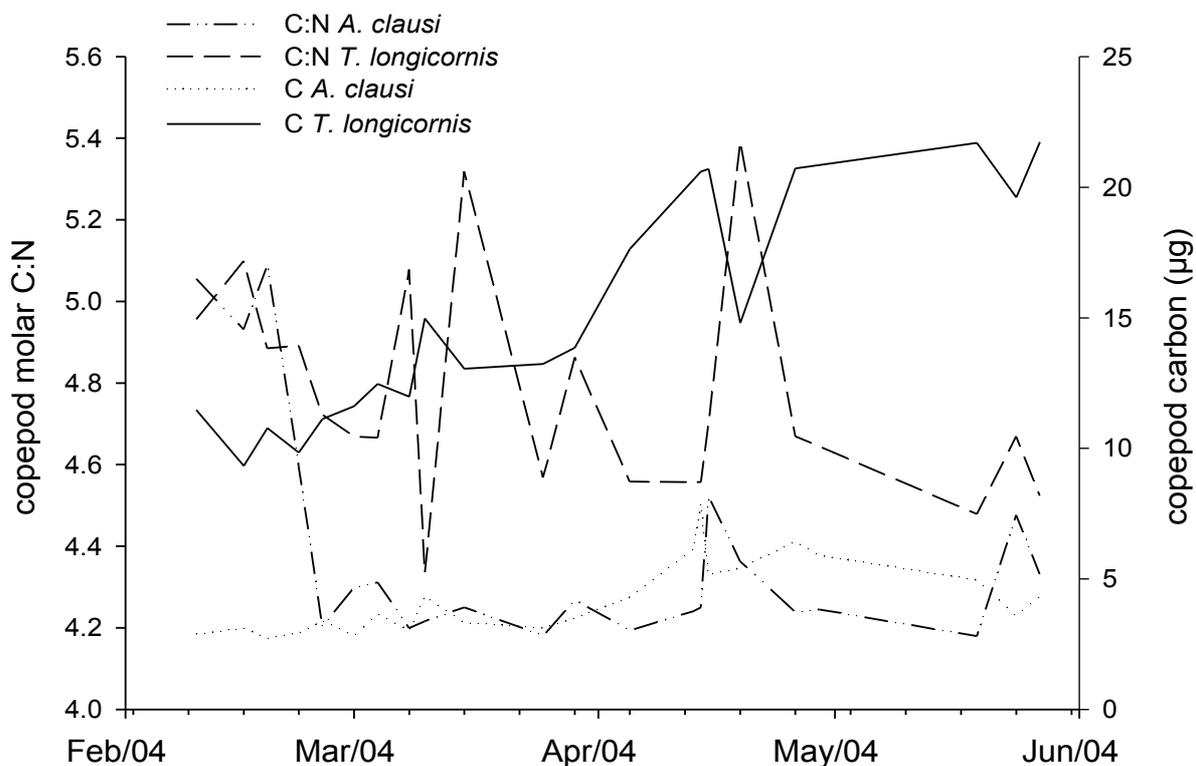


Fig. 6.1: Body C:N variability of *A. clausi* and *T. longicornis* during spring 2004 and absolute carbon contents that were taken to determine the average stoichiometric ratio in both species during spring.

Chapter 6: General discussion

or caused by the imbalanced food, and a result of the animal not being able to maintain its homeostasis when the food shows severe imbalances (Van Nieuwerburgh et al. 2004; Malzahn 2006). In fact, As *T. longicornis* eggs contain higher lipid proportions than adult copepods (Helland et al. 2003), the increase in body C:N in both species in April may not necessarily indicate a decreasing fitness of adult animals but rather an increase in ovary production because in both species maximal egg production rates were recorded in mid-April.

Secondly, the body specific ingestion of elements was calculated from bulk seston and its relative amounts on ingested particles in two size classes. However, especially the stable $\delta^{13}\text{C}$ ratios of copepods showed that *A. clausi* and *T. longicornis* feed selectively on different food sources at least during spring (chapter 5). Bulk seston C:N was positively correlated with seston $\delta^{13}\text{C}$. However, differentiating the seston signal into size classes showed how diverse the stable isotope signatures in size classes were (Fig. 6.2). Nevertheless, bulk seston parameters do reflect the condition and reproductive ability of 2 different copepod species.

Third, by regulation of enzyme activity the digestive system has the potential to increase C excretion and thus counterbalance imbalances from higher proportions of

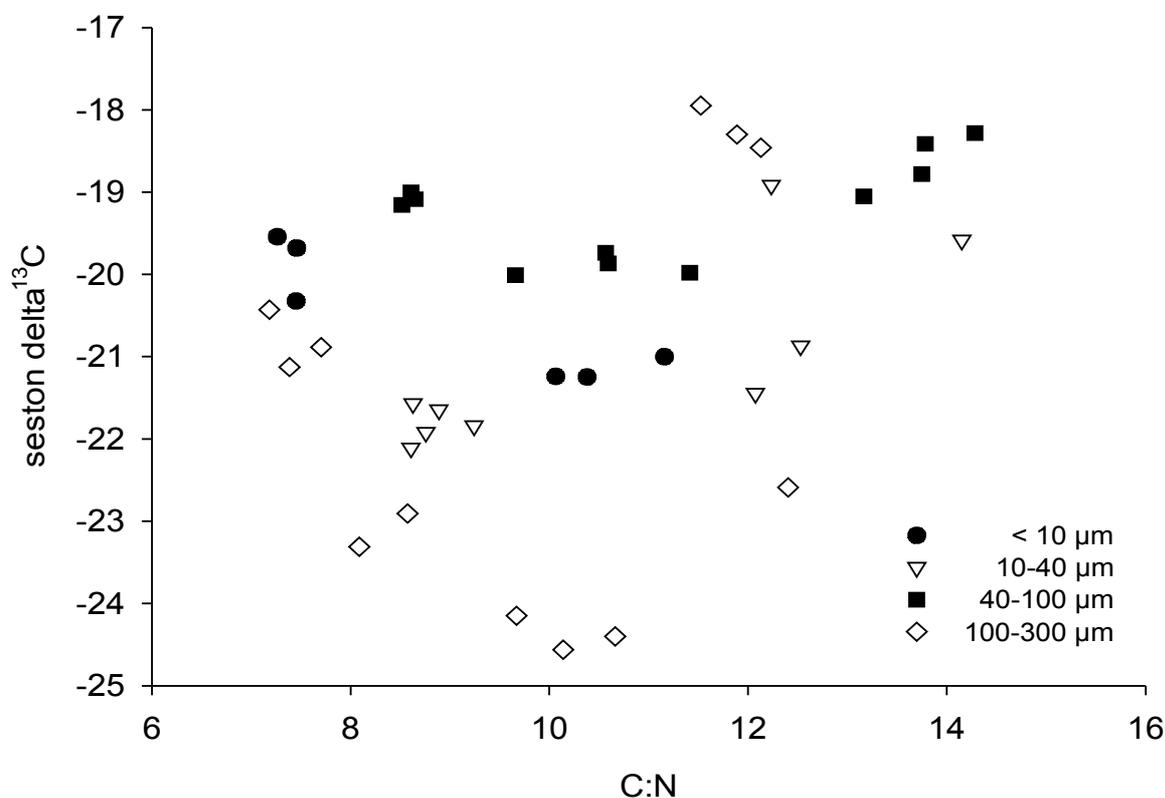


Fig. 6.2: Seston $\delta^{13}\text{C}$ versus seston C:N in size classes.

Chapter 6: General discussion

C compared to other nutrients (Katechakis et al. 2002; Darchambeau 2005). In the present study a positive correlation between *T. longicornis* C:N and chitinase activity was found and a negative correlation to arginine aminopeptidase activity. Chitinase is a carbohydrase that digests chitin, a chitin degradation product but also similar substrates such as aminosugars derived from glycopeptides. Exoskeletons of arthropods such as crustaceans but also diatom shells contain chitin. Copepods may be utilising diatom chitin as was suggested for Antarctic krill (Saborowski and Buchholz 1999). The correlation of chitinase activity with a high relative C content of *T. longicornis* indicates C assimilation through this pathway. Arginine aminopeptidase was negatively correlated to copepod C:N and therefore rather indicates N assimilation. It is an exopeptidase that releases arginine and lysine from oligopeptides that have been cleaved by endopeptidases.

Both enzymes display activity changes in opposite ways and differ between the two spring seasons (Fig. 6.3a, b). The bulk seston C:N pattern differs from the copepod body C:N pattern (Fig. 6.4). That displays that the copepods do not assimilate food in

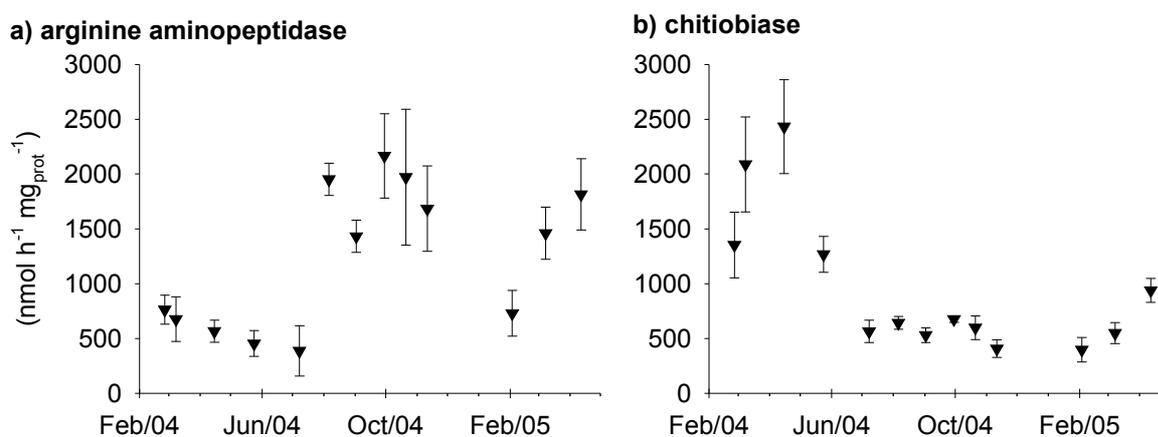


Fig. 6.3: Specific a) arginine aminopeptidase and b) chitinase activity of *T. longicornis* during the year.

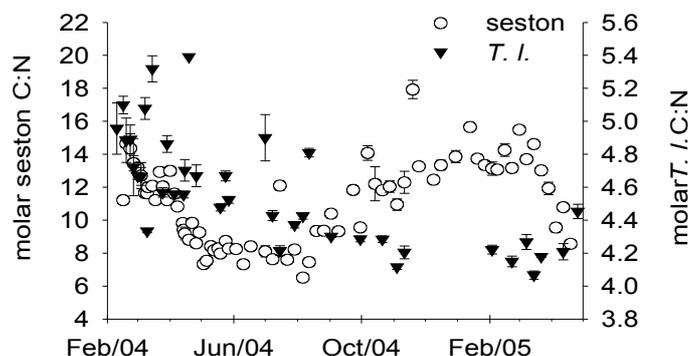


Fig. 6.4: Seston and *T. longicornis* molar C:N during the year.

Chapter 6: General discussion

stoichiometric ratios as encountered but that they use distinct food sources within bulk seston.

In spring 2004 the food source of *T. longicornis* has a similar stoichiometric pattern as bulk seston, while in summer and spring 2005, obviously more N over C (compared to seston) is assimilated. This is reflected by high arginine aminopeptidase activities compared to chitinase activities during these periods. Hence, copepods clearly do adjust their digestive system to the food they choose out of the total seston.

Starving and nitrogen limited zooplankton organism with low amounts of storage lipids, such as the copepods under study, catabolise body protein as shown by a reduction of protein under limiting conditions, and high activities of enzymes that are correlated with high relative body nitrogen contents (chapter 2). Metabolising internal protein leads to an over-proportional excretion of light ^{14}N compared to ^{15}N (Minagawa and Wada 1984). Therefore, the body $\delta^{15}\text{N}$ should not only increase in animals feeding on higher trophic levels but also in starved and nitrogen limited copepods. Both situations can easily be differentiated by concomitant evaluation of the body C:N ratios, being low in well conditioned copepods utilizing easily digestible, protein rich material and high in starved or nutrient limited animals. Indeed, the latter situation has been shown experimentally in daphnids feeding on nitrogen limited algae (Adams and Sterner 2000).

