Trophic interactions in the pelagic

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Susanna Knotz
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Koreferent:	PD Dr. M. Boersma
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Content

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 *Bacillariophycea* (diatoms) – which range in size from 5-500 µm – mainly during spring and autumns blooms. Some important representatives of diatoms are the genera *Chaetoceros*, *Thalassiosira*, *Coscinodiscus*, *Rhizosolenia*, *Odontella, Leptocylindrus* and *Skeletonema*

(Hoppenrath 2004). *Haptophyceae* or *Prymnesiophycea* (*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 *Dinophycea* (dinoflagellates) (*Ceratium*, *Gonyaulax*, *Alexandrium*, *Dynophysis*) are the main producers during the summer months (5-200 µm). During the same period, *Cryptophycea* are abundant in coastal communities as well as in the Central North Sea (3-20 µm). *Crysophycea* are usually represented by silicoflagellates such as *Distephanus* but only form minor components of the phytoplankton community in the North Sea. Small procaryotic *Cyanophycea* (~ 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 detrivorous 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, euphausids (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.).

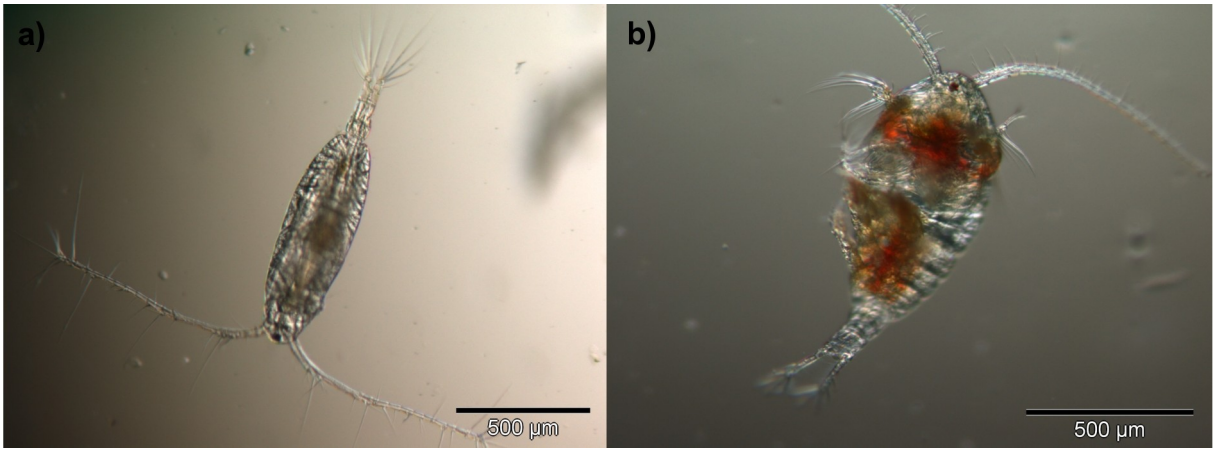


Fig. 1.2: a) Acartia clausi *female, b)* Temora longicornis *female.*

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

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 10^6 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 (Kiørboe 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 hetereotrophic 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

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

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 poly unsaturated 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 homeostastis 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).

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 (Kiørboe et al. 1999; Kiørboe 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,

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

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, chitobiase, 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.

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
Corycaeus anglicus	Ca	carnivorous	neritic, warm to cold temperate, Atlantic and Pacific
Acartia clausi	Ac	omnivorous	oceanic to neritic, warm to cold temperate, Atlantic and Pacific
Centropages typicus	Ct	omnivorous	oceanic, marine, warm to cold temperate, Atlantic
Temora longicornis	Tl	omnivorous	neritic, marine to estuarine, warm to cold temperate, Atlantic,
Paracalanus parvus	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.

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	McIlvaine	5
Beta-glucosidase	4-Methylumbelliferyl beta-D-glucoside	Sigma 3633	McIlvaine	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.

Carbon, nitrogen and dry mass (dm) were analysed in pools of 25 to 50 freeze dried females with a CHN analyser (Fisions 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 °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 °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 ± 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* (µg ind^{-1})	Cs length (µm ind^{-1})	Cs width (µm 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)
Tl	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 µmol L^{-1} in a total volume of 500 µL 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 (nmol h^{-1} mg_{prot}^{-1}). The linearity of the assay was tested with extracts of *A. clausi* and

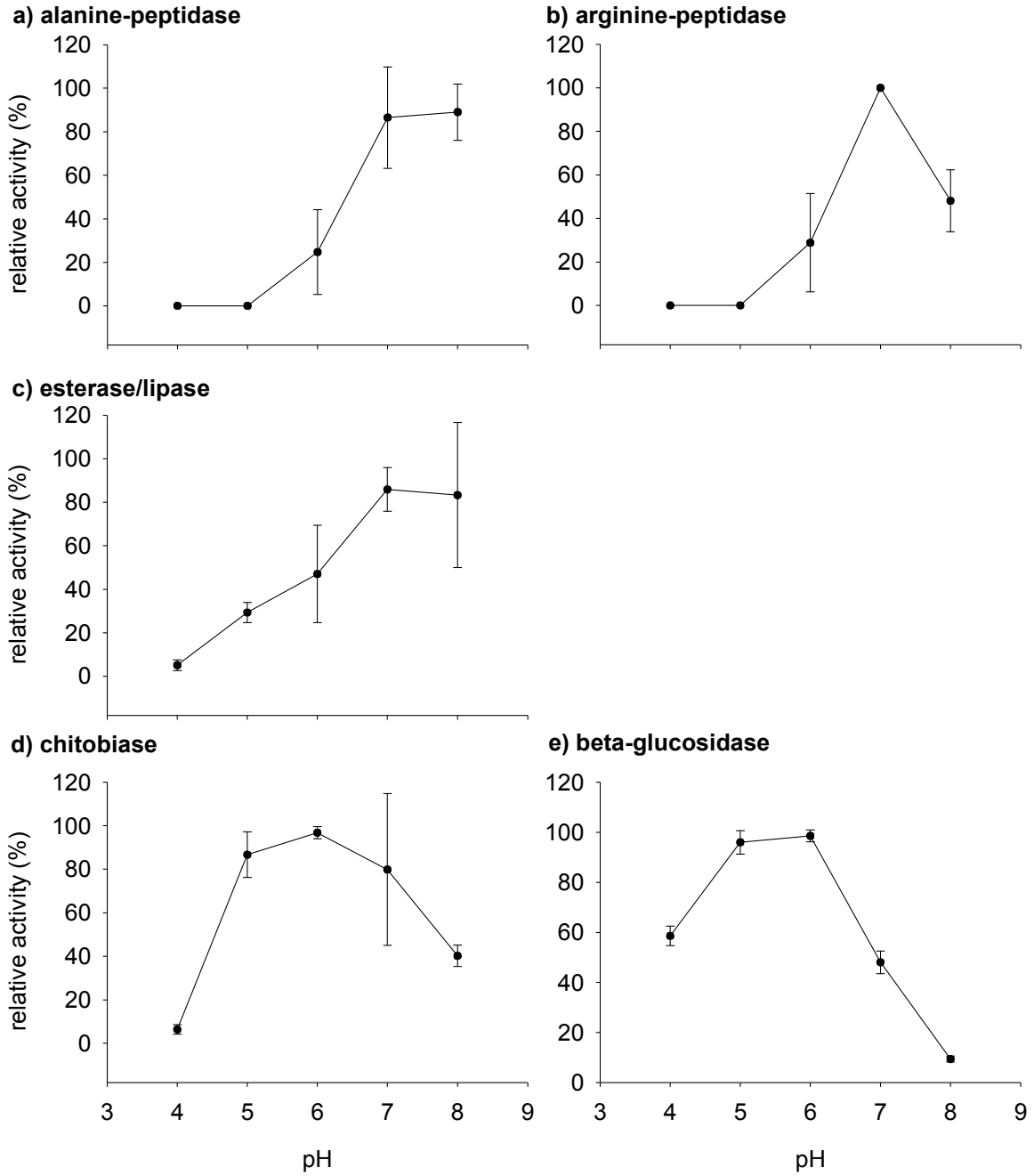


Fig. 2.1: pH-profiles of all enzymes measured with extracts of T. longicornis *females (n = 3; means ± SD).*

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).

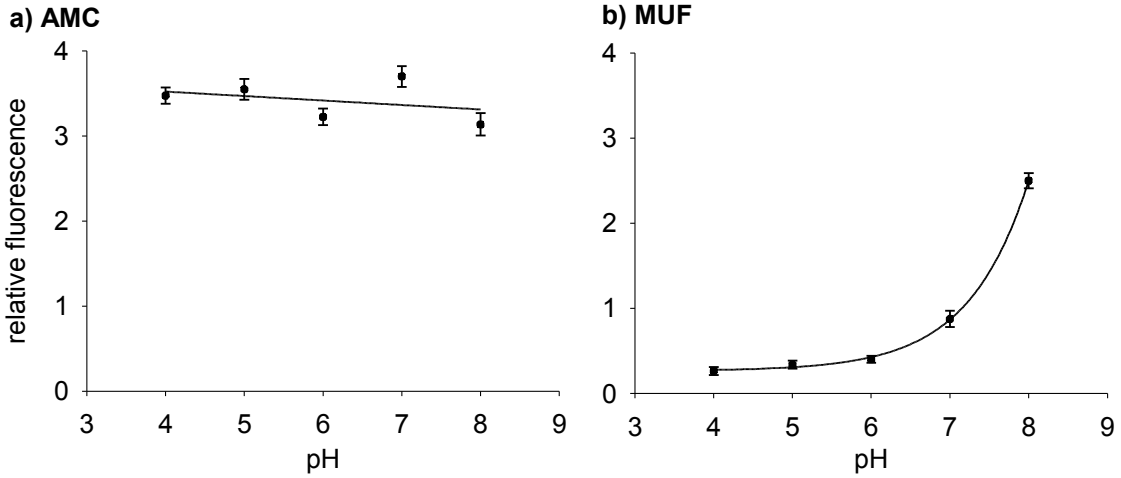


Fig. 2.2: Effects of pH on the fluorescence of a) 7-amino-4-methylcoumarin (AMC); linear regression and b) 4-beta-methylumbelliferone (MUF); exponential regression (n = 4; means ± SD).

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

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 nmol h^{-1} mg_{prot}^{-1} in *C. anglicus* to 5000 nmol h^{-1} mg_{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 chitobiase. It hydrolyses oligomers of N-acetyl-glucosamines (NAG) derived from chitin degradation to NAG monomers. Total activities of chitobiase ranged between 90 and 930 nmol h^{-1} mg_{prot}^{-1}. Surprisingly, species considered more carnivorous showed low chitobiase activities, while the more herbivorous species expressed elevated activities. Therefore, herbivorous copepods may be capable of utilising diatom chitin by elevated chitobiase 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 nmol h^{-1} mg_{prot}^{-1} in *C. anglicus* to 1100 nmol h^{-1} mg_{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.

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

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.*

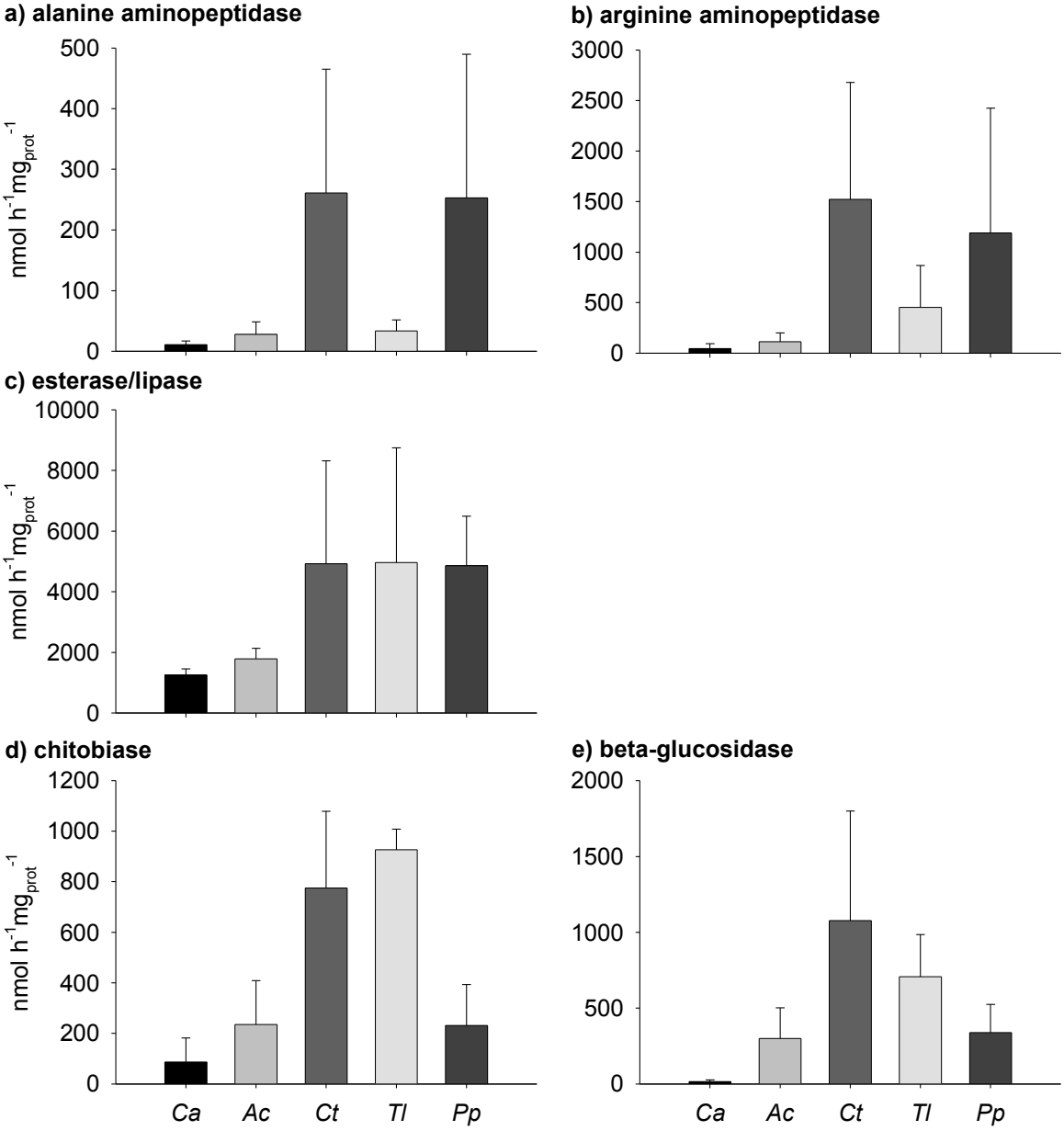


Fig. 2.3: Specific activities of enzymes (n = 4-6; means ± SD). For abbreviations refer to Table 2.1.