A. clausi and *T. longicornis* feed on different food sources during the phytoplankton spring bloom as depicted by stable isotope analyses and digestive enzyme studies. Surprisingly, these different feeding strategies lead to the same reproductive success. Both species included small nanoplankton in their nutrition, but *T. longicornis* to a lesser extent than *A. clausi*. However, both selected for larger particles, but these are scarce. As a result, both species acquired most of their minerals from plankton smaller than 10 μm . Particle counter based grazing rate determinations in bottle experiments bare some methodological problems. An increase in small particles in the grazed bottles can be caused by the growth of small nanoplankton but can also be a result of sloppy feeding. Both processes underestimate feeding on small particles. When large food particles are rare, individual feeding on these particles may be underestimated in bottle experiments because the grazer concentration in experiments greatly exceeds that in the field. Field concentrations of adult calanoid copepods were less than 1 per litre during

Chapter 6: General discussion

spring 2004 (Laakmann 2004). Furthermore, copepods may scan and cruise larger areas than just a one litre volume in the field (Paffenhöfer and Lewis 1990). Further, competitive heterotroph protozoans that slip the pre-sieving of natural seawater that is used for grazing experiments may outcompete copepod grazers, resulting in lower concentrations of the small particles in control bottles than in bottles containing copepods (Paffenhöfer 1998a).

However, even if the grazing results have to be interpreted with some caution, the differences between the size classes were such that the conclusion seems justified that even though copepods feed selectively on larger particles they obtain most of their nutrients from the smaller particles.

Both species show a hovering, sinking and hopping rather than cruising behaviour to find food. Copepods adjust their feeding habit to the size of particles they sense with their antennules (Price et al. 1983). A size of about 5-10 μm appears to be the boarder for individual prey handling in many species. Smaller particles cannot be rejected singly but only as bulk when toxicity or low quality is sensed by the chemoreceptors (Price et al. 1983). Getting rid of small particles may therefore cost more than it pays off when low food quality is sensed. Since the smallest size fraction of seston contains most of the nutrients that are available, the ingestion of these particles determines the stoichiometric ratio of the ingested food.

Food ingestion is the first, food assimilation the next step in energy transfer from lower trophic levels to copepods. What is assimilated is indicated by stable isotope analyses of the copepods. How copepods assimilate this food, and which enzymes they use to do so, was analysed with a method specially adapted to (mostly single individuals of) copepods using fluorogenic substrates that greatly increase sensitivity of an enzyme assay (chapter 2). Each copepod species was equipped with a unique set of proteases. Sexes did not differ in their principle enzyme equipment and only minor protease isozyme pattern variability occurred between individuals of one species. The principal pattern did not switch in response to short-term starvation. Enzyme production is obviously rather genetically determined than by environmental parameters. Therefore, the digestive system probably determines selective feeding and chemosensory abilities in copepods, rather than the reverse. This study indicates that the combination of stable isotope analyses with enzymatic analyses has large potential to explain selective ingestion and assimilation abilities of different species. Despite conserved protease isozyme patterns the activities of some enzymes can

Chapter 6: General discussion

change upon short-term starvation in two *Acartia* species (chapter 3). Chitinase activity was reduced under starvation. Therefore, this enzyme is probably not involved in catabolising internal resources and may denote a general food limitation situation, also in other copepod and zooplankton species. The other enzymes (alanine and arginine aminopeptidase, esterase/lipase and beta-glucosidase) are probably needed for catabolic processes (chapter 3) as well as feeding, which might explain why their activity was not reduced or even had higher activities under starvation. A low chitinase to arginine aminopeptidase (or also esterase/lipase and beta-glucosidase) ratio may, therefore, indicate a food limiting situation. High ratios of chitinase activity to arginine aminopeptidase activity were found at the German Bight stations combined with high relative protein contents. Unfortunately, the ratio may also reflect the exploitation of a specific food source that needs adjusted enzyme activities as mentioned above.

Each copepod species has obviously highly conserved enzyme patterns, especially the proteases. A genetic determination of protein digestion and amino acid assimilation is supported by the findings that copepod reproductive performance is highest when the amino acid composition of ingested food meets the species-specific amino acid composition of the grazer (Guisande et al. 2000). In contrast, reports exist that in other enzyme classes (carbohydrases or esterases and lipases) or species the isozyme production can be environmentally regulated (Guérin and Kerambrun 1982).

In summary, stoichiometric food quality (C:N) is a major determining factor for reproductive success of different copepod species during the year. The seston C:N ratio is correlated to the $\delta^{13}\text{C}$ signature of seston. Temporal and spatial patterns of food quantity and quality are highly variable. Copepods can adjust their grazing behaviour and digestive enzyme system in a compensatory mechanism to assimilate elements in ratios that suit them best, also foraging on the $< 10 \mu\text{m}$ seston size class to a large extent. However, their nutrient body stoichiometry is not completely homeostatic. Different species do ingest and assimilate food selectively. Food selectivity is probably mediated by their genetically determined equipment with digestive tools that are highly species specific. The regulation of enzyme activity however is determined by the specific needs of copepods. Arginine aminopeptidase and chitinase may be good indicators of the elemental assimilation of N and C, respectively, although it remains to be seen how this works mechanistically. Despite

Chapter 6: General discussion

the selective feeding of the copepods, bulk seston C:N or also $\delta^{13}\text{C}$ are good predictors of egg production rates.

6.2. Outlook

Temperature is only a basic delimiter of secondary production in the field together with food concentration. Thus, including food quality, digestive enzyme action, and grazing patterns, and combining these values with stable isotope analyses, is the way to increase our predictive power on the processes that regulate the interactions between zooplankton and phytoplankton.

In future, population dynamics studies in food webs should include an evaluation of the influence of food quality on hatching success, developmental success and predation risk.

Applying physiological micromethods to study digestive enzymes, such as presented here, can dramatically reduce the sampling effort in field studies. Other enzymes should be screened to find an equivalent for phosphorus assimilation to round up the picture of mineral transfer through selective ingestion and assimilation.

The results from the field need to be confirmed by experimental approaches that define the real prey field as compared to the total potential prey field as given by bulk seston of different copepod species. The influence of mineral deficiencies, eutrophication, toxic substances and the interaction with temperature on stable isotope signatures, grazer physiology and behaviour need to be defined to further refine the present knowledge.

Chapter 7: Summary

The aim of this study was to identify internal physiological factors and external nutritional factors that have the potential to influence the population dynamics of dominant copepod species in the North Sea. Seston quality in terms of C:N:P stoichiometry correlated significantly with reproduction of copepods. Grazing behaviour and the digestive system were found to interact in mediating the prominent effect of food quality on copepod condition and reproduction. Food quality changed considerably over time and space during the 2004-2005 sampling carried out in this study. Stable isotope ratios of the seston indicated major seston composition shifts in spring and a strong heterotroph component in summer.

Assay protocols were developed to measure a set of 5 digestive enzymes and water soluble protein in individual copepods weighing between 5 and 35 µg dry mass. The methods were applied to 5 abundant North Sea species. They differed in bulk nutrient contents and stoichiometric ratio. The specific enzyme activities were high and showed high individual variability.

In a short-term starvation experiment sublethal effects on digestive enzymes were evaluated in *A. clausi* and *A. tonsa*. The resulting patterns were complex. Chitinase activity decreased in starved individuals of both species while the other enzymes showed no change or even an activity increase (alanine aminopeptidase in *A. clausi*). Results are discussed as a possible adaptation to a patchy food environment (chapter 2). Protease isozyme patterns did not change as a result of short time starvation. Instead, highly conserved species-specific patterns were revealed. Genetic regulation of the enzyme equipment and the possible influence of the digestive system on food selectivity are discussed (chapter 3).

A temporal study at Helgoland and a spatial study in the North Sea examined the nutritional value of bulk seston for copepods. Food quantity was of minor importance in influencing copepod reproduction, although, high concentrations of phytoplankton supported highest egg production rates. Bulk seston quality as measured by C:N ratio and the proportion of particulate organic phosphorus (POP) on total particulate phosphorus (TPP) were significantly correlated to egg production of *A. clausi* and *T. longicornis*. Grazing behaviour hinted to compensatory feeding at low specific food concentrations in both species. Mineral uptake rates in March were high and rather independent of phytoplankton concentration. The largest proportion of elements was bound in the < 10 µm size class and copepods covered their mineral demand mainly

Chapter 7: Summary

by feeding on this smallest size class. Copepods took up seston N:P in ratios matching their body ratios. Body specific N and P uptake did only rarely exceed a ratio of 1:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$) and then were only slightly higher. However, C uptake reached values of up to 2.5:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{ day}^{-1}$). Ingested C:N and POP proportion followed the seston signature. Therefore, with increasing seston quality (decreasing C:N) the C:N imbalance decreased (chapter 4).

Stable isotope ratio analyses of carbon and nitrogen displayed that *A. clausi* and *T. longicornis* were feeding on similar high trophic levels compared to seston, as deduced from the $\delta^{15}\text{N}$ signal, but they utilised different carbon sources within the seston as displayed by $\delta^{13}\text{C}$. Over the year seston $\delta^{13}\text{C}$ was inversely correlated to egg production in *A. clausi* and *T. longicornis*, indicating that food sources containing a lower proportion of heavy ^{13}C isotope as encountered in spring are favourable for copepods. The lowest $\delta^{13}\text{C}$ values in *T. longicornis* coincided with highest $\delta^{15}\text{N}$ values. Chitinase activity of *T. longicornis* was positively correlated to body C:N and negatively to body $\delta^{13}\text{C}$, while arginine aminopeptidase, esterase/lipase and beta-glucosidase were negatively correlated to body C:N and positively to body $\delta^{13}\text{C}$. Therefore, the two enzyme types denote assimilation of C and N, respectively. A high relative body N content indicates good fitness. A low body $\delta^{13}\text{C}$ could stand for high turnover rates of carbon (chapter 5).

Combining enzymatic with stable isotope and stoichiometric analyses in field studies can explain processes on the zooplankton-phytoplankton interaction level far better than using just one approach. The methods could be further refined by looking closer at species-specific interactions between trophic community levels. Experimental approaches should clarify the so far hypothetical link between seston stable isotope parameters, digestive enzyme action and copepod condition (reproductive ability).

Chapter 8: Zusammenfassung

Mit dieser Arbeit sollten interne physiologische und externe Ernährungsfaktoren identifiziert werden, die potentiell die Populationsdynamik dominanter Copepodenarten in der Nordsee beeinflussen. Es wird gezeigt, dass Sestonqualität, ausgedrückt als C:N:P Stöchiometrie, signifikant mit der Reproduktion von Copepoden korrelierte. Fraßverhalten und Verdauungssystem interagierten bei der Übertragung eines deutlichen Futterqualitätseffekts auf die Tiere, ihren Ernährungszustand und ihre Fortpflanzungsfähigkeit. Die Futterqualität schwankte deutlich in Raum und Zeit während der Probenkampagne für diese Studie in den Jahren 2004 und 2005. Die Analyse der stabilen Isotopenverhältnisse zeigte, dass sich im Frühling die Zusammensetzung des Sestons jeweils stark veränderte und dass während des Sommers Heterotrophe dominierten.

Analyseprotokolle wurden entwickelt, mit denen 5 verschiedene Verdauungsenzyme gleichzeitig oder wasserlösliche Proteine in einzelnen Copepoden gemessen werden können, die nur 5-35 µm Trockenmasse wiegen. Die Methoden wurden dann auf 5 häufige Nordseearten angewendet. Die Copepoden unterschieden sich in Nährstoffgehalten und stöchiometrischen Verhältnissen. Die spezifischen Enzymaktivitäten zeigten hohe Aktivitäten und intra-spezifische Variabilität (Kapitel 2). Sublethale Effekte auf das Enzymsystem von *A. clausi* und *A. tonsa* wurden in Kurzzeit-Hunger-Versuchen untersucht. Die Ergebnisse waren komplex: Die Chitobiaseaktivität sank in gehungerten Individuen beider Arten, während die anderen Enzyme keine Veränderungen zeigten oder eine erhöhte Aktivität aufwiesen (Alanin-Aminopeptidase in *A. clausi*). Die Ergebnisse werden als Anpassung an eine hoch-variable Umwelt diskutiert. Protease Isoenzym-Muster veränderten sich nicht durch die Kurzzeit-Hunger-Situation. Es traten jedoch hoch-konservierte interspezifische Muster zu Tage. Die genetische Regulierung der Enzymausstattung und mögliche Einflüsse des Verdauungssystems auf Fraßselektivität werden diskutiert (Kapitel 3).

Eine Zeitreihenstudie vor Helgoland und eine räumliche Studie in der Nordsee untersuchte den Ernährungswert von Seston für Copepoden. Die Futterquantität beeinflusste die Fortpflanzung kaum, obwohl hohe Konzentrationen an Phytoplankton höchste Eiproduktionsraten unterstützen. Die Gesamtsestonqualität, ausgedrückt als C:N Verhältnis und als Anteil von organischem Phosphor (POP) am

Chapter 8: Zusammenfassung

Gesamtphosphor (TPP), korrelierte signifikant mit der Eiproduktionsrate von *A. clausi* und *T. longicornis*. Bei niedriger spezifischer Futterkonzentration wies das Fraßverhalten auf kompensatorischen Fraß in beiden Arten hin. Die kleinste Seston-Größenklasse, < 10 µm, enthielt die höchsten Konzentrationen an jeweiligen Elementen, und Copepoden nutzten diese Größenklasse als Hauptfutterquelle. Copepoden nahmen Seston N:P in Verhältnissen auf, die ihren Körperverhältnissen entsprachen. Körpermineral-spezifische Aufnahmeraten für N und P überstiegen nur selten und unwesentlich ein Verhältnis von 1:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{Tag}^{-1}$). Jedoch erreichte die C-Aufnahmerate Werte von bis zu 2.5:1 ($\mu\text{mol } \mu\text{mol}^{-1} \text{Tag}^{-1}$). Das C:N-Verhältnis und der POP-Anteil der aufgenommenen Nahrung entsprachen der Sestonsignatur. Mit ansteigender Sestonqualität (niedrigerem C:N) verminderte sich das Ungleichgewicht zwischen Körper-C:N und Seston C:N (Kapitel 4).

Aus den $\delta^{15}\text{N}$ -Signalen ging hervor, dass *A. clausi* und *T. longicornis* auf ähnlich hohen trophischen Ebenen gegenüber dem Gesamtseston fraßen. Jedoch nutzten sie unterschiedliche Kohlenstoffquellen innerhalb des Sestons, angezeigt durch die $\delta^{13}\text{C}$ -Signaturen. Über das Jahr war Seston $\delta^{13}\text{C}$ invers mit der Eiproduktion von *A. clausi* und *T. longicornis* korreliert, was darauf hinweist, dass Futterquellen mit niedrigem Anteil an schwerem ^{13}C , wie sie im Frühjahr auftreten, förderlich für Copepoden sind. Die niedrigsten $\delta^{13}\text{C}$ -Werte in *T. longicornis* traten zusammen mit den höchsten $\delta^{15}\text{N}$ -Werten auf. Die Chitobiaseaktivität von *T. longicornis* war positiv korreliert mit dem Körper C:N-Verhältnis und negativ mit dem Körper $\delta^{13}\text{C}$, während Arginin-Aminopeptidase, Esterase/Lipase und Beta-Glucosidase positiv mit dem Körper C:N-Verhältnis und negativ mit dem $\delta^{13}\text{C}$ -Wert korreliert waren. Beide Enzymtypen weisen damit auf eine Funktion in der C- bzw. N-Assimilation hin. Ein hoher relativer Körper-N-Gehalt zeigt eine gute Fitness der Copepoden an. Hohe Umsatzraten könnten das niedrige Körper $\delta^{13}\text{C}$ Verhältnis erklären (Kapitel 5).

Die Kombination von enzymatischen mit stabilen Isotopen und stöchiometrischen Analysen in Feldstudien kann Interaktionsprozesse auf der Zooplankton-Phytoplankton-Ebene besser erklären als nur einer der Analysetypen. Die Methodik könnte verfeinert werden, um noch mehr auf die artspezifischen Interaktionen zwischen trophischen Gemeinschaftsebenen einzugehen. Experimentelle Folgestudien sollten belegen, dass die bislang hypothetische Verbindung zwischen stabilen Isotopen, Verdauungsenzymen und Copepodenfitness (Fortpflanzungsfähigkeit) Bestand hat.

Chapter 9: References

- Acharya K, Kyle M, Elser JJ (2004) Effects of stoichiometric dietary mixing on *Daphnia* growth. *Oecologia* 138: 333-340
- Adams TS, Sterner RW (2000) The effect of dietary nitrogen content on trophic level ^{15}N enrichment. *Limnol Oceanogr* 45: 601-607
- Albaina A, Irigoien X (2004) Relationships between frontal structures and zooplankton communities along a cross-shelf transect in the Bay of Biscay (1995 to 2003). *Mar Ecol Prog Ser* 284: 65-75
- Alonzo, F., Mayzaud, P., Razoul, S. (2000) Egg production, population structure and biochemical composition of the subantarctic copepod *Paraeuchaeta antarctica* in the Kerguelen Archipelago. *Mar Ecol Prog Ser* 205: 207-217.
- Ambler JW (1986) Effect of food quantity and quality on egg production of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. *Estuar Coast Shelf Sci* 23: 183-196
- Anderson TR, Boersma M, Raubenheimer D (2004) Stoichiometry: linking elements to biochemicals. *Ecology* 85: 1193-1202
- Anderson TR, Hessen DO (1995) Carbon or nitrogen limitation in marine copepods? *J Plankton Res* 17: 317-331
- Anderson TR, Pond DW (2000) Stoichiometric theory extended to micronutrients: comparison of the roles of essential fatty acids, carbon, and nitrogen in the nutrition of marine copepods. *Limnol Oceanogr* 45: 1162-1167
- Arendt KE, Jónasdóttir SH, Hansen PJ, Gärtner S (2005) Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. *Mar Biol* 146: 513-530
- Arnaud J, Brunet M, Mazza J (1978) Studies on the midgut of *Centropages typicus* (copepod, calanoid). I. Structural and ultrastructural data. *Cell Tissue Res* 187: 333-353
- Arnaud, J., Brunet, M., Mazza, J. (1980) Comparative structure and ultrastructure of the midgut in several species of calanoid copepods. *Zoomorphologie* 95: 213-233.
- Aspila KI, Agemian H, Chau ASY (1976) A semi-automatic method for the determination of inorganic, organic and total phosphate in sediments. *The Analyst* 101: 187-197
- Augustin CB, Boersma M (2006) Effects of nitrogen stressed algae on different *Acartia* species. *J Plankton Res* 28: 429-436
- Baars MA, Oosterhuis SS (1984) Diurnal feeding rhythms in North Sea copepods measured by gut fluorescence, digestive enzyme activity and grazing on labelled food. *Neth J Sea Res* 18: 97-119
- Ban S, Burns C, Castel J, Chaudron Y, Christou E, Escribano R, Umani SF, Gasparini S, Ruiz FG, Hoffmeyer M, Ianora A, Kang H-K, Laabir M, Lacoste A, Miralto A, Ning X, Poulet S, Rodriguez V, Runge J, Shi J, Starr M, Uye S-i, Wang Y (1997) The paradox of diatom-copepod interactions. *Mar Ecol Prog Ser* 157: 287-293
- Barnard R, Batten SD, Beaugrand G, Buckland C, Conway DVP, Edwards M, Finlayson J, Gregory LW, Halliday NC, John AWG, Johns DG, Johnson AD, Jonas TD, Lindley JA, Nyman J, Pritchard P, Reid PC, Richardson AJ, Saxby RE, Sidey J, Smith MA, Stevens DP, Taylor CM, Tranter PRG, Walne AW, Wootton M, Wotton COM, Wright JC (2004) Continuous Plankton Records: Plankton Atlas of the North Atlantic Ocean (1958–1999). II. Biogeographical charts. *Mar Ecol Prog Ser Suppl*: 11-75
- Barnes, H. Blackstock, J. (1973) Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphanillin method for total lipids. *J Exp Biol Ecol* 12: 103-118.
- Bautista B, Harris RP, Rodriguez V, Guerrero F (1994) Temporal variability in copepod fecundity during two different spring bloom periods in coastal waters off Plymouth (SW England). *J Plankton Res* 16: 1367-1377
- Beardsley C, Pernthaler J, Wosniok W, Amann R (2003) Are readily culturable bacteria in Coastal North Sea waters suppressed by selective grazing mortality? *Appl Environ Microbiol* 69: 2624-2630
- Beaugrand G (2004) Continuous Plankton Records: Plankton Atlas of the North Atlantic Ocean (1958–1999). I. Introduction and methodology. *Mar Ecol Prog Ser Suppl*: 3-10
- Beaugrand G, Brander KM, Lindley JA, Souissi S, Reid PC (2003) Plankton effect on cod recruitment in the North Sea. *Nature* 426: 661-664
- Berdalet E, Marrasé C, Estrada M, Arin L, MacLean ML (1996) Microbial community responses to nitrogen- and phosphorus-deficient nutrient inputs: microplankton dynamics and biochemical characterization. *J Plankton Res* 18: 1627-1641

Chapter 9: References

- Billen G, Joiris C, Meyer-Reil L, Linderboom H (1990) Role of bacteria in the North Sea ecosystem. *Neth J Sea Res* 26: 265-293
- Bochdansky AB, Puskaric S, Herndl GJ (1995) Influence of zooplankton grazing on free dissolved enzymes in the sea. *Mar Ecol Prog Ser* 121: 53-63
- Boersma M (2000) The nutritional quality of P-limited algae for *Daphnia*. *Limnol Oceanogr* 45: 1157-1161
- Boersma M, Kreutzer C (2002) Life at the edge: is food quality really of minor importance at low quantities? *Ecology* 83: 2552-2561
- Bonnet D, Carlotti F (2001) Development and egg production in *Centropages typicus* (Copepoda: Calanoida) fed different food types: a laboratory study. *Mar Ecol Prog Ser* 224: 133-148
- Boucher, J. Samain, J. F. (1974) L'activité amylasique indice de la nutrition du zooplancton; mise en évidence d'un rythme quotidien en zone d'upwelling. *Tethys* 6: 179-188
- Bouillon S, Mohan PC, Sreenivas N, Dehairs F (2000) Sources of suspended organic matter and selective feeding by zooplankton in an estuarine mangrove ecosystem as traced by stable isotopes. *Mar Ecol Prog Ser* 208: 79-92
- Brockmann UH, Laane RWPM, Postma H (1990) Cycling of nutrient elements in the North Sea. *Neth J Sea Res* 26: 239-264
- Brunet, M., Arnaud, J., Mazza, J. (1994) Gut structure and digestive cellular processes in marine Crustacea. *Oceanogr. Mar. Biol. Annu. Rev.* 32: 335-367
- Bundy MH, Gross TF, Vanderploeg HA, Strickler JR (1998) Perception of inert particles by calanoid copepods: behavioral observations and a numerical model. *J Plankton Res* 20: 2129-2152
- Bundy MH, Paffenhöfer GA (1993) Innervation of copepod antennules investigated using laser scanning confocal microscopy. *Mar Ecol Prog Ser* 102: 1-14
- Bunker AJ, Hirst AG (2004) Fecundity of marine planktonic copepods: global rates and patterns in relation to chlorophyll a, temperature and body weight. *Mar Ecol Prog Ser* 279: 161-181
- Båmstedt, U. (1975) Studies on the deep-water pelagic community of Korsfjorden, Western Norway – Ecological aspects of individual variations in weight and protein and lipid content of *Euchaeta norvegica*. *Sarsia* 59: 31-46
- Båmstedt, U. (1986) Chemical composition and energy content. In: Corner, E. D. S. and O'Hara, S. C. M., (Eds), *The biological chemistry of marine copepods*. Clarendon Press, Oxford, pp. 1-58.
- Båmstedt, U. (1988) Ecological significance of individual variability in copepod bioenergetics. *Hydrobiol* 167/168, 43-59.
- Cahu CL, Zambonino Infante JL, Péres A, Quazuguel P, Le Gall MM (1998) Algal addition in sea bass (*Dicentrarchus labrax*) larvae rearing: effect on digestive enzymes. *Aquaculture* 161: 479-489
- Campbell RW, Head EJ (2000) Egg production rates of *Calanus finmarchicus* in the western North Atlantic: effect of gonad maturity, female size, chlorophyll concentration, and temperature. *Can J Fish Aquat Sci/J Can Sci Halieut Aquat* 57: 518-529
- Castro-Longoria E (2003) Egg production and hatching success of four *Acartia* species under different temperature and salinity regimes. *J Crust Biol* 23: 289-299
- Chan AS, Horn MH, Dickson KA, Gawlicka A (2004) Digestive enzyme activities in carnivores and herbivores: comparisons among four closely related prickleback fishes (Teleostei: Stichaeidae) from a California rocky intertidal habitat. *J Fish Biol* 65: 848-858
- Checkley DM, Jr. (1980a) The egg production of a marine planktonic copepod in relation to its food supply: laboratory studies. *Limnol Oceanogr* 25: 430-446
- Checkley DM, Jr. (1980b) Food limitation of egg production by a marine, planktonic copepod in the sea off southern California. *Limnol Oceanogr* 25: 991-998
- Chervin MB (1978) Assimilation of particulate organic carbon by estuarine and coastal copepods. *Mar Biol* 49: 265-275
- Colin SP, Dam HG (2002) Testing for toxic effects of prey on zooplankton using sole versus mixed diets. *Limnol Oceanogr* 47: 1430-1437
- Cordova-Murueta JH, García-Carreño FL, Navarrete-del-Toro MdIA (2003) Digestive enzymes present in crustacean feces as a tool for biochemical, physiological, and ecological studies. *J Exp Mar Biol Ecol* 297: 43-56
- Cowles TJ, Olson RJ, Chisholm SW (1988) Food selection by copepods: discrimination on the basis of food quality. *Mar Biol* 100: 41-49
- Cushing DH (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Adv Mar Biol* 26: 249-292
- Cyr H, Curtis JM (1999) Zooplankton community size structure and taxonomic composition affects size-selective grazing in natural communities. *Oecologia* 118: 306-315
- Dagg M (1977) Some effects of patchy food environments on copepods. *Limnol Oceanogr* 22: 99-107
- Dalsgaard J, St. John M, Kattner G, Müller-Navarra DC, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol* 45: 225-340