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 chitobiase 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. chitobiase, 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.

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 hydrolysation 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

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 ± 1°C in a 16:8 hours light:dark cycle (culturing conditions). They were fed ad libitum with *Rhodomonas* sp. (~500 µg 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 µg 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 (nmol h^{-1} mg_{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 ± 1°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 x 10 x 0.075 cm) for discontinuous SDS-PAGE (Laemmli 1970; Schägger 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 ~25°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 billiant 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.

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

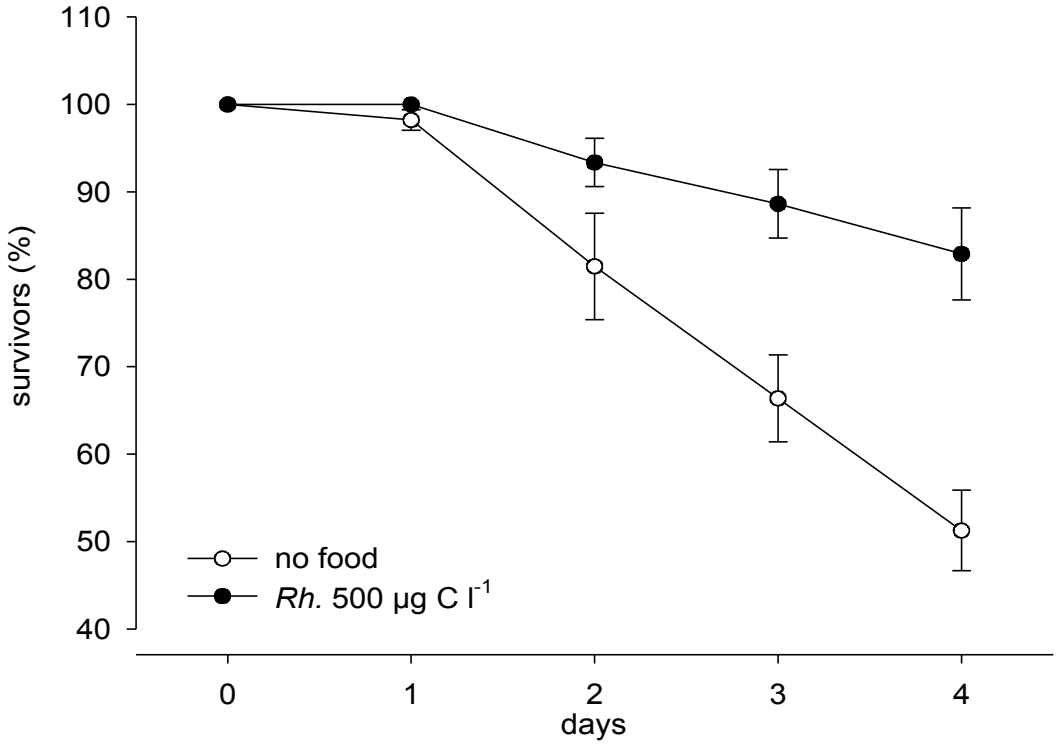


Fig. 3.1: Acartia clausi*: percent survivors without food or fed with* Rhodomonas *sp. (500 µg C l^{-1}), (means ± SE, N=9-10).*

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 ± 2% (means ± SE) as compared to the 6 ± 2% rate in the *Rhodomonas* sp. control. Mortality rates and survival were significantly different between treatments on all four days (LSD-Test, p≤0.05).

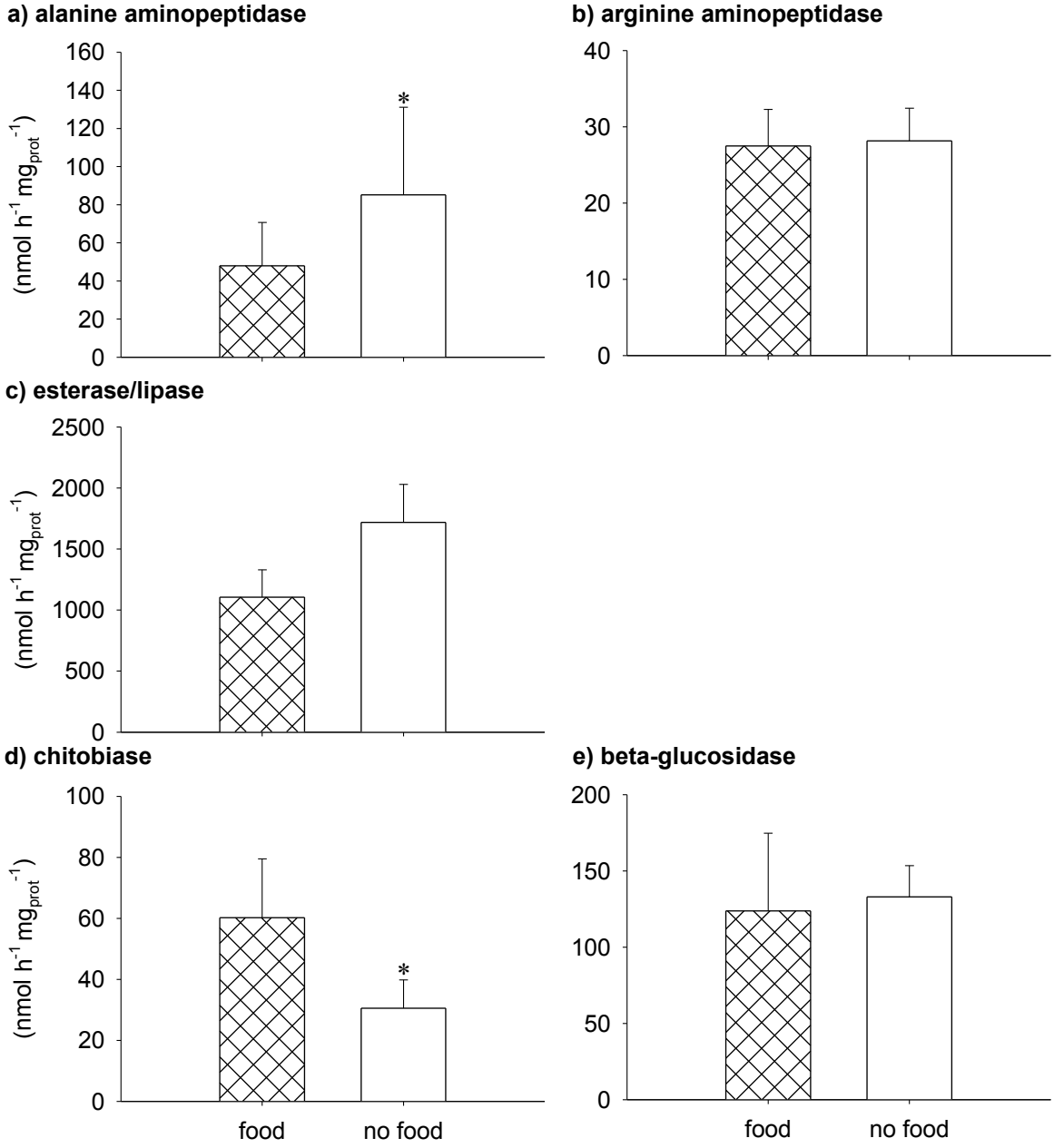


Fig. 3.2: Acartia clausi*: specific digestive enzyme activities of fed and non-fed individuals; (N=6 ± SE); *: treatments significantly different.*

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

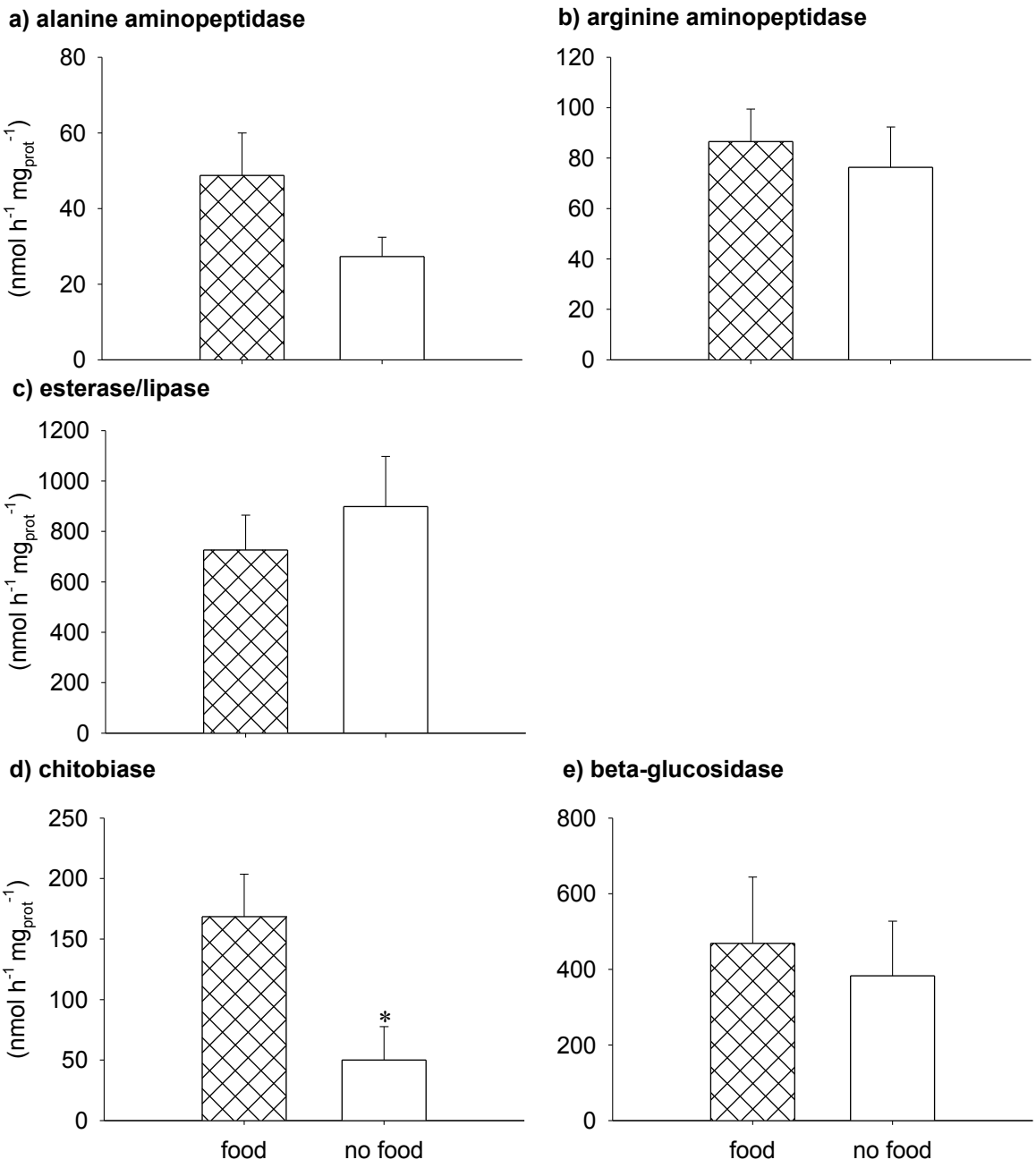


Fig. 3.3: Acartia tonsa*: specific digestive enzyme activities of fed and non-fed individuals (N=6 ± SE); *: treatments significantly different.*

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

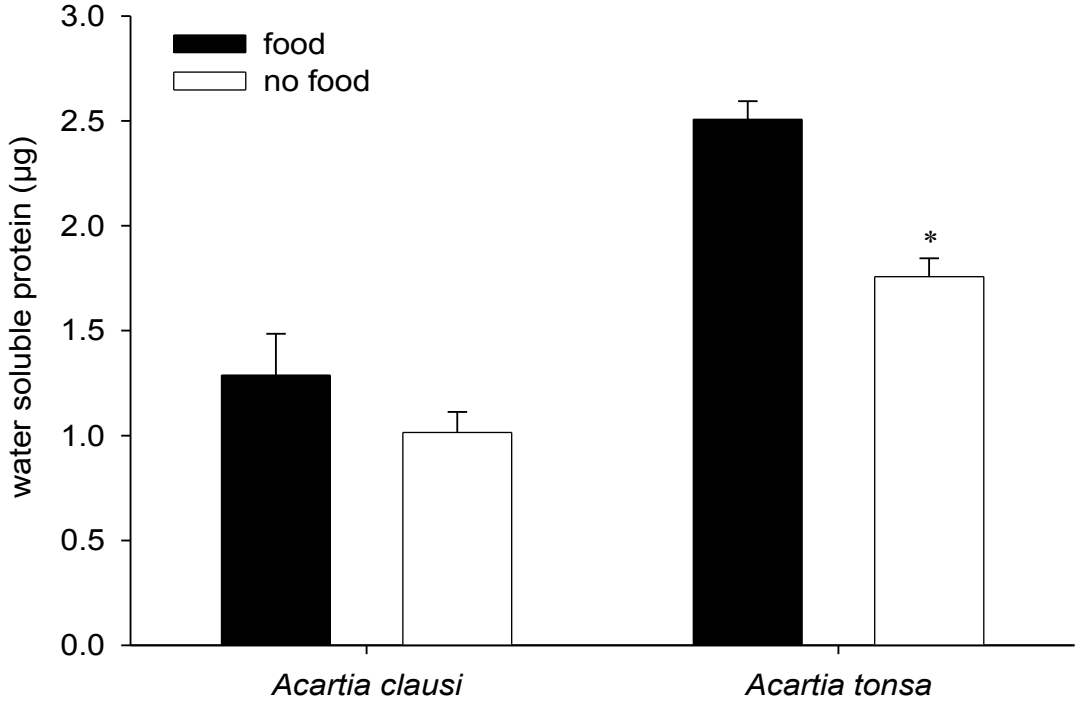


Fig. 3.4: Water soluble protein content of fed and non-fed Acartia clausi *and* A. tonsa *specimens (means ± SE, N=9-10); *: treatments significantly different.*

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.

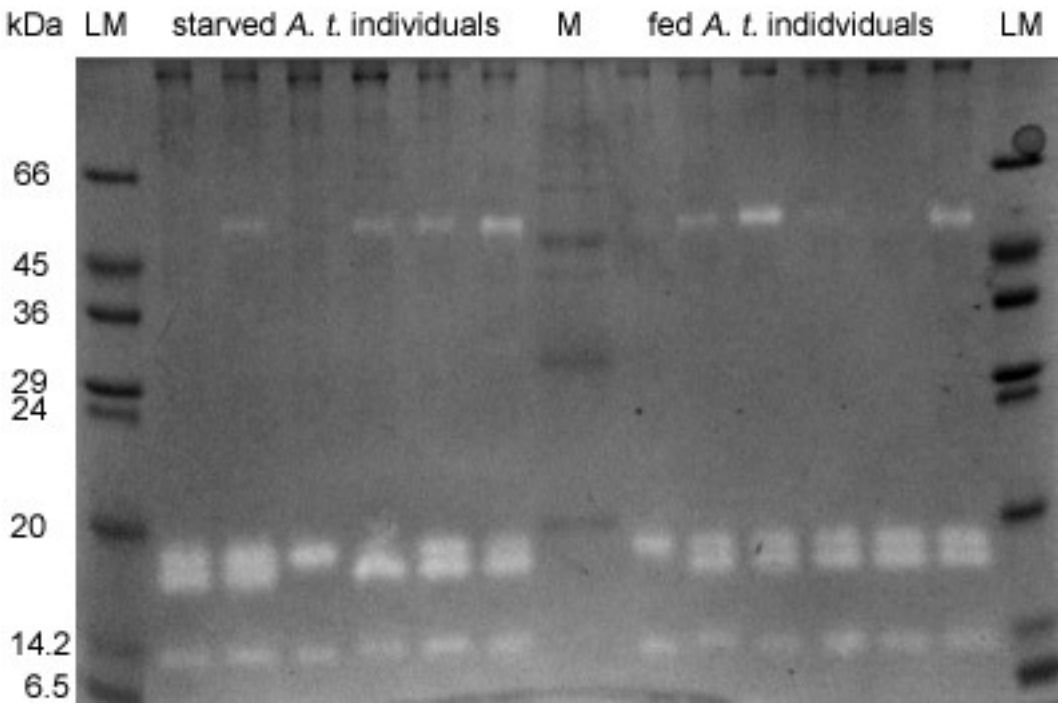


Fig. 3.5: Protease activity bands in 6 starved and 6 fed individuals of Acartia tonsa *(A. t.); LM – Sigma low range marker, M – 10 kDa step marker starting with 20 kDa; left column: molecular weights of LM in kDa.*

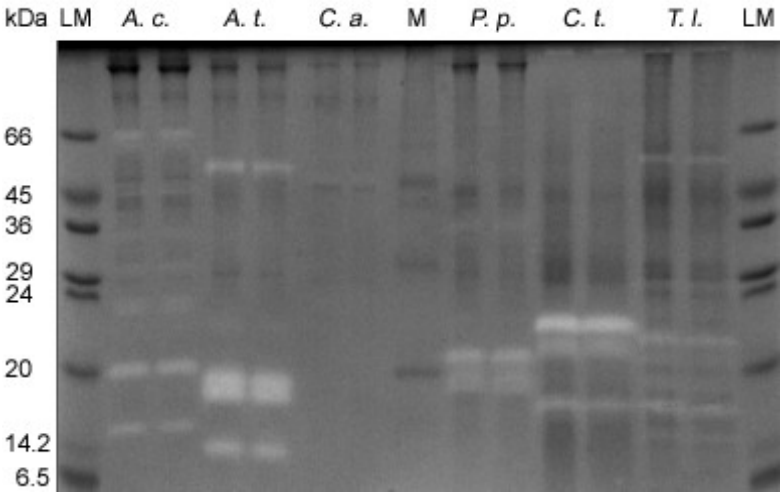
3.4. Discussion

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 wood be enhanced and the abundance of enzyme synthesising B- and F-cells of the copepod midgut (Arnaud et al. 1980) wood 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 chitobiase activity decreased in both species after two days. Different results between species were obtained for alanine-aminopeptidase of which

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. - T*emora 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

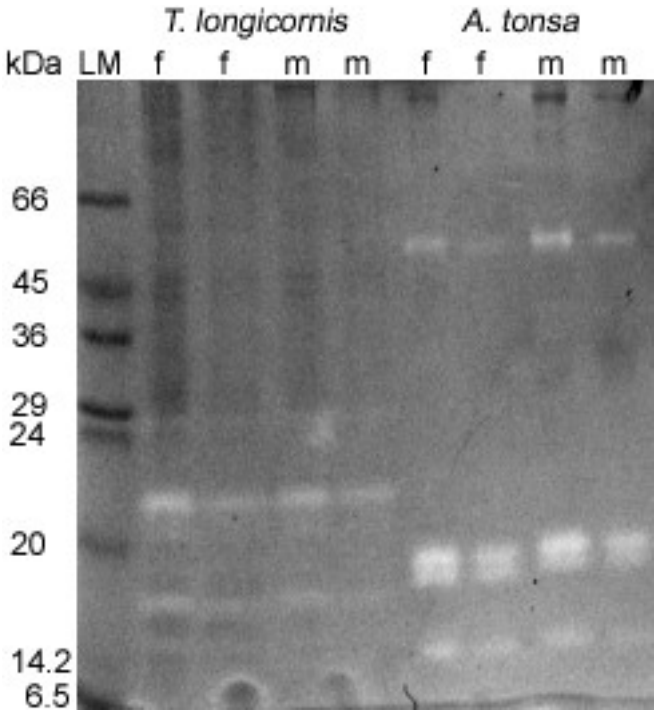


Fig. 3.7: Protease activity bands in Temora longicornis *and* Acartia tonsa*; LM – Sigma low range marker, f – females, m – males; left column: molecular weights of LM in kDa.*

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-

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 over-wintering 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

reaction of the digestive enzyme to the lack of suitable substrates. Similar reactions were observed in euphausids. Starvation caused a reduction of chitinolytic activity in the stomachs and in the midgut glands of Antarctic krill (Saborowski and Buchholz 1999). Chitobiase 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, endopetidases appeared in various isoforms. The analysis of endopeptidases by zymography is a suitable tool to reveal differences between species, sexes, or treatments (Einsele

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* (Schwenzer 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 relates 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

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.

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* (Ianora 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).

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 whithin 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

fractionated by gently pouring the water through a set of sieves. The fractions > 10 µm were resuspended in 450-500 mL filtered sea water (Whatman GF/C), gently mixed, and even volumes distributed between filters; the < 10 µm 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 µm 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 mL1N 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 %).

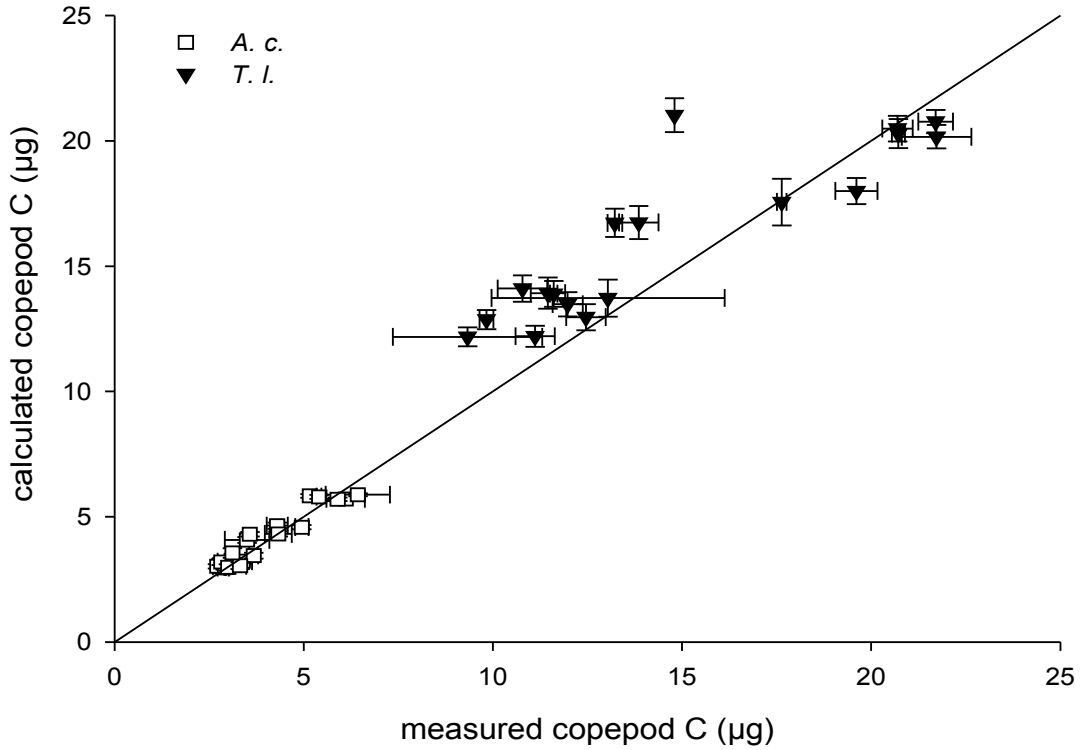


Fig. 4.1: Measured A. clausi *and* T. longicornis *carbon values against calculated carbon (see methods), means ± SE; line denoting 1:1 relationship.*

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 µm). 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 $Q_{10}=3$ (Kiørboe 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*: $\mu g\,C=0.0470*length-31.71$, $r=0.81$, $r^2=0.66$, $p<0.000001$, $N=118$ measured between 10/02/2004 and 24/05/2005; *A. clausi*:

$\mu g\, C = 0.01184 * length - 7.06$, $r = 0.93$, $r^2 = 0.87$, $p < 0.000001$, $N = 42$ 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): $GFR_C = \frac{(N_{control} - N_{grazing})}{(24\mathrm{h} * N_{ind} * C_{ind})}$.