Chapter 9: References

- Darchambeau F (2005) Filtration and digestion responses of an elementally homeostatic consumer to changes in food quality: a predictive model. *Oikos* 111: 322-336
- DeMott WR (1986) The role of taste in food selection by freshwater zooplankton. *Oecologia* 69: 334-340
- DeMott WR (1988) Discrimination between algae and detritus by freshwater and marine zooplankton. *Bull Mar Sci* 43: 486-499
- DeMott WR (1989) Optimal foraging theory as a predictor of chemically mediated food selection by suspension-feeding copepods. *Limnol Oceanogr* 34: 140-154
- DeMott WR, Watson MD (1991) Remote detection of algae by copepods: responses to algal size, odors and motility. *J Plankton Res* 13: 1203-1222
- Devreker D, Souissi S, Seuront L (2005) Effects of chlorophyll concentration and temperature variation on the reproduction and survival of *Temora longicornis* (Copepoda, Calanoida) in the Eastern English Channel. *J Exp Mar Biol Ecol* 318: 145-162
- Donaghay PL (1988) Role of temporal scales of acclimation, food quality and trophic dominance in controlling the evolution of copepod feeding behavior. *Bull Mar Sci* 43: 469-485
- Donaghay PL, Small LF (1979) Food selection capabilities of the estuarine copepod *Acartia clausi*. *Mar Biol* 52: 137-146
- Downing JA, Osenberg CG, Sarnelle O (1999) Meta-analysis of marine nutrient-enrichment experiments: variation in the magnitude of nutrient limitation. *Ecology* 80: 1157-1167
- Dutz J (1998) Repression of fecundity in the neritic copepod *Acartia clausi* exposed to the toxic dinoflagellate *Alexandrium lusitanicum*: relationship between feeding and egg production. *Mar Ecol Prog Ser* 175: 97-107
- Dutz J, Klein Breteler WCM, Kramer G (2005) Inhibition of copepod feeding by exudates and transparent exopolymer particles (TEP) derived from a *Phaeocystis globosa* dominated phytoplankton community. *Harmful Algae* 4: 929-940
- Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. *Limnol Oceanogr* 40: 860-867
- Edwards M, Richardson AJ (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature* 430: 881-884
- Einsle UK (1988) Taxonomy of the genus *Megacyclops* (Crustacea, Copepoda): Morphometry and the use of enzyme electrophoresis *Hydrobiologia* 167-168: 387-391
- Elser JJ, Hassett RP (1994) A stoichiometric analysis of the zooplankton-phytoplankton interaction in marine and freshwater ecosystems. *Nature* 370: 211-213
- Estep KW, Nejtgaard JC, Skjoldal HR, Rey F (1990) Predation by copepods upon natural populations of *Phaeocystis pouchetii* as a function of the physiological state of the prey. *Mar Ecol Prog Ser* 67: 235-249
- Evjemo, J. O., Reitan, K. I., Olsen, Y. (2003) Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. *Aquaculture* 227: 1-4
- Fehling J, Davidson K, Bolch CJ, Bates SS (2004) Growth and domoic acid production by *Pseudo-nitzschia seriata* (Bacillariophyceae) under phosphate and silicate limitation. *J Phycol* 40: 674-683
- Fock HO, Greve W (2002) Analysis and interpretation of recurrent spatio-temporal patterns in zooplankton dynamics: a case study on *Noctiluca scintillans* (Dinophyceae) in the German Bight (North Sea). *Mar Biol* 140: 59-73
- Fogg GE (1995) Some comments on picoplankton and its importance in the pelagic ecosystem. *Aquat Microb Ecol* 9: 33-39
- Frangópulos M, Guisande C, DeBlas E, Maneiro I (2004) Toxin production and competitive abilities under phosphorus limitation of *Alexandrium* species. *Harmful Algae* 3: 131-139
- Franke HD, Buchholz F, Wiltshire KH (2004) Ecological long-term research at Helgoland (German Bight, North Sea): retrospect and prospect - an introduction. *Helgol Mar Res* 58: 223-229
- Fransz HG, Colebrook JM, Gamble JC, Krause M (1991) The zooplankton of the North Sea. *Neth J Sea Res* 28: 1-52
- Frost PC, Evans-White MA, Finkel ZV, Jensen TC, Matzek V (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos* 109: 18-28
- Fry B, Anderson RK, Entzeroth L, Bird JL, Parker PL (1984) ¹³C enrichment and oceanic food web structure in the northwestern Gulf of Mexico. *Contrib Mar Sci* 27: 49-63
- Fry B, Wainright SC (1991) Diatom sources of ¹³C-rich carbon in marine food webs. *Mar Ecol Prog Ser* 76: 149-157

Chapter 9: References

- Fukami K, Watanabe A, Fujita S, Yamaoka K, Nishijima T (1999) Predation on naked protozoan microzooplankton by fish larvae. *Mar Ecol Prog Ser* 185: 285-291
- García-Carreño FL, Dimes LE, Haard NF (1993) Substrate-gel electrophoresis for composition and molecular weight of proteinases or proteinaceous proteinase inhibitors. *Anal Biochem* 214: 65-69
- Gasparini S, Daro MH, Antajan E, Tackx M, Rousseau V, Parent JY, Lancelot C (2000) Mesozooplankton grazing during the *Phaeocystis globosa* bloom in the southern bight of the North Sea. *J Sea Res* 43: 345-356
- Gatten, R. R., Sargent, J. R., Forsberg, T. E. V., O'Hara, S. C. M., Corner, E. D. S. (1980) On the nutrition and metabolism of zooplankton. XIV. Utilization of lipid by *Calanus helgolandicus* during maturation and reproduction. *J Mar Biol Assoc UK* 60: 391-399
- Geider RJ (1987) Light and temperature dependence of the carbon to chlorophyll a ratio in microalgae and cyanobacteria: implications for physiology and growth of phytoplankton. *New Phytologist* 106: 1-34
- Geider RJ, La Roche J (2002) Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *Eur J Phycol* 37: 1-17
- Gerdtts G, Wichels A, Döpke H, Klings K-W, Gunkel W, Schütt C (2004) 40-year long-term study of microbial parameters near Helgoland (German Bight, North Sea): historical view and future perspectives. *Helgol Mar Res* 58: 230-242
- Gervais F, Riebesell U (2001) Effect of phosphorus limitation on elemental composition and stable carbon isotope fractionation in a marine diatom growing under different CO₂ concentrations. *Limnol Oceanogr* 46: 497-504
- Gismervik I (2006) Top-down impact by copepods on ciliate numbers and persistence depends on copepod and ciliate species composition. *J Plankton Res* 28: 499-507
- Gowen R, McCullough G, Kleppel G, Houchin L, Elliott P (1999) Are copepods important grazers of the spring phytoplankton bloom in the western Irish Sea? *J Plankton Res* 21: 465-483
- Granéli E, Johansson N (2003) Effects of the toxic haptophyte *Prymnesium parvum* on the survival and feeding of a ciliate: The influence of different nutrient conditions. *Mar Ecol Prog Ser* 254: 49-56
- Granum E, Krikvold S, Mykkestad SM (2002) Cellular and extracellular production of carbohydrates and amino acids by the marine diatom *Skeletonema costatum*: diel variations and effects of N depletion. *Mar Ecol Prog Ser* 242: 83-94
- Grasshoff K, Erhardt M, Kremling K (1983) Methods of seawater analysis. Verlag Chemie, Weinheim
- Greve W (1994) The 1989 German Bight invasion of *Muggiaea atlantica*. *ICES J Mar Sci* 51: 355-358
- Greve W, Reiners F, Nast J, Hoffmann S (2004) Helgoland Roads meso- and macrozooplankton time-series 1974 to 2004: lessons from 30 years of single spot, high frequency sampling at the only off-shore island of the North Sea. *Helgol Mar Res* 58: 274-288
- Guarna MM, Borowsky RL (1993) Genetically controlled food preference: biochemical mechanisms. *Proc Natl Acad Sci USA* 90: 5257-5261
- Guérin J-P, Kerambrun P (1982) Effects of diet on esterases, alkaline phosphatase, malate dehydrogenase and phosphoglucomutase activity observed by polyacrylamide gel electrophoresis in *Tisbe holothuriae* (harpacticoid copepod). *Comp Biochem Physiol B* 73: 761-770
- Guerrini F, Mazzotti A, Boni L, Pistocchi R (1998) Bacterial-algal interactions in polysaccharide production. *Aquat Microb Ecol* 15: 247-253
- Guildford SJ, Hecky RE (2000) Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnol Oceanogr* 45: 1213-1223
- Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum Press, New York
- Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can J Microbiol* 8: 229-239
- Guisande C, Maneiro I, Riveiro I, Barreiro A, Pazos Y (2002) Estimation of copepod trophic niche in the field using amino acids and marker pigments. *Mar Ecol Prog Ser* 239: 147-156
- Guisande C, Riveiro I, Maneiro I (2000) Comparisons among the amino acid composition of females, eggs and food to determine the relative importance of food quantity and food quality to copepod reproduction. *Mar Ecol Prog Ser* 202: 135-142
- Hallberg E, Hirche H-J (1980) Differentiation of mid-gut in adults and over-wintering copepodids of *Calanus finmarchicus* (Gunnerus) and *C. helgolandicus* Claus. *J Exp Mar Biol Ecol* 48: 283-295
- Han X, Wang R, Wang J (2002) Digestive gut structure and activity of protease, amylase, and alkaline phosphatase in *Calanus sinicus* during summer in the Yellow Sea and the East China Sea. *J Exp Mar Biol Ecol* 270: 131-146

Chapter 9: References

- Halsband-Lenk C, Carlotti F, Greve W (2004) Life-history strategies of calanoid congeners under two different climate regimes: a comparison. *ICES J Mar Sci* 61: 709-720
- Halsband-Lenk C, Hirche HJ, Carlotti F (2002) Temperature impact on reproduction and development of congener copepod populations. *J Exp Mar Biol Ecol* 271: 121-153
- Halsband C, Hirche HJ (2001) Reproductive cycles of dominant calanoid copepods in the North Sea. *Mar Ecol Prog Ser* 209: 219-229
- Hansen BW, Bjørnson PK, Hansen PJ (1994) The size ratio between planktonic predators and their prey. *Limnol Oceanogr* 39: 395-403
- Hansen FC (1995) Trophic interactions between zooplankton and *Phaeocystis cf. globosa*. *Helgoländer Meeresunters* 49: 283-293
- Harris RP, Samain JF, Moal J, Martin-Jezequel V, Poulet SA (1986) Effects of algal diet on digestive enzyme activity in *Calanus helgolandicus*. *Mar Biol* 90: 353-361
- Hassett RP (2004) Supplementation of a diatom diet with cholesterol can enhance copepod egg-production rates. *Limnol Oceanogr* 49: 488-494
- Hassett RP, Blades-Eckelbarger P (1995) Diel changes in gut-cell morphology and digestive activity of the marine copepod *Acartia tonsa*. *Mar Biol* 124: 59-69
- Hassett RP, Cardinale B, Stabler LB, Elser JJ (1997) Ecological stoichiometry of N and P in pelagic ecosystems: Comparison of lakes and oceans with emphasis on the zooplankton-phytoplankton interaction. *Limnol Oceanogr* 42: 648-662
- Hassett RP, Landry MR (1983) Effects of food-level acclimation on digestive enzyme activities and feeding behavior of *Calanus pacificus*. *Mar Biol* 75: 47-55
- Hassett RP, Landry MR (1988) Short-term changes in feeding and digestion by the copepod *Calanus pacificus*. *Mar Biol* 99: 63-74
- Hassett RP, Landry MR (1990) Effects of diet and starvation on digestive enzyme activity and feeding behavior of the marine copepod *Calanus pacificus*. *J Plankton Res* 12: 991-1010
- Hays GC, Richardson AJ, Robinson C (2005) Climate change and marine plankton. *Trends Ecol Evol* 20: 337-344
- Head EJH, Wang R, Conover RJ (1984) Comparison of diurnal feeding rhythms in *Temora longicornis* and *Centropages hamatus* with digestive enzyme activity. *J Plankton Res* 6: 543-551
- Helland S, Terjesen BF, Berg L (2003) Free amino acid and protein content in the planktonic copepod *Temora longicornis* compared to *Artemia franciscana*. *Aquaculture* 215: 213-228
- Hessen DO, Færøvig PJ, Andersen T (2002) Light, nutrients, and P:C ratios in algae: grazer performance related to food quality and quantity. *Ecology* 83: 1886-1898
- Hidalgo, M. C., Urea, E., Sanz, A. (1999) Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture* 170: 267-283
- Hillebrand H, Dürselen C-D, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. *J Phycol* 35: 403-424
- Hirche HJ (1981) Digestive enzymes of copepodids and adults of *Calanus finmarchicus* and *C. helgolandicus* in relation to particulate matter. *Kiel Meeresforsch* 5: 174-185
- Hirche HJ (1996) Diapause in the marine copepod, *Calanus finmarchicus* - a review. *Ophelia* 44: 129-143
- Hirche HJ, Meyer U, Niehoff B (1997) Egg production of *Calanus finmarchicus*: effect of temperature, food and season. *Mar Biol* 127: 609-620
- Hirst AG, Bunker AJ (2003) Growth of marine planktonic copepods: Global rates and patterns in relation to chlorophyll a, temperature, and body weight. *Limnol Oceanogr* 48: 1988-2010
- Holste L, Peck M (2006) The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation. *Mar Biol* 148: 1061-1070
- Hoppe, H. G. (1983) Significance of exoenzymatic activities in the ecology of brackish water: Measurements by means of methylumbelliferyl-substrates. *Mar Ecol Progr Ser* 11: 299-308
- Hoppenrath M (2004) A revised checklist of planktonic diatoms and dinoflagellates from Helgoland (North Sea, German Bight). *Helgol Mar Res* 58: 243-251
- Hwang JS, Costello JH, Strickler JR (1994) Copepod grazing in turbulent flow: elevated foraging behavior and habituation of escape responses. *J Plankton Res* 16: 421-431
- Hwang JS, Strickler R (2001) Can copepods differentiate prey from predator hydromechanically? *Zool Stud* 40: 1-6
- Ianora A, Miralto A, Poulet SA, Carotenuto Y, Buttino I, Romano G, Casotti R, Pohnert G, Wichard T, Colucci-D'Amato L, Terrazzano G, Smetacek V (2004) Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. *Nature* 429: 403-407

Chapter 9: References

- Irigoien X, Harris RP, Verheye HM, Joly P, Runge J, Starr M, Pond D, Campbell R, Shreeve R, Ward P, Smith AN, Dam HG, Peterson W, Davidson R, et al. (2002) Copepod hatching success in marine ecosystems with high diatom concentrations. *Nature* 419: 387-389
- Johansson N, Granéli E (1999) Cell density, chemical composition and toxicity of *Chrysochromulina polylepsis* (Haptophyta) in relation to different N:P supply ratios. *Mar Biol* 135: 209-217
- John EH, Flynn KJ (2000) Growth dynamics and toxicity of *Alexandrium fundyense* (Dinophyceae): the effect of changing N:P supply ratios on internal toxin and nutrient levels. *Eur J Phycol* 35: 11-23
- Johnston DJ (2003) Ontogenetic changes in digestive enzyme activity of the spiny lobster, *Jasus edwardsii* (Decapoda; Palinuridae). *Mar Biol* 143: 1071-1082
- Johnston, D. and Freeman, J. (2005) Dietary preference and digestive enzyme activities as indicators of trophic resource utilization by six species of crab. *Biol Bull Mar Biol Lab Woods Hole* 208, 36-46.
- Jónasdóttir SH (1994) Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. *Mar Biol* 121: 67-81
- Jónasdóttir SH, Fields D, Pantoja S (1995) Copepod egg production in Long Island Sound, USA, as a function of the chemical composition of seston. *Mar Ecol Prog Ser* 119: 87-98
- Jones DA, Kumlu M, Le Vay L, Fletcher DJ (1997) The digestive physiology of herbivorous, omnivorous and carnivorous crustacean larvae: a review. *Aquaculture* 155: 289-299
- Jones RH, Flynn KJ (2005) Nutritional status and diet composition affect the value of diatoms as copepod prey. *Science* 307: 1457-1459
- Kang H-K, Poulet SA (2000) Reproductive success in *Calanus helgolandicus* as a function of diet and egg cannibalism. *Mar Ecol Prog Ser* 201: 241-250
- Katechakis A, Stibor H, Sommer U, Hansen T (2002) Changes in the phytoplankton community and microbial food web of Blanes Bay (Catalan Sea, NW Mediterranean) under prolonged grazing pressure by doliolids (Tunicata), cladocerans or copepods (Crustacea). *Mar Ecol Prog Ser* 234: 55-69
- Kattner G, Krause M (1989) Seasonal variations of lipids (wax esters, fatty acids and alcohols) in calanoid copepods from the North Sea. *Mar Chem* 26: 261-275
- Kattner G, Krause M, Trahms J (1981) Lipid composition of some typical North Sea copepods. *Mar Ecol Prog Ser* 4: 69-74.
- Kerambrun P, Champalbert G (1975) Analyse électrophorétique en gel de polyacrylamide des protéines d'*Anomalocera patersoni* (Copépode Pontellidé) et mise en évidence des activités estérasique. *Elements d'un dimorphisme sexuel biochimique. C R Hebd Séances Acad Sci Paris* 281: 1019-1022
- Kerambrun P, Champalbert G (1993) Evidence for a dial rhythm of digestive enzyme activity in the neustonic copepod *Anomalocera patersoni*: relation with population densities. *Biochem Syst Ecol* 21: 575-582
- Kilham SS, Kreeger DA, Goulden CE, Lynn SG (1997) Effects of nutrient limitation on biochemical constituents of *Ankistrodesmus falcatus*. *Freshwat Biol* 38: 591-596
- Kjørboe T, Møhlenberg F, Nicolajsen H (1982) Ingestion rate and gut clearance in the planktonic copepod *Centropages hamatus* (Lilljeborg) in relation to food concentration and temperature. *Ophelia* 21: 181-194
- Kjørboe T, Sabatini M (1995) Scaling of fecundity, growth and development in marine planktonic copepods. *Mar Ecol Prog Ser* 120: 285-298
- Kjørboe T, Saiz E (1995) Planktivorous feeding in calm and turbulent environments, with emphasis on copepods. *Mar Ecol Prog Ser* 122: 135-145
- Kjørboe T, Saiz E, Viitasalo M (1996) Prey switching behaviour in the planktonic copepod *Acartia tonsa*. *Mar Ecol Prog Ser* 143: 65-75
- Kjørboe T, Saiz E, Visser A (1999) Hydrodynamic signal perception in the copepod *Acartia tonsa*. *Mar Ecol Prog Ser* 179: 97-111
- Kjørboe T, Visser AW (1999) Predator and prey perception in copepods due to hydromechanical signals. *Mar Ecol Prog Ser* 179: 81-95
- Klausmeier CA, Litchman E, Daufresne T, Levin SA (2004) Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature* 429: 171-174
- Klein Breteler W, Koski M (2003) Development and grazing of *Temora longicornis* (Copepoda, Calanoida) nauplii during nutrient limited *Phaeocystis globosa* blooms in mesocosms. *Hydrobiologia* 491: 185-192
- Klein Breteler WCM (1980) Continuous breeding of marine pelagic copepods in the presence of heterotrophic dinoflagellates. *Mar Ecol Prog Ser* 2: 229-233