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

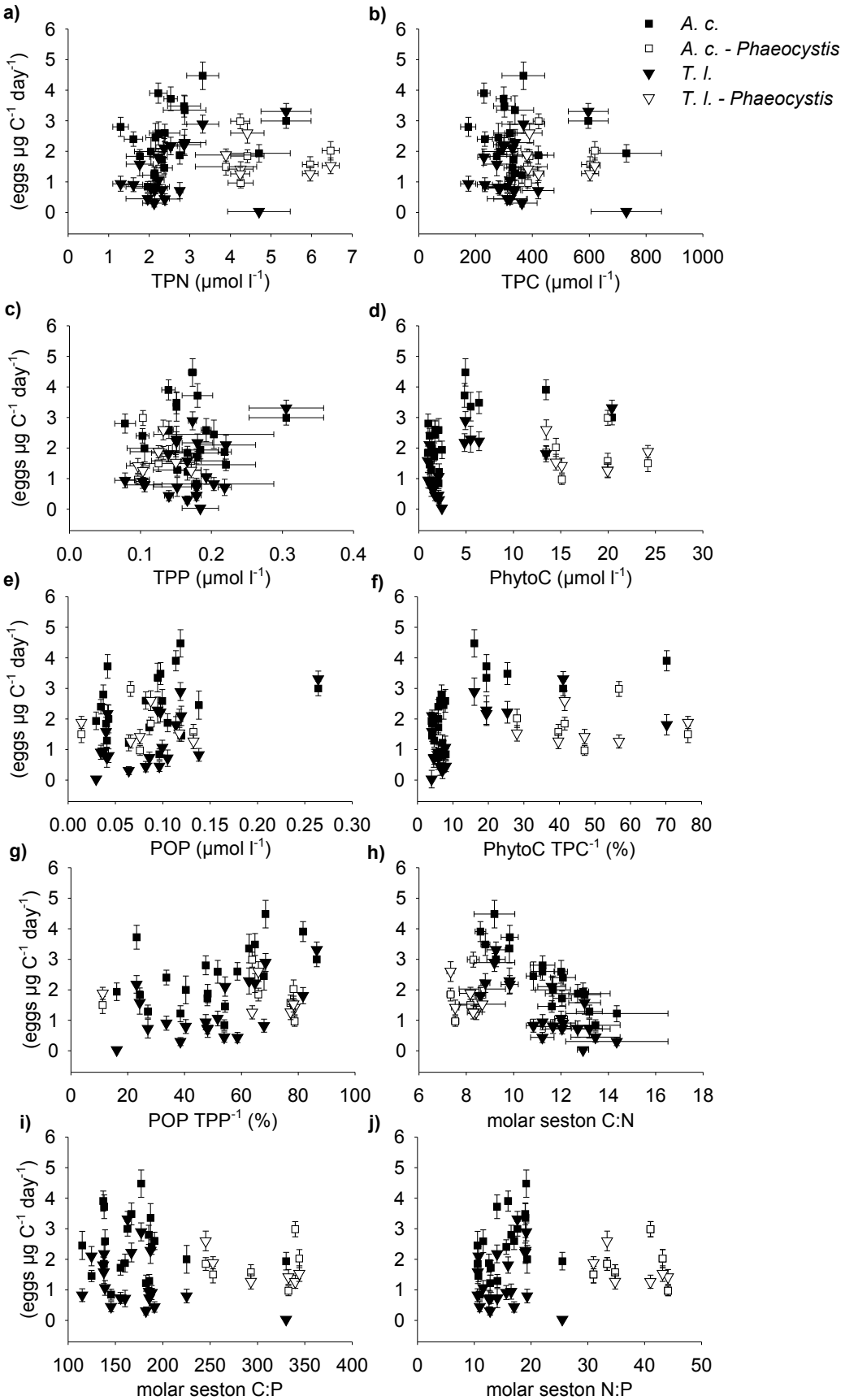


Fig. 4.2: Standardised egg production (means ± SE) versus food quantity a) TPN, b) TPC, c) TPP, and quality d) PhytoC, e) POP, f) percentage of PhytoC on TPC, g) percentage of POP on TPP, h) molar seston C:N, i) molar C:P, j) molar N:P; open symbols represent data obtained in May when Phaeocystis *sp.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

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 µm and 10-40 µm 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 ± SE, N=22) and *T. longicornis* (4.78 ± 0.06) (means ± 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 ($C:N_{ing}$ /$C:N_{body}$).

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 °C) during the survey period was recorded in early March. Afterwards, temperature rose constantly up to 10.8 °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 (

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

Table 4.1: Food Quantity in size fractions (µmol l^{-1}) and fraction percentages on the total particulate material < 300 µm (N=3-8; means ± SD).

Fraction Date	TPC µmol L^{-1}	SD	%	TPN µmol L^{-1}	SD	%	TPP µmol 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 µm 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*

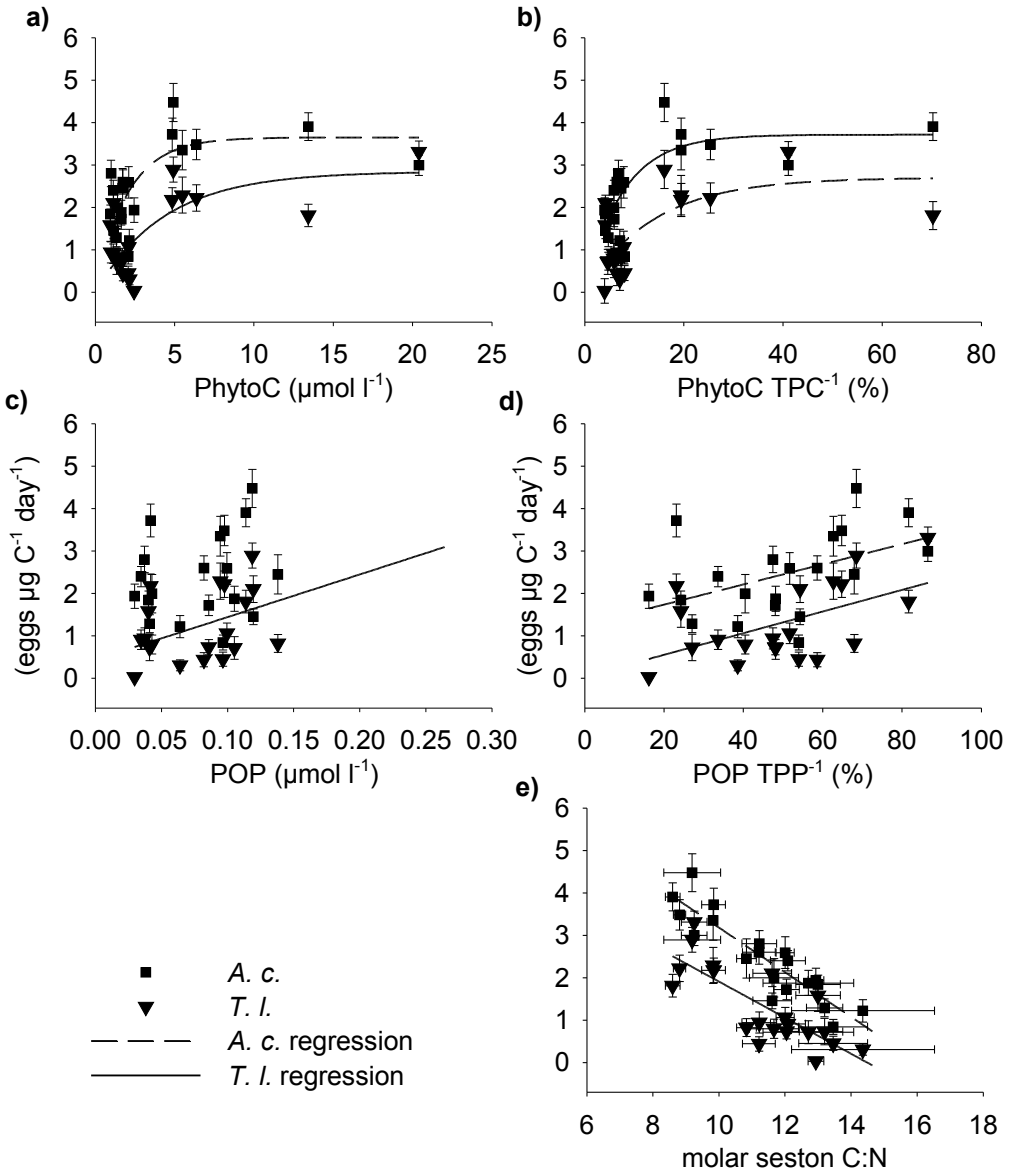


Fig. 4.3: Regression plots: significant correlations of egg production with nutritional parameters as in Table 4.1 (only May data); in a) + b) regression function $f(x)=a*(1-e^{-bx})$, otherwise linear regression.

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).

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 (µmol L^{-1})	TPN (µmol L^{-1})	TPP (µmol L^{-1})	POP (µmol L^{-1})	PIP (µmol 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

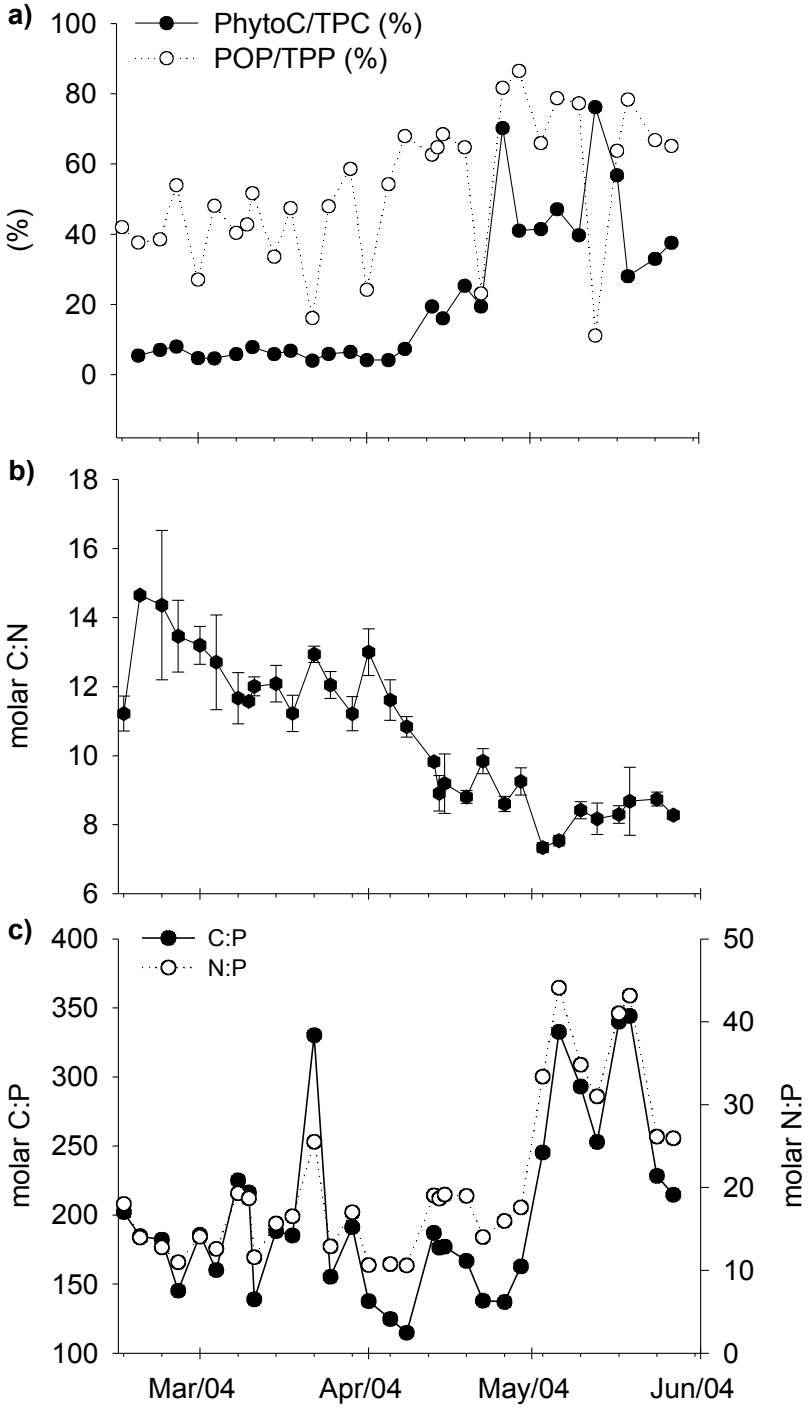


Fig. 4.4: Development of quality parameters during spring: a) percentage of POP TPP^{-1} and of PhytoC TPC^{-1} (calculated from means), b) particulate molar C:N (N=3-6; mean ± SD), c) particulate molar C:P and N:P (calculated from means); note different scales.

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

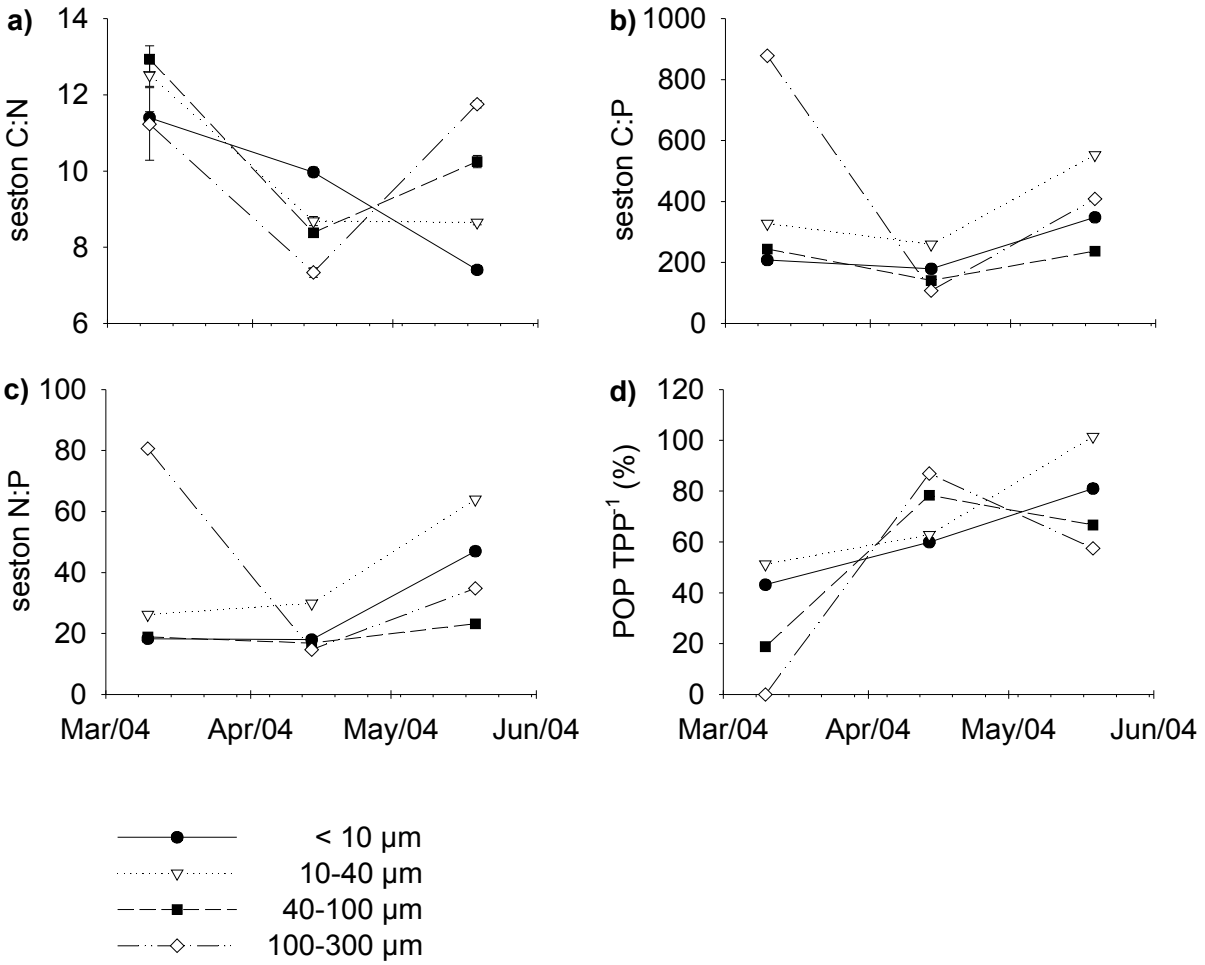


Fig. 4.5: Quality in size fractions (< 10 µm, 10-40 µm, 40-100 µm, 100-300 µm) a) molar seston C:N (N=3-6; means ± SE), b) molar seston C:P, c) molar seston N:P, d) percentage of POP TPP^{-1} (calculated from averages).

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

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≤0.05) in bold.

Parameter	r(X,Y)	r²	t	p	r(X,Y)	r²	t	p
	Acartia clausi				*Temora longicornis*			
	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 athecate 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 analyis.

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 µmol 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 $b=0.23$

($r^2=0.49$, $p=0.0006$), 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 ± 4% of the smallest size class, and was above expected values (58 ± 2%), in *T. longicornis* with 46 ± 5% ingestion it was below expected values (60 ± 2%) (Fig. 4.6a). Average availability of the 5-10 µm size class was 33 and 34 ± 1% for *A. clausi* and *T. longicornis*, while they fed more than expected with 43 ± 5% and 52 ± 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 ± 1% for *A. clausi* and 6 ± 1% for *T. longicornis*, while *A. clausi* fed 18 ± 5 % and *T. longicornis* 23 ± 5 % (Fig. 4.6c). The largest size class of 20-40 µm made up only 0.9 ± 0.1% for *A. clausi* and 0.7 ± 0.1% for *T. longicornis*; it was preyed upon with very high rates of 3 ± 1% and 8 ± 4% (Fig. 4.6d).

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

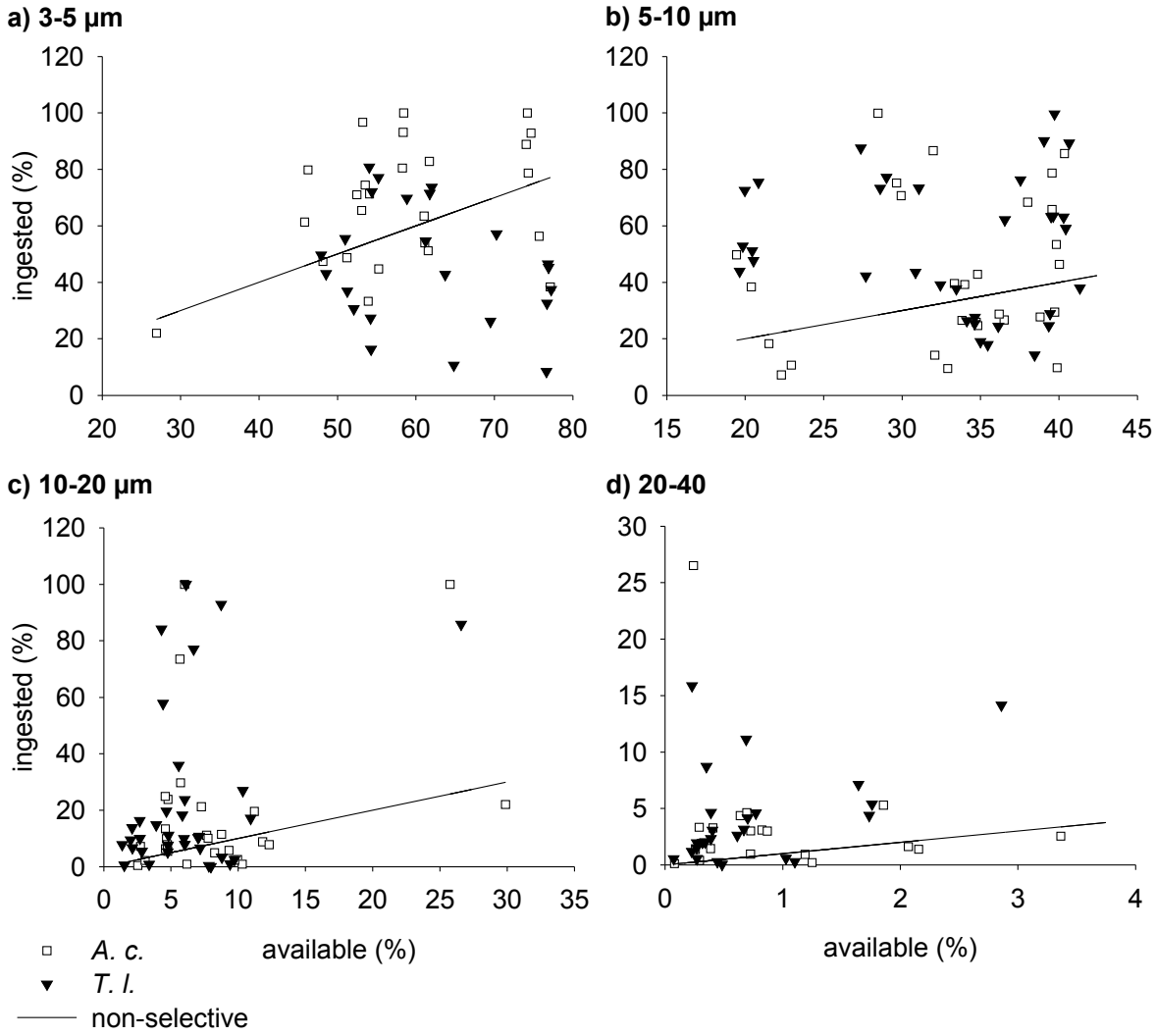


Fig. 4.6: Carbon specific grazing on a) 3-5 µm size class b), 5-10 µm size class c), 10-20 µm size class, d) 20-40 µm size class, ingested particles and available particle concentration, excluding zero ingestion data.

$N=75$). 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

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

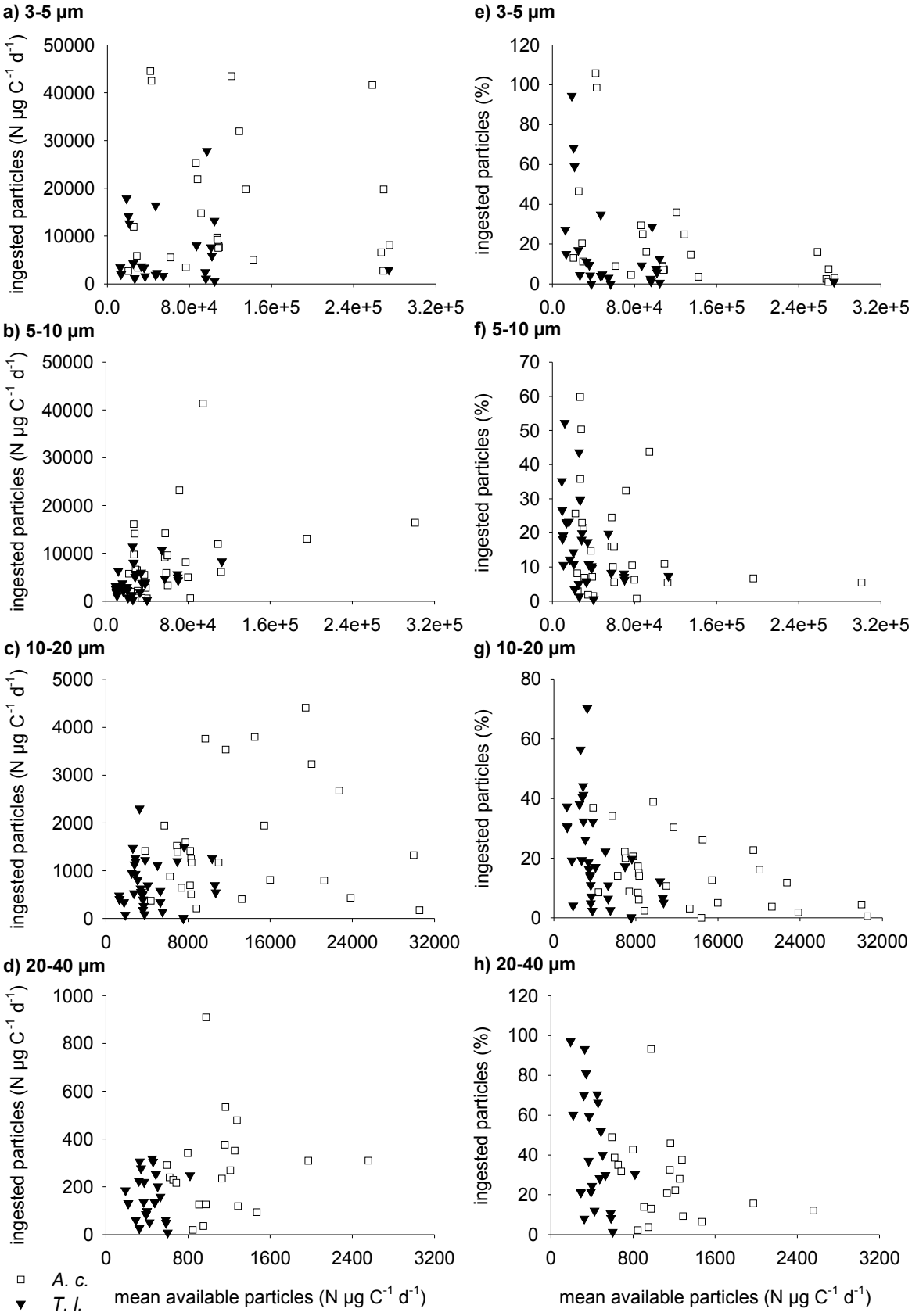


Fig. 4.7: Carbon specific particle ingestion (a-d) and percent of ingested on available particles (e-h) per µg copepod carbon; neighbouring graphs display results for the same size class (same x-axis).

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.