Chapter 9: References

- Klein Breteler WCM, Gonzalez SR (1988) Influence of temperature and food concentration on body size, weight and lipid content of two *Calanoid* copepod species. *Hydrobiologia* 167-168: 201-210
- Klein Breteler, W. C. M., H. G. Fransz, and S. R. Gonzalez (1982) Growth and development of four calanoid copepod species under experimental and natural conditions. *Neth J Sea Res* 16:195-207
- Klein Breteler WCM, Grice K, Schouten S, Kloosterhuis HT, Damste JSS (2002) Stable carbon isotope fractionation in the marine copepod *Temora longicornis*: Unexpectedly low $\delta^{13}\text{C}$ value of faecal pellets. *Mar Ecol Prog Ser* 240: 195-204
- Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. *Mar Biol* 135: 191-198
- Klein Breteler WCM, Schogt N, Rampen S (2005) Effect of diatom nutrient limitation on copepod development: role of essential lipids. *Mar Ecol Prog Ser* 291: 125-133
- Kleppel GS (1993) On the diets of calanoid copepods. *Mar Ecol Prog Ser* 99: 183-195
- Kleppel GS, Burkart CA (1995) Egg production and the nutritional environment of *Acartia tonsa*: the role of food quality in copepod nutrition. *ICES J Mar Sci* 52: 297-304
- Kleppel GS, Burkart CA, Houchin L (1998) Nutrition and the regulation of egg production in the calanoid copepod *Acartia tonsa*. *Limnol Oceanogr* 43: 1000-1007
- Koski M (1999) Carbon:nitrogen ratios of Baltic Sea copepods - indication of mineral limitation? *J Plankton Res* 21: 1565-1573
- Koski M, Dutz J, Breteler W (2005) Selective grazing of *Temora longicornis* in different stages of a *Phaeocystis globosa* bloom - a mesocosm study. *Harmful Algae* 4: 915-927
- Koski M, Breteler WK, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). *Mar Ecol Prog Ser* 170: 169-187
- Koski M, Klein Breteler WCM (2003) Influence of diet on copepod survival in the laboratory. *Mar Ecol Progr Ser* 264: 73-82
- Koski M, Kuosa H (1999) Short communication. The effect of temperature, food concentration and female size on the egg production of the planktonic copepod *Acartia bifilosa*. *J Plankton Res* 21: 1779-1789
- Kouwenberg, J. H. M., 1993. Sex ratio of calanoid copepods in relation to population composition in the Northwestern Mediterranean. *Crustaceana* 64, 281-299.
- Krause G, Budeus G, Gerdes D, Schaumann K, Hesse K (1986) Frontal systems in the German Bight and their physical and biological effects. In: Nihoul JCJ (ed) *Marine Interfaces Ecohydrodynamics*, pp 119-140
- Krause M (1995) A review of hydrographic controls on the distribution of zooplankton biomass and species in the North Sea with particular reference to a survey conducted in January-March 1987. *Prog Oceanogr* 35: 81-152
- Krause M, Radach G (1980) On the succession of developmental stages of herbivorous zooplankton in the northern North Sea during FLEX '76. 1. First statements about the main groups of the zooplankton community. *Meteor Forschungsergeb A Phys. Chem. Meeres* 133-149
- Krylov PI, Alekseev VR, Frenkel OA (1996) Feeding and digestive activity of cyclopoid copepods in active diapause. *Hydrobiologia* 320: 71-79
- Kumlu M (1997) The effect of feed types on survival and trypsin activity in *Temora longicornis* (Crustacea:Copepoda). *Isr J Aquacult/Bamidgeh* 49: 199-204
- Laakmann S (2004) Abundanz und Reproduktionserfolg ausgewählter calanoider Copepoden während der Frühjahrsplanktonblüte um Helgoland. Masters Thesis, Bremen
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685
- Landry MR, Hassett RP (1985) Time scales in behavioral, biochemical, and energetic adaptations to food-limiting conditions by a marine copepod. *Arch Hydrobiol Beih Ergeb Limnol/Adv Limnol* 21: 209-221
- Leboulanger C, Descolas-Gros C, Fontugne MR, Bentaleb I, Jupin H (1995) Interspecific variability and environmental influence on particulate organic carbon $\delta^{13}\text{C}$ in cultured marine phytoplankton. *J Plankton Res* 17: 2079-2091
- Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. *Mar Ecol Prog Ser* 307: 273-306
- Legrand C, Rengefors K, Fistarol GO, Granéli E (2003) Allelopathy in phytoplankton - biochemical, ecological and evolutionary aspects. *Phycologia* 42: 406-419

Chapter 9: References

- Lester LJ, Cook JP (1987) Ontogenic changes in isozyme patterns of *Penaeus* species. *Comp Biochem Physiol B* 86: 253-258
- Le Vay, L., Jones, D. A., Puello-Cruz, A. C., Sangha, R. S., Ngamphongsai, C. (2001) Digestion in relation to feeding strategies exhibited by crustacean larvae. *Comp Biochem Physiol A* 128: 621-628
- Libourel-Houde SE, Roman MR (1987) Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. *Mar Ecol Progr Ser* 40: 60-77
- Lindley JA (1982) Continuous plankton records: Geographical variations in numerical abundance, biomass and production of euphausiids in the North Atlantic Ocean and the North Sea. *Mar Biol* 71: 7-10
- Long JD, Hay ME (2006) When intraspecific exceeds interspecific variance: effects of phytoplankton morphology and growth phase on copepod feeding and fitness. *Limnol Oceanogr* 51: 988-996
- Lonsdale DJ, Hassett RP, Dobbs FC, Yen J (1998) Physiological traits associated with a reproductive-resting stage in *Coullana canadensis* (Copepoda: Harpacticoida). *Mar Biol* 131: 123-131
- Maar M, Nielsen TG, Richardson K, Christaki U, Hansen OS, Zervoudaki S, Christou ED (2002) Spatial and temporal variability of food web structure during the spring bloom in the Skagerrak. *Mar Ecol Prog Ser* 239: 11-29
- Maguer J-F, L'Helguen S, Le Corre P (2000) Nitrogen uptake by phytoplankton in a shallow water tidal front. *Estuar Coast Shelf Sci* 51: 349-357
- Malzahn AM (2006) Larval fish dynamics in changing environments. Dissertation. Leibniz Institute for Marine Sciences IfM/GEOMAR, Kiel
- Maps F, Runge JA, Zaradjian B, Joly B (2005) Egg production and hatching success of *Temora longicornis* (Copepoda, Calanoida) in the southern Gulf of St. Lawrence. *Mar Ecol Prog Ser* 285: 117-128
- Mauchline, J., 1998. The biology of calanoid copepods. *Advances in Marine Biology*. Vol. 33. Academic Press, London.
- Mayzaud, P., Conover, R. J., 1976. Influence of potential food supply on the activity of digestive enzymes of neritic zooplankton. *Proceedings of the 10th European Symposium on Marine Biology*. 2, 415-427.
- Mayzaud P, Mayzaud O (1981) Kinetic properties of digestive carbohydrases and proteases of zooplankton. *Can J Fish Aquat Sci* 38: 535-543
- Mayzaud P, Poulet SA (1978) The importance of the time factor in the response of zooplankton to varying concentrations of naturally occurring particulate matter. *Limnol Oceanogr* 23: 1144-1154
- Mayzaud P, Roche-Mayzaud O, Razouls S (1992) Medium term time acclimation of feeding and digestive enzyme activity in marine copepods: influence of food concentration and copepod species. *Mar Ecol Prog Ser* 89: 197-212
- Mayzaud P, Tirelli V, Bernard JM, Roche-Mayzaud O (1998) The influence of food quality on the nutritional acclimation of the copepod *Acartia clausi*. *J Mar Syst* 15: 483-493
- McIlvaine, T. C. (1921) A buffer solution for colorimetric comparison. *J Biol Chem* 49: 183-186
- Micheli F (1999) Eutrophication, fisheries, and consumer-resource dynamics in marine pelagic ecosystems. *Science* 285: 1396-1398
- Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. *Geochim Cosmochim Acta* 48: 1135-1140
- Miralto A, Barone G, Romano G, Poulet SA, Ianora A, Russo GL, Buttino I, Mazzarella G, Laabir M, Cabrini M, Giacobbe MG (1999) The insidious effect of diatoms on copepod reproduction. *Nature* 402: 173-176
- Mitra A (2006) A multi-nutrient model for the description of stoichiometric modulation of predation in micro- and mesozooplankton. *J Plankton Res* 28: 597-611
- Mitra A, Flynn KJ (2005) Predator-prey interactions: is 'ecological stoichiometry' sufficient when good food goes bad? *J Plankton Res* 27: 393-399
- Morris RJ, McCartney MJ, Joint IR, Robinson GA (1985) Further studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. *J Exp Mar Biol Ecol* 86: 151-170
- Morris RJ, McCartney MJ, Robinson GA (1983) Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. I. Biochemical changes in relation to the nutrient chemistry of water. *J Exp Mar Biol Ecol* 70: 249-262
- Müller-Navarra DC (1995) Biochemical versus mineral limitation in *Daphnia*. *Limnol Oceanogr* 40: 1209-1214
- Müller-Navarra DC, Brett MT, Park S, Chandra S, Ballantyne AP, Zorita E, Goldman CR (2004) Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature* 427: 69
- Niehoff B (2000) Effect of starvation on the reproductive potential of *Calanus finmarchicus*. *ICES J Mar Sci* 57: 1764-1772

Chapter 9: References

- Niehoff B (2003) Gonad morphology and oocyte development in *Pseudocalanus* spp. in relation to spawning activity. *Mar Biol* 143: 759-768
- Niehoff B (2004) The effect of food limitation on gonad development and egg production of the planktonic copepod *Calanus finmarchicus*. *J Exp Mar Biol Ecol* 307: 237-259
- Nielsen TG, Munk P (1998) Zooplankton diversity and the predatory impact by larval and small juvenile fish at the Fisher Banks in the North Sea. *J Plankton Res* 20: 2313-2332
- Niermann U, Bingel F, Ergün G, Greve W (1998) Fluctuation of dominant mesozooplankton species in the Black Sea, North Sea and the Baltic Sea: Is a general trend recognisable? *Turk J Zool* 22: 63-81
- Oosterhuis, S. S., Baars, M. A. (1985) On the usefulness of digestive enzyme activity as index for feeding activity in copepods. *Hydrobiol Bull* 19: 89-100
- Oosterhuis, S. S., Baars, M. A., Klein-Breteler, W. C. M. (2000) Release of the enzyme chitinase by the copepod *Temora longicornis*: Characteristics and potential tool for estimating crustacean biomass production in the sea. *Mar Ecol Prog Ser* 196: 195-206
- Otto L, Zimmerman JTF, Furnes GK, Mork M, Saetre R, Becker G (1990) Review of the physical oceanography of the North Sea. *Neth J Sea Res* 26: 161-238
- Paffenhöfer G-A, Lewis KD (1990) Perceptive performance and feeding behavior of calanoid copepods. *J Plankton Res* 12: 933-946
- Paffenhöfer GA (1998a) Heterotrophic protozoa and small metazoa: feeding rates and prey-consumer interactions. *J Plankton Res* 20: 121-133
- Paffenhöfer GA (1998b) On the relation of structure, perception and activity in marine planktonic copepods. *J Mar Syst* 15: 457-473
- Paffenhöfer GA, Van Sant KB (1985) The feeding response of a marine planktonic copepod to quantity and quality of particles. *Mar Ecol Prog Ser* 27: 55-65
- Pedersen L, Jensen HM, Burmeister AD, Hansen BW (1999) The significance of food web structure for the condition and tracer lipid content of juvenile snail fish (Pisces: *Liparis* spp.) along 65-72° N off West Greenland. *J Plankton Res* 9: 1593-1611
- Penna A, Berluti S, Penna N, Magnani M (2000) Influence of nutrient ratios on the in vitro extracellular polysaccharide production by marine diatoms from the Adriatic Sea. *Biol Mar Mediterr* 7: 278-283
- Pertola S, Koski M, Viitasalo M (2002) Stoichiometry of mesozooplankton in N- and P-limited areas of the Baltic Sea. *Mar Biol* 140: 425-434
- Peters J, Renz J, Beusekom Jv, Boersma M, Hagen W (2006) Trophodynamics and seasonal cycle of the copepod *Pseudocalanus acuspes* in the Central Baltic Sea (Bornholm Basin): evidence from lipid composition. *Mar Biol* DOI: 10.1007/s00227-006-0290-8
- Peters, G., Saborowski, R., Buchholz, F., Mentlein, R., 1999. Two distinct forms of the chitin-degrading enzyme N-acetyl-beta-D-glucosaminidase in the Antarctic krill: specialists in digestion and moult. *Mar. Biol.* 134, 697-703.
- Peterson BJ (1999) Stable isotopes as tracers of organic matter input and transfer in benthic food webs: a review. *Acta Oecologica* 20: 479-487
- Plath K, Boersma M (2001) Mineral limitation of zooplankton: stoichiometric constraints and optimal foraging. *Ecology* 82: 1260-1269
- Pond, D. W., Harris, R. P., Brownlee, C. (1995) A microinjection technique using a pH-sensitive dye to determine the gut pH of *Calanus helgolandicus*. *Mar Biol* 123: 75-79
- Poulet SA, Ouellet G (1982) The role of amino acids in the chemosensory swarming and feeding of marine copepods. *J Plankton Res* 4: 341-361
- Price HJ, Paffenhöfer GA (1986) Effects of concentration on the feeding of a marine copepod in algal monocultures and mixtures. *J Plankton Res* 8: 119-128
- Price HJ, Paffenhöfer GA, Strickler JR (1983) Modes of cell capture in calanoid copepods. *Limnol Oceanogr* 28: 116-123
- Prince E, Lettieri L, McCurdy K, Kubanek J (2006) Fitness consequences for copepods feeding on a red tide dinoflagellate: deciphering the effects of nutritional value, toxicity, and feeding behavior. *Oecologia* 147: 479-488
- Raabe TU, Brockmann UH, Dürselen C-D, Krause M, Rick H-J (1997) Nutrient and plankton dynamics during a spring drift experiment in the German Bight. *Mar Ecol Prog Ser* 156: 275-288
- Redfield AC (1958) The biological control of chemical factors in the environment. *Amer Sci* 46: 205-221
- Reid PC, Lancelot C, Gieskes WWC, Hagmeier E, Weichart G (1990) Phytoplankton of the North Sea and its dynamics: a review. *Neth J Sea Res* 26: 295-331
- Reitan KI, Rainuzzo JR, Øie G, Olsen Y (1997) A review of the nutritional effects of algae in marine fish larvae. *Aquaculture* 155: 207-222