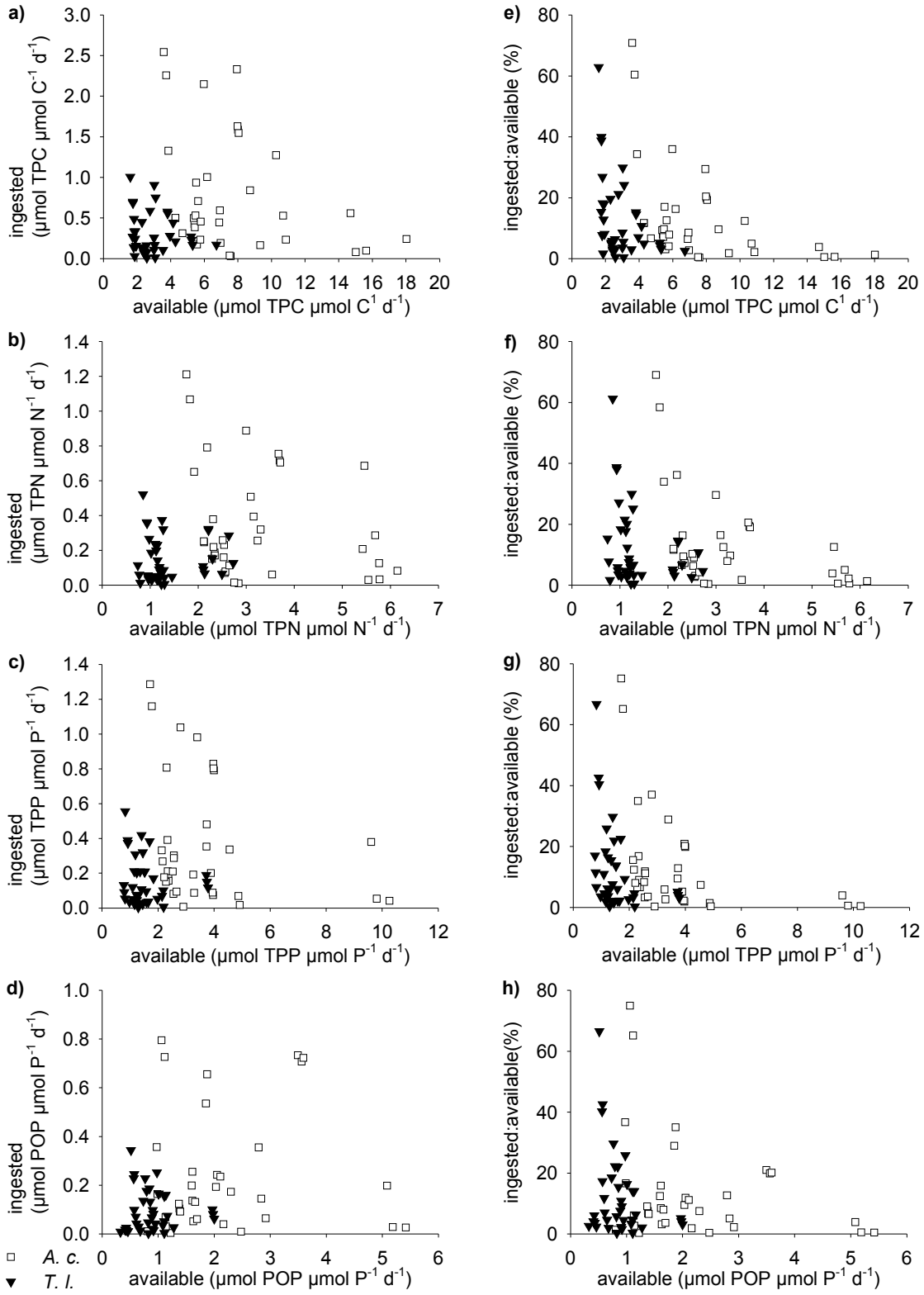


Fig. 4.8: Body nutrient specific grazing on seston nutrients: a-d) ingestion rates, e-h) % ingested of available nutrients per body nutrients.

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 (µmol µmol^{-1} day^{-1}) and reached maximum rates of 2.5 (µmol µmol^{-1} day^{-1}) (Fig. 4.8a). Nutrient uptake of N, P and organic P rarely exceeded 1:1 (µmol µmol^{-1} 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 (µmol µmol^{-1} day^{-1}) (Fig. 4.8a), specific N and P uptake was not higher than 0.6:1 (µmol µmol^{-1} day^{-1})(Fig. 4.8b,c), and the uptake of organic P per body P did not exceed 0.4:1 (µmol µmol^{-1} 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 ± SE).

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

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

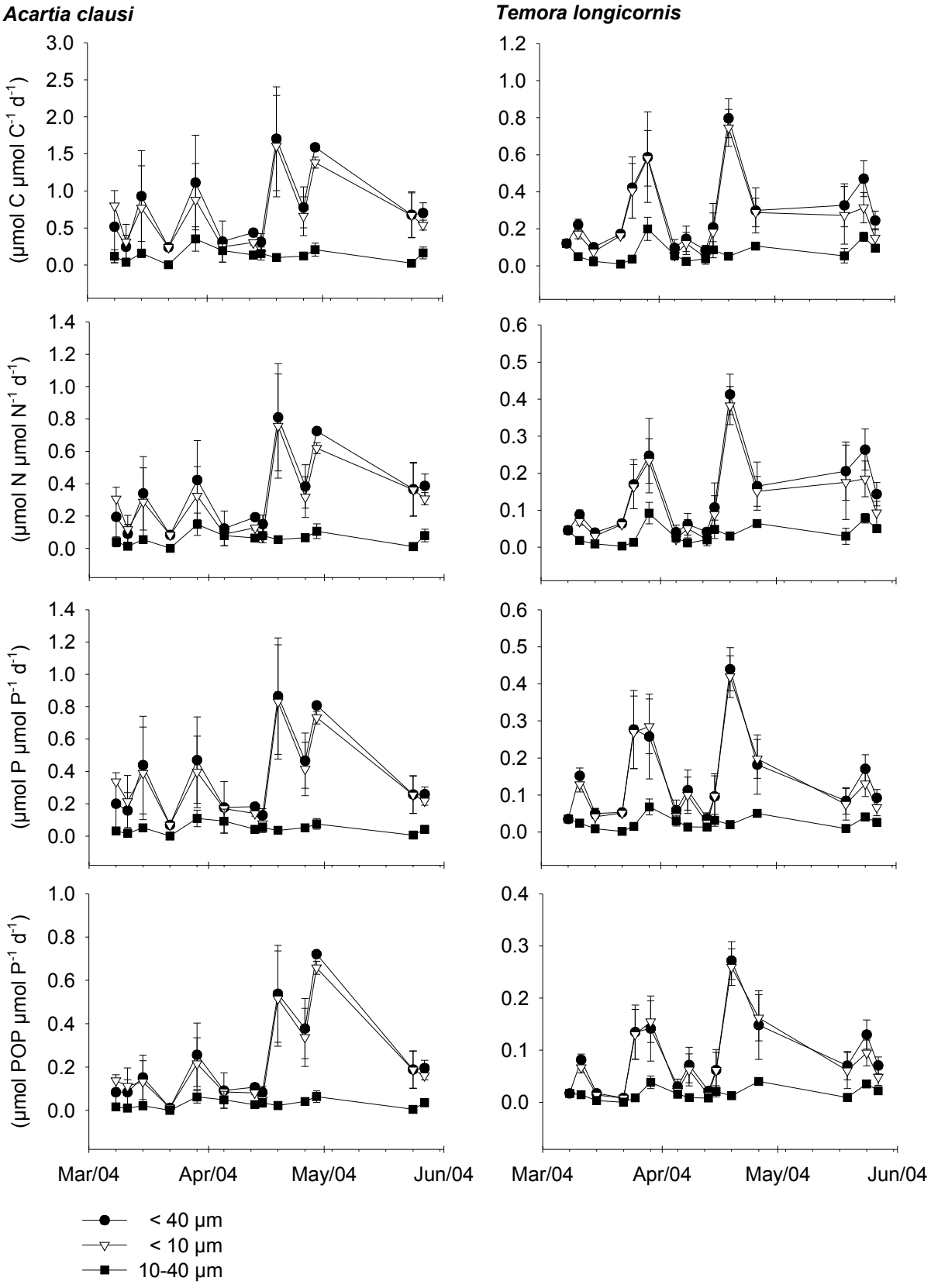


Fig. 4.9: Body specific nutrient uptake of seston nutrients (C, N, P, POP) in the size class < 10 µm and 10-40 µm and < 40 µm in A. clausi and T. longicornis during spring (no grazing measurements during the first half of May).

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

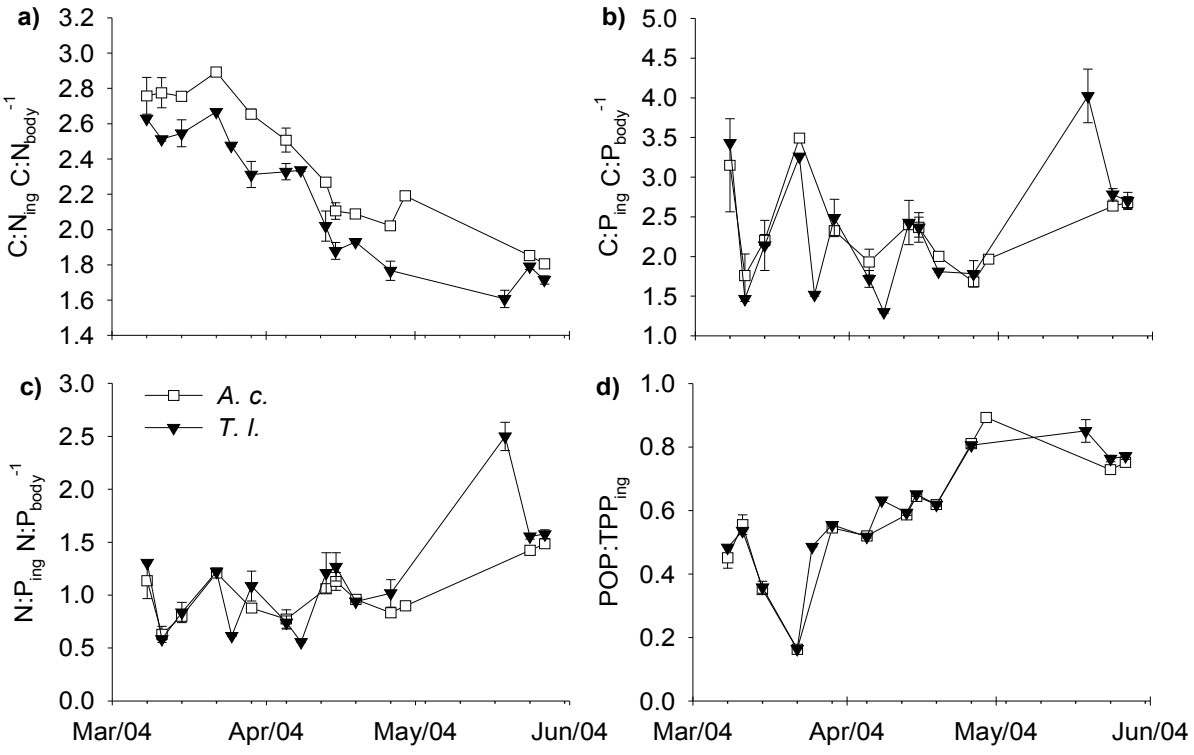


Fig. 4.10: Elemental ratios of ingested food versus body ratios over time: a) $C:N_{ing}$ $C:N_{body}^{-1}$, b) $C:P_{ing}$ $C:P_{body}^{-1}$, c) $N:P_{ing}$ $N:P_{body}^{-1}$, d) POP TPP_{ing}^{-1}.

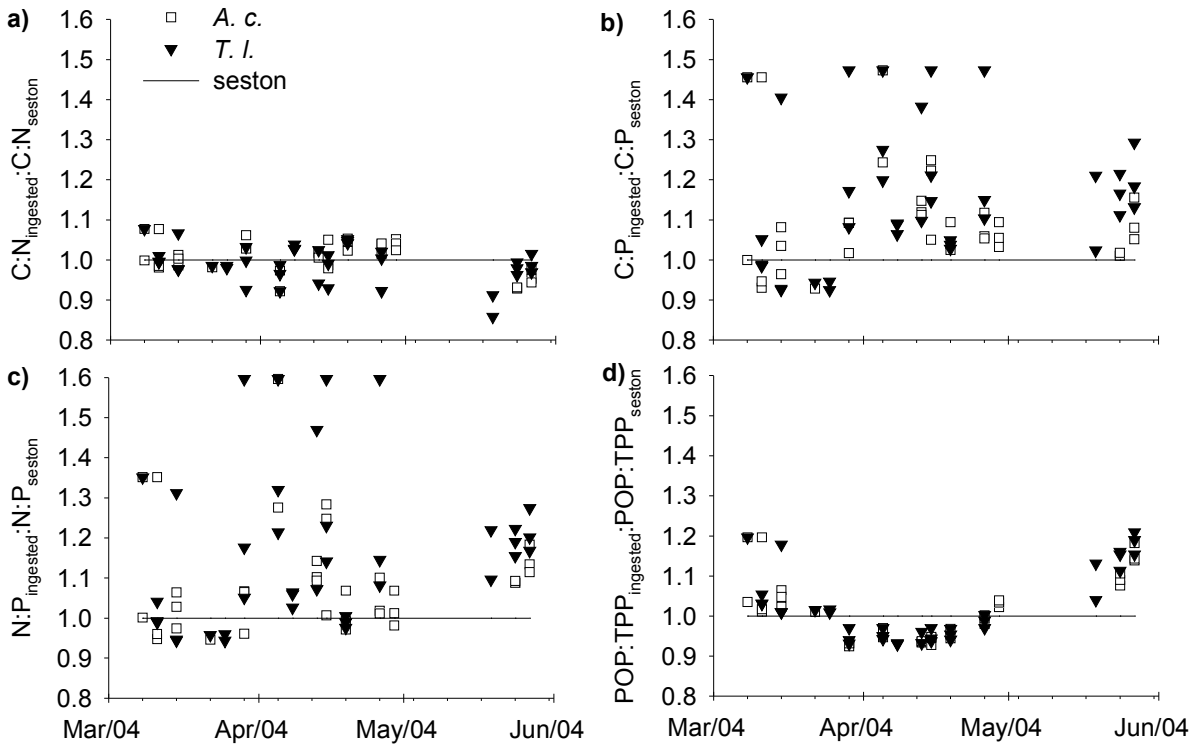


Fig. 4.11: Individual uptake (based on copepod C) of nutrient ratios in relation to seston ratios: a) $C:N_{ingestsed}$:$C:N_{seston}$, b) $C:P_{ingested}$:$C:P_{seston}$, c) $N:P_{ingested}$:$N:P_{seston}$, d) $POP:TPP_{ingested}$:$POP:TPP_{seston}$.

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 (Kiørboe 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

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 (µmol µmol^{-1} day^{-1}) and slightly above. In carbon terms, most values stayed around 1:1 (µmol

µmol^{-1} day^{-1}) but uptake rates could rise up to 2.5:1 (µmol µmol^{-1} 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).