Chapter 9: References

- Rey-Rassat C, Irigoien X, Harris R, Head R, Carlotti F (2002a) Growth and development of *Calanus helgolandicus* reared in the laboratory. *Mar Ecol Prog Ser* 238: 125-138
- Rey-Rassat C, Irigoien X, Harris R, Head R, Carlotti F (2002b) Egg production rates of *Calanus helgolandicus* females reared in the laboratory: variability due to present and past feeding conditions. *Mar Ecol Prog Ser* 238: 139-151
- Richardson AJ, Schoeman DS (2004) Climate Impact on plankton ecosystems in the Northeast Atlantic. *Science* 305: 1609-1612
- Richardson AJ, Verheye HM (1998) The relative importance of food and temperature to copepod egg production and somatic growth in the southern Benguela upwelling system. *J Plankton Res* 20: 2379-2399
- Riviere D (1983) Biochemical comparison of four species of planktonic copepods of the Northwest Mediterranean by means of enzymograms of esterases, leucine aminopeptidase, alkaline phosphatase, malate dehydrogenase and the malic enzyme. *Mar Biol* 75: 19-23
- Roche-Mayzaud O, Mayzaud P, Biggs DC (1991) Medium-term acclimation of feeding and of digestive and metabolic enzyme activity in the neritic copepod *Acartia clausi*. 1. Evidence from laboratory experiments. *Mar Ecol Progr Ser* 69: 25-40
- Rodriguez, A., Le Vay, L., Mourente, G., Jones, D. A. (1994) Biochemical composition and digestive enzyme activity in larvae and postlarvae of *Penaeus japonicus* during herbivorous and carnivorous feeding. *Mar Biol* 118: 45-51
- Roessler PG (1990) Environmental control of glycerolipid metabolism in microalgae: commercial implications and future research directions. *J Phycol* 26: 393-399
- Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of global warming on wild animals and plants. *Nature* 421: 57-60
- Saborowski, R., Buchholz, F. (1999) A laboratory study on digestive processes in the Antarctic krill, *Euphausia superba*, with special regard to chitinolytic enzymes. *Polar Biol* 21: 295-304
- Sainz JC, García-Carreno FL, Córdova-Murueta JH, Cruz-Hernández P (2005) Whiteleg shrimp (*Litopenaeus vannamei*, Boone, 1931) isotrypsins: their genotype and modulation. *J Exp Mar Biol Ecol* 326: 105-113
- Sañudo-Wilhelmy SA, Tovar-Sanchez A, Fu F-X, Capone DG, Carpenter EJ, Hutchins DA (2004) The impact of surface-adsorbed phosphorus on phytoplankton Redfield stoichiometry. *Nature* 432: 897-900
- Sastri, A. R., Roff, J. C. (2000) Rate of chitinase degradation as a measure of development rate in planktonic crustacea. *Can J Fish Aquat Sci* 57: 1965-1968
- Savage VM, Gillooly JF, Brown JH, West GB, Charnov EL (2004) Effects of body size and temperature on population growth. *Am Nat* 163: 429-441
- Schägger H, von Jagow G (1987) Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Anal Biochem* 166: 368-379
- Schlitzer, R. (2006) Ocean Data View, Version 3.1.0-2006, <http://odv.awi.de>
- Schmidt K, Kähler P, Bodungen B (1998) Copepod egg production rates in the Pomeranian Bay (Southern Baltic Sea) as a function of phytoplankton abundance and taxonomic composition. *Mar Ecol Prog Ser* 174: 183-195
- Schütte T (2006) Untersuchungen zur Nahrungselektivität und zu chemosensorischen Fähigkeiten von *Temora longicornis*. Masters Thesis. Biologische Anstalt Helgoland, Oldenburg
- Schwenzer DE, Bulnheim HP (1991) Enzyme electrophoretic studies as a contribution to the systematics of the taxon *Tisbe* (Copepoda, Harpacticoida). *Zool Syst Evolut-Forsch* 29: 409-432
- Shaw BA, Harrison PJ, Andersen RJ (1995) Feeding deterrence properties of apo-fucoanthinoids from marine diatoms. 2. Physiology of production of apo-fucoanthinoids by the marine diatoms *Phaeodactylum tricorutum* and *Thalassiosira pseudonana*, and their feeding deterrent effects on the copepod *Trigriopus californicus*. *Mar Biol* 124: 473-481
- Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., Klenk, D. C. (1985) Measurement of protein using bicinchoninic acid. *Anal Biochem* 150: 76-85
- Sommer U (1988) Phytoplankton succession in microcosm experiments under simultaneous grazing pressure and resource limitation. *Limnol Oceanogr* 33: 1037-1054
- Sommer U, Sommer F (2006) Cladocerans versus copepods: the cause of contrasting top-down controls on freshwater and marine phytoplankton. *Oecologia* 147: 183-194
- Sommer U, Stibor H, Katschik A, Sommer F, Hansen T (2002) Pelagic food web configurations at different levels of nutrient richness and their implications for the ratio fish production:primary production. *Hydrobiologia* 484: 11-20
- Sotka EE (2003) Genetic control of feeding preference in the herbivorous amphipod *Ampithoe longimana*. *Mar Ecol Prog Ser* 256: 305-310

Chapter 9: References

- St. John MA, Clemmesen C, Lund T, Köster T (2001) Diatom production in the marine environment: implications for larval fish growth and condition. *ICES J Mar Sci* 58: 1106-1113
- Sterner RW, Elser JJ (2002) Ecological stoichiometry: the biology of elements from molecules to the biosphere. University Press, Princeton
- Sterner RW, Schulz KL (1998) Zooplankton nutrition: recent progress and a reality check. *Aquat Ecol* 32: 261-279
- Tande KS, Slagstad D (1982) Ecological investigation on the zooplankton community of Balsfjorden, Northern Norway. Seasonal and short-time variations in enzyme activity in copepodite stage V and VI males and females of *Calanus finmarchicus* (Gunnerus). *Sarsia* 67: 63-68
- Tang KW, Jakobsen HH, Visser AW (2001) *Phaeocystis globosa* (Prymnesiophyceae) and the planktonic food web: feeding, growth, and trophic interactions among grazers. *Limnol Oceanogr* 46: 1860-1870
- Teegarden GJ (1999) Copepod grazing selection and particle discrimination on the basis of PSP toxin content. *Mar Ecol Prog Ser* 181: 163-176
- Thibault D, Gaudy R, Le Fevre J (1994) Zooplankton biomass, feeding and metabolism in a geostrophic frontal area (Almeria-Oran Front, western Mediterranean). Significance to pelagic food webs. *J Mar Syst* 5: 297-311
- Thor P (2000) Relationship between specific dynamic action and protein deposition in calanoid copepods. *J Exp Mar Biol Ecol* 245: 171-182
- Thor P (2003) Elevated respiration rates of the neritic copepod *Acartia tonsa* during recovery from starvation. *J Exp Mar Biol Ecol* 283: 133-143
- Thor P, Dam HG, Rogers DR (2003) Fate of organic carbon released from decomposing copepod fecal pellets in relation to bacterial production and ectoenzymatic activity. *Aquat Microb Ecol* 33: 279-288
- Tirelli V, Mayzaud P (2005) Relationship between functional response and gut transit time in the calanoid copepod *Acartia clausi*: role of food quantity and quality. *J Plankton Res* 27: 557-568
- Tiselius P (1998) Short term feeding responses to starvation in three species of small calanoid copepods. *Mar Ecol Prog Ser* 168: 119-126
- Tiselius P (1992) Behaviour of *Acartia tonsa* in patchy food environments. *Limnol Oceanogr* 37: 1640-1651
- Turner, J. T., 1984. The feeding ecology of some zooplankters that are important prey items of larval fish. NOAA Tech. Rep. NMFS 7, 1-28.
- Turner JT, Doucette GJ, Powell CL, Kulis DM, Keafer BA, Anderson DM (2000) Accumulation of red tide toxins in larger size fractions of zooplankton assemblages from Massachusetts Bay, USA. *Mar Ecol Prog Ser* 203: 95-107
- Utermöhl H (1931) Neue Wege in der quantitativen Erfassung des Planktons (Mit besonderer Berücksichtigung des Ultraplanktons). *Ver Int Verein Theor Angew Limnol* 5: 567-595
- Van Duyl FC, Bak RPM, Kop AJ, Nieuwland G (1990) Bacteria, auto- and heterotrophic nanoflagellates, and their relations in mixed, frontal and stratified waters of the North Sea. *Neth J Sea Res* 26: 97-109
- Van Nieuwerburgh L, Wänstrand I, Snoeijis P (2004) Growth and C:N:P ratios in copepods grazing on N- or Si-limited phytoplankton blooms. *Hydrobiologia* 514: 57-72
- Van Wambeke F, Christaki U, Gaudy R (1996) Carbon fluxes from the microbial food web to mesozooplankton. An approach in the surface layer of a pelagic area (NW Mediterranean Sea). *Oceanologica Acta* 19: 57-66
- Vanderploeg HA, Paffenhöfer GA (1985) Modes of algal capture by the freshwater copepod *Diaptomus sicilis* and their relation to food-size selection. *Limnol Oceanogr* 30: 871-885
- Vanderploeg HA, Scavia D, Liebig JR (1984) Feeding rate of *Diaptomus sicilis* and its relation to selectivity and effective food concentration in algal mixtures and in Lake Michigan. *J Plankton Res* 6: 919-941
- Veloza AJ, Chu F-LE, Tang KW (2006) Trophic modification of essential fatty acids by heterotrophic protists and its effects on the fatty acid composition of the copepod *Acartia tonsa*. *Mar Biol* 148: 779-788
- Viitasalo M, Kiørboe T, Flinkman J, Pedersen LW, Visser AW (1998) Predation vulnerability of planktonic copepods: consequences of predator foraging strategies and prey sensory abilities. *Mar Ecol Prog Ser* 175: 129-142
- Villar-Argaiz M, Medina-Sánchez JM, Carrillo P (2002) Linking life history strategies and ontogeny in crustacean zooplankton: implications for homeostasis. *Ecology* 83: 1899-1914
- Visser AW, Saito H, Saiz E, Kiørboe T (2001) Observations of copepod feeding and vertical distribution under natural turbulent conditions in the North Sea. *Mar Biol* 138: 1011-1019

Chapter 9: References

- Vrba J, Callieri C, Bittl T, Šimek K, Bertoni R, Filandr P, Hartman P, Hejzlar J, Macek M, Nedoma J (2004) Are bacteria the major producers of extracellular glycolytic enzymes in aquatic environments? *Internat Rev Hydrobiol* 89: 102-117
- Walve J, Larsson U (1999) Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. *J Plankton Res* 21: 2309-2321
- Wichels A, Gerds G, Schütt C (2002) *Pseudoalteromonas* spp. phages, a significant group of marine bacteriophages in the North Sea. *Aquat Microb Ecol* 27: 233-239
- Wiltshire KH, Manly BFJ (2004) The warming trend at Helgoland Roads, North Sea: Phytoplankton response. *Helgol Mar Res* 58: 269-273
- Wynne D, Rhee GY (1986) Effects of light intensity and quality on the relative N and P requirement (the optimum N:P ratio) of marine planktonic algae. *J Plankton Res* 8: 91-103
- Zöllner, N., Kirsch, K. (1962) Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoide) gemeinsamen Sulfosphovanillin-Reaktion. *Z Ges Exp Med* 135: 545-561
- Zubkov MV, Sleigh MA, Burkill PH (2001) Heterotrophic bacterial turnover along the 20° W meridian between 59° N and 37° N in July 1996. *Deep Sea Res II* 48: 987-1001

Danksagung

Ich danke meinen beiden Betreuern P. D. Dr. Maarten Boersma und Dr. Reinhard Saborowski, die vor Ort auf Helgoland an der Biologischen Anstalt Helgoland in der Stiftung AWI (HGF) meine Ansprechpartner waren und meinem betreuenden Professor Dr. Ulrich Sommer an der Christian-Albrechts-Universität zu Kiel am Leibnitz-Institut IfM/Geomar, sowie den Mitgliedern meiner Prüfungskommission für ihr Interesse und ihre Zeit und Frau Heike Herden für Ihre Freundlichkeit.