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 $\delta^{13}C$ and $\delta^{15}N$ 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 $d^{15}N$ signature was significantly negatively correlated with the molar *T. longicornis* C:N ratio. *A. clausi* showed the same trend. Over the year the seston $\delta^{13}C$ 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 ($\delta^{15}N$ signature) during a spring bloom but they utilized different carbon sources ($\delta^{13}C$ 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 $\delta^{15}N$ and also in $\delta^{13}C$. 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 $\delta^{13}C$ 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).

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).

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}C:^{12}C and ^{15}N:^{14}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

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 ^{13}C and ^{15}N are widely used in ecology. The ratio between ^{13}C and ^{12}C gives an indication of the carbon source that was foraged. The enrichment of the stable nitrogen isotope ^{15}N 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:

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

$\delta^{15}N$ was calibrated versus air, $\delta^{13}C$ 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.

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 (nmol h^{-1} mg_{prot}^{-1}).

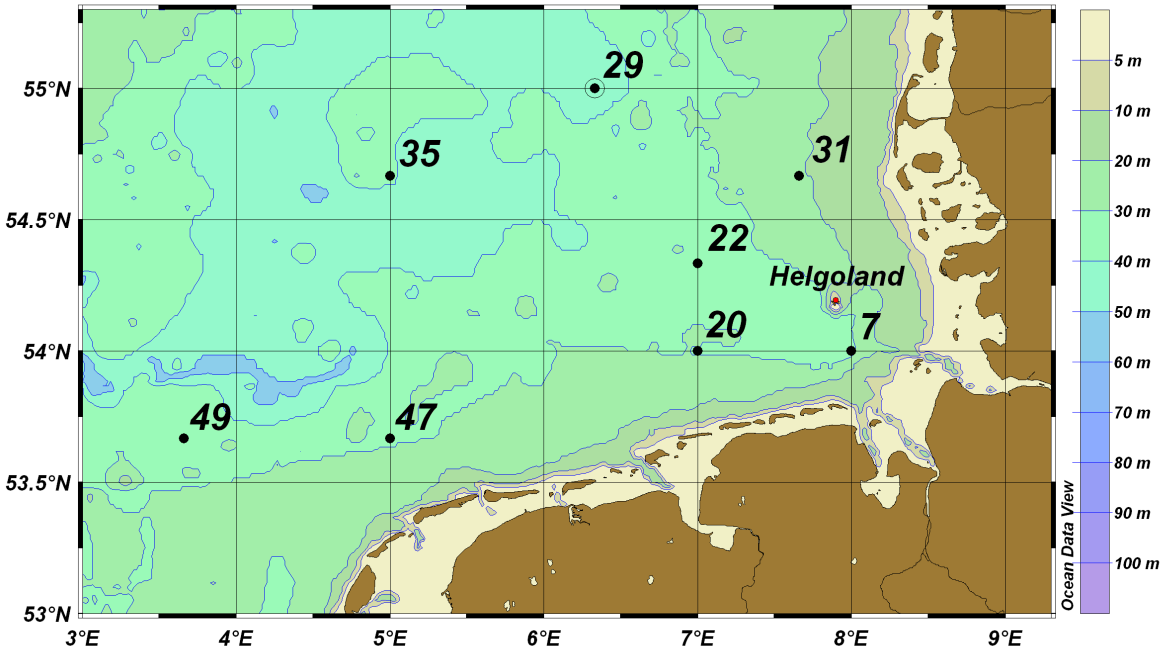


Fig. 5.1: Helgoland (red dot) and GLOBEC sampling stations (station numbers) in the North Sea (map created with Ocean Data View Version 3.1.0, Schlitzer 2006).

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) Wathman 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.

Table 5.1: Ranges of stable isotope values in seston and copepod samples (N=3-6, means ± 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 $\delta^{15}N$				
Total	4.9 ± 0.1	26/04/2004	11.9 ± 0.4	06/08/2004
Spring 04	4.9 ± 0.1	26/04/2004	10.4 ± 0.5	14/04/2004
Spring 05	7.5 ± 1.6	01/03/2005	10.0 ± 0.6	10/04/2005
***T. l.* $\delta^{15}N$**				
total	8.5 ± 0.7	19/02/2004	15.6 ± 0.4	22/03/2005
Spring 04	8.5 ± 0.7	19/02/2004	15.0 ± 0.1	29/03/2004
Spring 05	12.7 ± 0.7	08/03/2005	15.6 ± 0.4	22/03/2005
***A. c.* $\delta^{15}N$**				
Spring 04	7.7 ± 0.0	29/04/2004	13.8 ± 0.3	13/04/2004
Seston $\delta^{13}C$				
Total	-23.7 ± 0.3	05/04/2004	-12.8 ± 0.4	19/11/2004
Spring 04	-22.3 ± 0.1	13/05/2004	-16.7 ± 0.2	22/03/2004
Spring 05	-23.7 ± 0.3	05/04/2005	-16.8 ± 0.1	15/02/2005
***T. l.* $\delta^{13}C$**				
Total	-21.1 ± 0.3	01/07/2004	16.7 ± 0.10	04/11/2004
Spring 04	-20.8 ± 0.0	19/04/2004	-17.8 ± 0.03	27/05/2004
Spring 05	-20.1 ± 0.2	12/04/2005	-18.7 ± 0.20	22/02/2004
***A. c.* $\delta^{13}C$**				
Spring 04	-23.5 ± 0.0	15/04/2004	-17.4 ± 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 $\delta^{15}N$ (Fig. 5.2a, Table 5.1) ranged from 4.9 ± 0.07 ‰ to 11.9 ± 0.4 ‰ in seston (means ± SD, also the following values), from 8.5 ± 0.7 to 15.6 ± 0.4 in *T. longicornis*, and from 7.7 to 13.8 in *A. clausi* which was only measured during spring

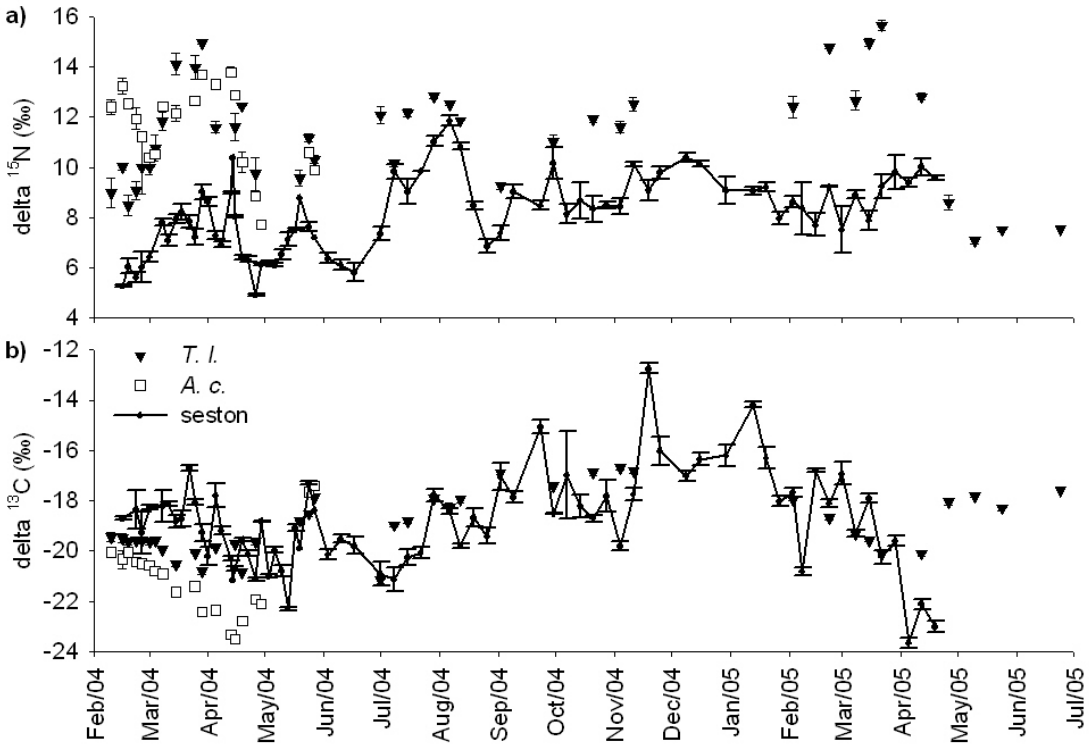


Fig. 5.2: Seston and copepod stable isotopes during a year including 2 spring times (N=3-6, means ± SE), Acartia clausi was only sampled during spring 2004.

2004. Seston $\delta^{15}N$ 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 $\delta^{15}N$ signature stayed relatively stable at a medium level of 8.9 ± 0.8 (N=18; mean ± SD). Values started to rise again in March 2005. The copepods more or less follow the seston pattern but on a higher level.

Seston $\delta^{13}C$ 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 $\delta^{13}C$ stayed relatively high, with highest values from mid-November till mid-January. The copepod $\delta^{13}C$ ratios were at similar levels as seston. The $\delta^{13}C$ 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

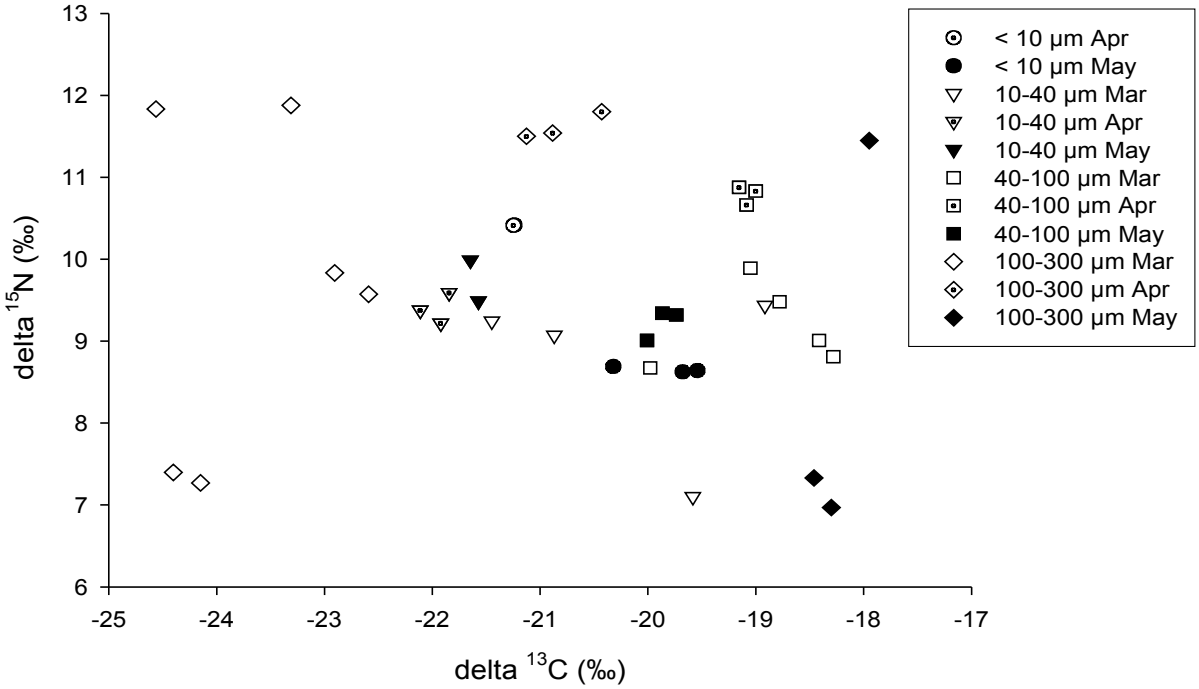


Fig. 5.3: Stable isotopes in size fractions during 3 spring phases of phytoplankton development.