Ich danke Maarten Boersma für die Sicherung meiner Finanzierung während des größten Zeitraums meiner Arbeit über das BMBF-finanzierte Projekt GLOBEC-Deutschland, für die letzten paar Monate des Zusammenschreibens auch dem Arbeitsamt Pinneberg. AWI, GLOBEC-Deutschland und MARBEF danke ich für die zusätzliche finanzielle Unterstützung für Reisekosten, die es mir ermöglichte, an nationalen und internationalen Konferenzen in Gijon, Spanien und in Hammamet, Tunesien und an einem MARBEF-Copepoden-Kurs in Bizerte, Tunesien teilzunehmen.

Den vielen Kollegen und Freunden von Foodweb, GLOBEC und der Biologischen Anstalt Helgoland danke ich für die nette Zusammenarbeit, besonders den Hauptakteuren der Projekte nebst meinen Betreuern also Jürgen Alheit, Justus van Beusekom, Fritz Buchholz, Gunnar Gerds, Antje Wichels und Karen Wiltshire. Für tatkräftige Unterstützung und Diskussionen danke ich besonders Christian Agurto, Kristina Barz, Karin und Ulf Bickmeyer, Jörg Dutz, Andrea Gerech, Magnus Hengevoß, Jens-Peter Hermann, Silvia Janisch, André Keunecke, Alexandra Kraberg, Sonja Leidenberger, Silke Laakmann, Anne Langer, Nina Mühlenbeck, Eva Nossek, Janna Peters, Jasmin Renz, Tatyana Romanova, Katrin Schachmann, Tanja Schütte, Anne Wesche und ganz besonders auch den Crews von Aade, Uthörn, Heincke, Alkor und Dana.

Den Betreuern des Copepoda-Kurses in Tunesien danke ich für den erweiterten Horizont auf die Copepodenforschung: Geoff Boxshall, Jiang Shiou Hwang, Thomas Kiørboe, Carol Lee, Claude Razoul, François Schmitt, Sami Souissi, Rudi Strickler und den anderen Kursteilnehmern aus aller Welt, besonders Veronica, Neila und Mohammad.

Für nette gemeinsame Stunden während und nach der Arbeit danke ich besonders Anne W. und Melanie, Gabriela und Luis, Tanja und Christina, Anne L. und Anne S., Arne und Nicole, Alex, Folke, Isa, Britta, Karin, Reinhold und den Volleyballern vom VfI-Fosite.

Nicht zuletzt danke ich meinem lieben Wolf, meinen lieben Eltern im Schwarzwald und meinen Freunden in Hamburg, Köln und Freiburg und sonstwo in der Welt für die moralische Unterstützung.

Curriculum Vitae von Susanna Knotz

TÄTIGKEITEN UND WEITERBILDUNG

- Oktober 2002 – dato Doktorandin der **Christian-Albrechts Universität zu Kiel** bei Prof. Dr. U. Sommer, P. D. Dr. M. Boersma und Dr. R. Saborowski an der **Biologischen Anstalt Helgoland in der Stiftung Alfred-Wegener-Institut für Polar- und Meeresforschung (Helmholtz-Gemeinschaft)** in dem BMBF-geförderten Projekt **Globec-Deutschland** eingebunden in das **Foodweb Projekt Helgoland**
- Juli 2005 MARBEF-Training Course in the Biology and Systematics of Copepods, 04.-08.07.2005, **University of Bizerte, Tunesien**
- September 2002 Öffentlicher Vortrag an der **Fachhochschule Kiel** über wissenschaftsjournalistisch aufbereitetes Gesundheitsthema
- Mai – Juni 2002 P&R-Arbeit zur Einführung der EU-Wasser-rahmenrichtlinie für das **Umweltministerium Schleswig-Holstein** im Auftrag der **Agentur XÄM**
- März 2001 – September 2002 Mitarbeit im **EU-Projekt Aqua-Chip** in Zusammenarbeit mit Dr. G. Kasimir vom **HYDRA-Institut Wien**
- Oktober – November 2001 Datenaufbereitung und grafische Gestaltung von Makrozoobenthosdaten im Auftrag des **HYDRA-Instituts Konstanz**, Büro P. Rey; Mitarbeit bei der Elektrofischung verschiedener Fließgewässer in Liechtenstein und der Schweiz
- Juni – August 2001 Regionalbearbeiterin für die Niederländische und Deutsche Nordsee für den **ADAC-Sommerservice** im Auftrag des **HYDRA-Instituts Konstanz**, Büro Peter Rey
- Mai 2001 Biologisch-ökologische Fließgewässeruntersuchungen nach DIN 38410, Teil 2 für das **Limnologische Institut Dr. Nowak**
- Mai 2001 Übersetzungsarbeiten für **IBS-Multimedia**, Todtnau
- März – Mai 2001 Büroassistentin, **Bürstenfabrik Knotz**, Todtnau
- September, November 2000 Übersetzungsarbeiten für **IBS-Multimedia**, Todtnau
- März – April 2000 Übersetzungsarbeiten für **IBS-Multimedia**, Todtnau

- August 1999 – Februar 2000 Gestaltungsaufträge für **Greenpeace Hamburg**
- September 1999 Übersetzungsarbeiten für den **Verlag MMK/ad press**, Hamburg
- August 1999 – Februar 2001 Wissenschaftliche Angestellte am **Limnologischen Institut Dr. Nowak** in Ottersberg, bis Februar 2000 als Teilzeitkraft, ab März 2000 als Vollzeitkraft und Bereichsleiterin der Abteilung **Mikrobiologie/Ökotoxikologie**
- Mai 1999 Werkvertrag bei Dr. Gesine Schütte, **BIOGUM (Biologie, Umwelt, Technik), Institut für Allgemeine Botanik der Universität Hamburg**
- November 1998 – März 1999 Wissenschaftliche Hilfskraft am **Institut für Hydrobiologie und Fischereiwissenschaft** bei Dr. Anno Faubel an der **Universität Hamburg**
- Juni – September 1998 **Leonardo da Vinci-Stipendium** für ein ENTRANTS-Projekt an der **Universität Oslo und der Landwirtschaftlich-Technischen Hochschule Ås** in Norwegen bei Jonathan E. Colman, Dr. Eigil Reimers und Dr. Stein R. Moe
- April – Mai 1998 Zusammenarbeit mit **Dipl.-Forstw. Gerhard Kihm** für das Forstamte Ettlingenweier bei Karlsruhe
- Februar 1998 Praktikum beim **Verlag interconnections** in Freiburg
- Dezember 1997 – Jan. 1998 Wissenschaftliche Hilfskraft am **Institut für Bodenkunde der Forstwissenschaftlichen Fakultät der Universität Freiburg** bei Dr. Stefan Raspe
- Oktober 1997 Meeresbiologiekurs "Ökosystem Mittelmeer" mit Tauchausbildung, 01.-11.10.1997, **HYDRA Institut für Meereswissenschaften, Elba, Italien**
- September – November 1997 Praktikum bei **impuls/UmTourS**, einem Unternehmen für Landschaftsplanung und Umwelttouristik, Freiburg
- Juni – August 1997 Mitarbeit bei der Neugestaltung des **BUND-Waldschadenslehrpfades** am Schauinsland bei Freiburg in Zusammenarbeit mit der **Forstverwaltung** und der **Forstlichen Versuchsanstalt Freiburg**
- August 1995 Erstellen eines Warenpräsentationskataloges für die **Bürstenfabrik Knotz**, Todtnau

- Oktober – November 1994 **Praktikum** in einem Telemetrieprojekt der **Aktion Fischotterschutz e. V.** in Demen (Mecklenburger Seenplatte) in Kooperation mit dem **Umweltministerium Mecklenburg-Vorpommern**
- Oktober 1993 – Juni 1997 **Abschluss des Biologiestudiums an der Albert-Ludwigs-Universität** in Freiburg
Diplomprüfungen in den Fächern Tierphysiologie/Verhaltensbiologie; Neurobiologie/Biophysik; Zellbiologie; Neuropathologie
Diplomarbeit "Neuronale Kontrolle der Verstärkung in einem Gelenkstellungsregelkreis der Stabheuschrecke *Carausius morosus*" bei Dr. Rolf Kittmann am Lehrstuhl Prof. Dr. K. Vogt in der Abteilung Tierphysiologie des Instituts für Zoologie
- September 1992 – Mai 1993 **Studium an der Brock University** in St. Catharines, Ontario/Kanada
Honours BSc Thesis "The effect of dopamine on second messenger systems in the hindgut of the crayfish *Procambarus clarkii*" bei A. Joffre Mercier
Kurse in Datenverarbeitung, Meeresbiologie, Paläontologie
- Mai – Juli 1992 Wissenschaftliche Hilfskraft in der **Abteilung Tierphysiologie des Instituts für Zoologie der Universität Freiburg** bei Dr. K.-G. Collatz
- Oktober 1989 – August 1992 **Biologiestudium an der Albert-Ludwigs-Universität** in Freiburg im Breisgau
- 1980 – 1989 **Gymnasium** in Schönau im Schwarzwald
Abiturprüfungsfächer: Chemie, Englisch, Mathematik, Geschichte/Gemeinschaftskunde, Deutsch, Preis der Chemischen Industrie, Mitglied der Schülermitverwaltung und der Schülerzeitungsredaktion

SPRACHKENNTNISSE

Englisch mündlich und schriftlich fließend; **Spanisch, Französisch, Norwegisch** ausbaufähige Kenntnisse

Pinneberg, 31.5.2006, Susanna Knotz

Description of the scientific contributions to multiple author manuscripts

- 1) S. Knotz, M. Boersma, R. Saborowski "Microassays for a set of enzymes in individual small marine copepods" *Comparative Biochemistry and Physiology, Part A* 145/3: 406-411 (2006)

All analyses, the text writing and graphical presentation were done by Susanna Knotz under the supervision of PD Dr. Maarten Boersma and Dr. Reinhard Saborowski.

- 2) S. Knotz, C. B. Augustin, M. Boersma, R. Saborowski "Sublethal physiological effects of food limitation in copepods"

All analyses, the text writing and graphical presentation were done by Susanna Knotz under the supervision of PD Dr. Maarten Boersma and Dr. Reinhard Saborowski. C. B. Augustin assisted in gaining mortality data.

- 3) S. Knotz, S. Laakmann, J. J. E. van Beusekom, R. Saborowski, K. H. Wiltshire, M. Boersma "Food quality, quantity and hydrographic influence on *Acartia clausi* and *Temora longicornis* egg production during a spring phytoplankton bloom"

All analyses, the text writing and graphical presentation were done by Susanna Knotz under the supervision of PD Dr. Maarten Boersma and Dr. Reinhard Saborowski. Silke Laakmann provided data on egg production of *Acartia clausi* and *Temora longicornis*. Justus van Beusekom provided lab space and advised on phosphorus measurements, Karen Wiltshire provided data on phytoplankton, temperature and salinity.

- 4) S. Knotz, A. Malzahn, A. Wesche, J. J. E. van Beusekom, R. Saborowski, M. Boersma "Stable isotopes as predictors of copepod reproductive success"

All analyses, the text writing and graphical presentation were done by Susanna Knotz under the supervision of PD Dr. Maarten Boersma and Dr. Reinhard Saborowski. Arne Malzahn assisted in seston sampling and stable isotope sample preparation and Anne Wesche provided egg production data of four dominant copepods at Helgoland in 2004.

Erklärung

Die vorliegende Dissertation wurde selbstständig von mir angefertigt und ist nach Form und Inhalt meine eigene Arbeit. Sie wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein bisher einziges und erstes Promotionsverfahren.

Die Promotion soll im Fach Meereskunde erfolgen.

Ich lasse Zuhörer für die Disputation zu.

Pinneberg, den 31. Mai, 2006

Susanna Knotz