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 $\delta^{15}N$ were reached by the largest size fraction (diamonds), but also four out of five of the lowest $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{13}C$ ratios combined with highest $\delta^{15}N$. May samples (filled diamonds) were among those with highest $\delta^{13}C$ 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 $\delta^{15}N$ about 9 ‰ and a $\delta^{13}C$ 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 $\delta^{13}C$ ratios compared to April

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

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

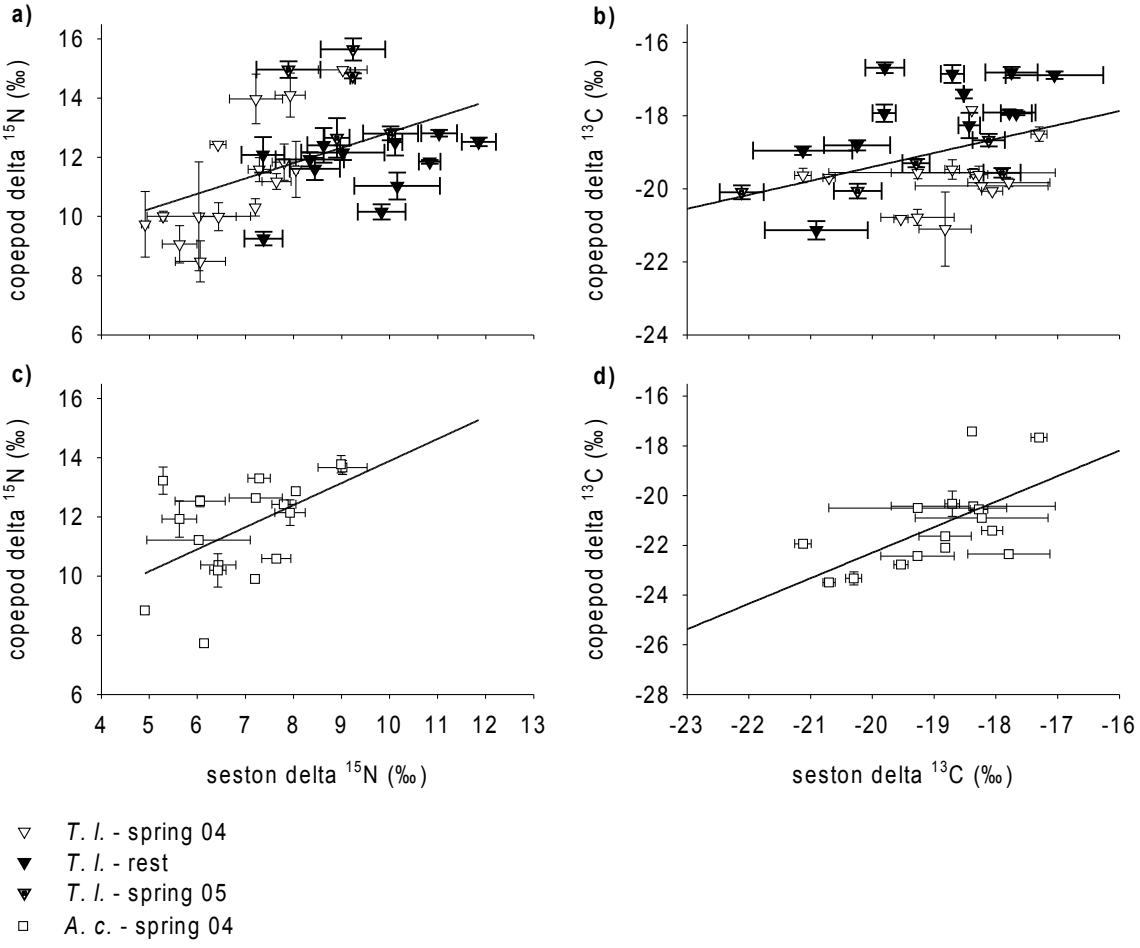


Fig. 5.4: Copepod stable isotopes versus seston stable isotopes with regression lines (N=3-6, means ± SD); occasionally only one parallel.

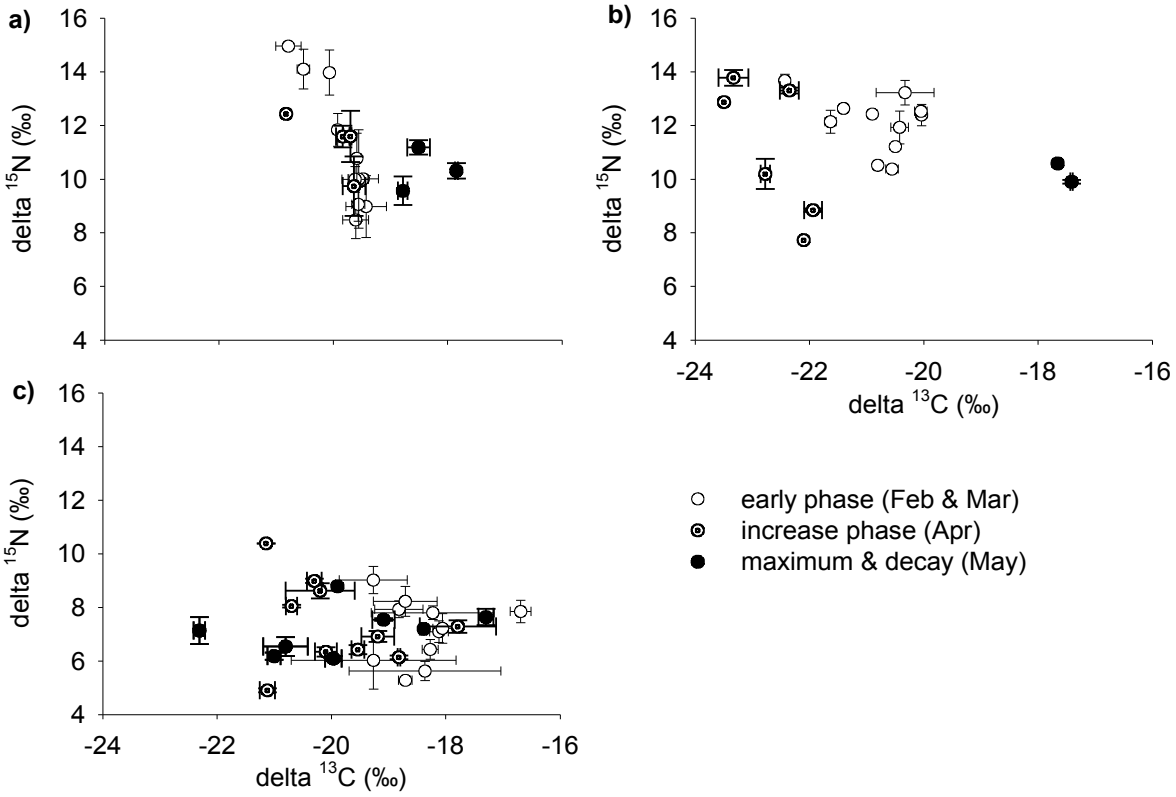


Fig. 5.5: Stable isotopes of a) Temora longicornis*, b)* Acartia clausi *and c) seston during spring 2004, phytoplankton bloom phases signified by different symbols.*

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

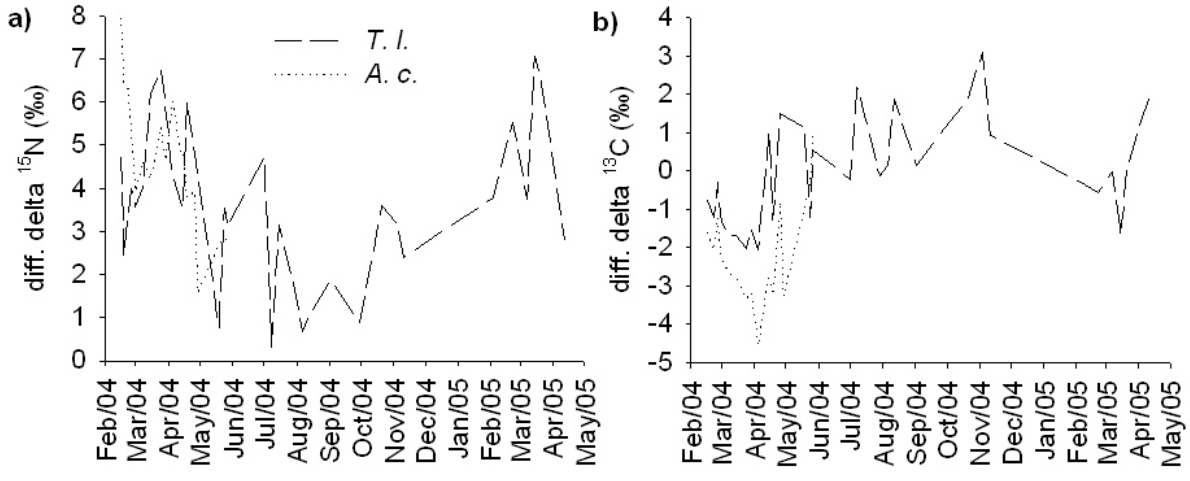


Fig. 5.6 Difference between Temora longicornis/Acartia clausi *and seston a) $\delta^{15}N$ and b) $\delta^{13}C$; (N=3, means, no copepod results in December and January).*

^{13}C depleted carbon than at other times. The two copepods had a similar $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{13}C$ as *T. longicornis* during early spring 2004. The negative distance between *T. longicornis* and seston $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ in *T. longicornis* ($r^2=0.17$, $p=0.02$) and *A. clausi*

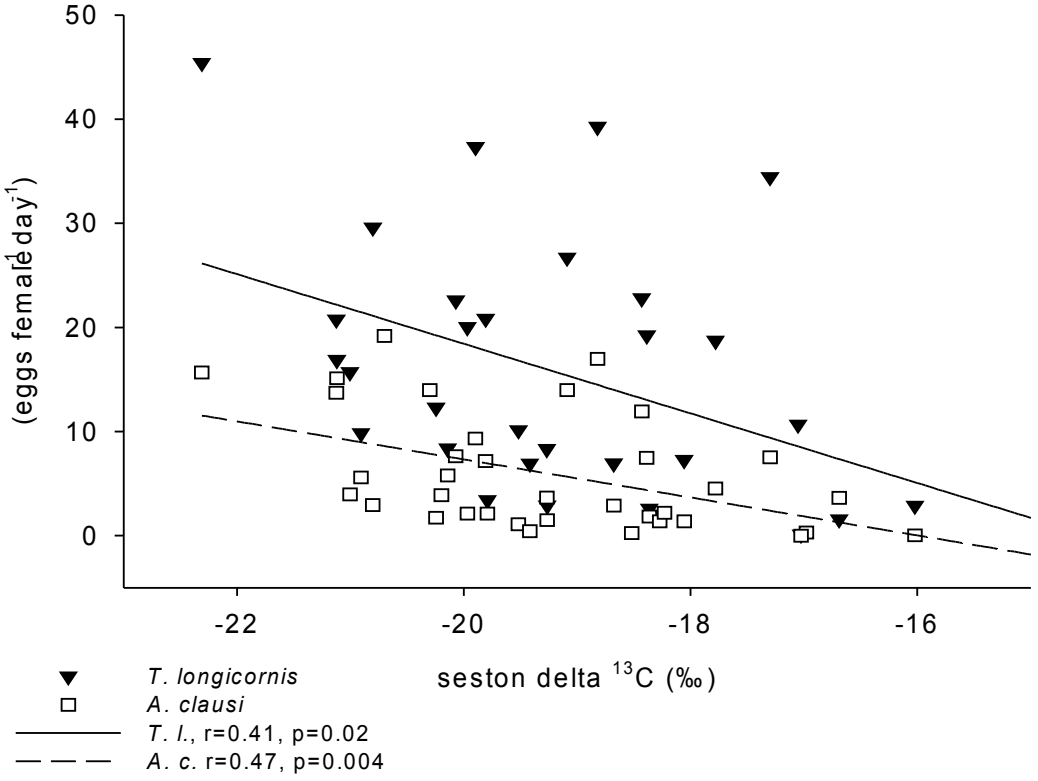


Fig. 5.7: Egg production of copepods is negatively correlated to seston $\delta^{13}C$ over the year.

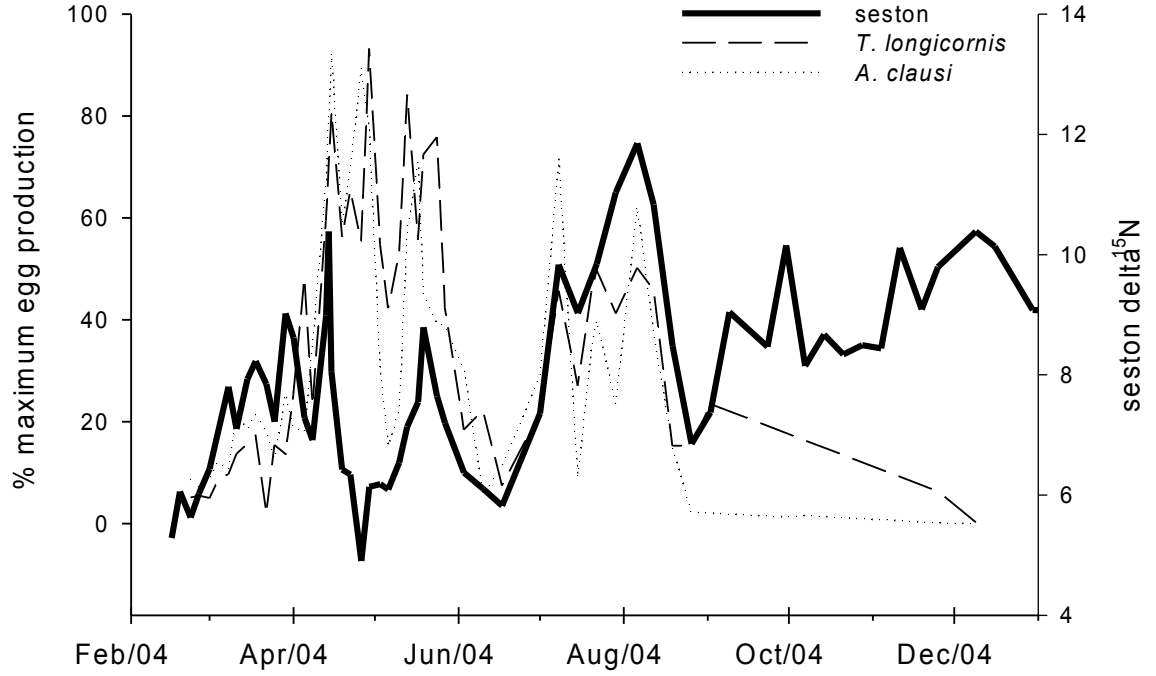


Fig. 5.8: Egg production pattern of A. clausi *and* T. longicornis *during 2004 as compared with seston δ[15]N.*

($r^2=0.22$, $p=0.004$) (Fig. 5.7). The seston C:N correlated with the seston $\delta^{13}C$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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.

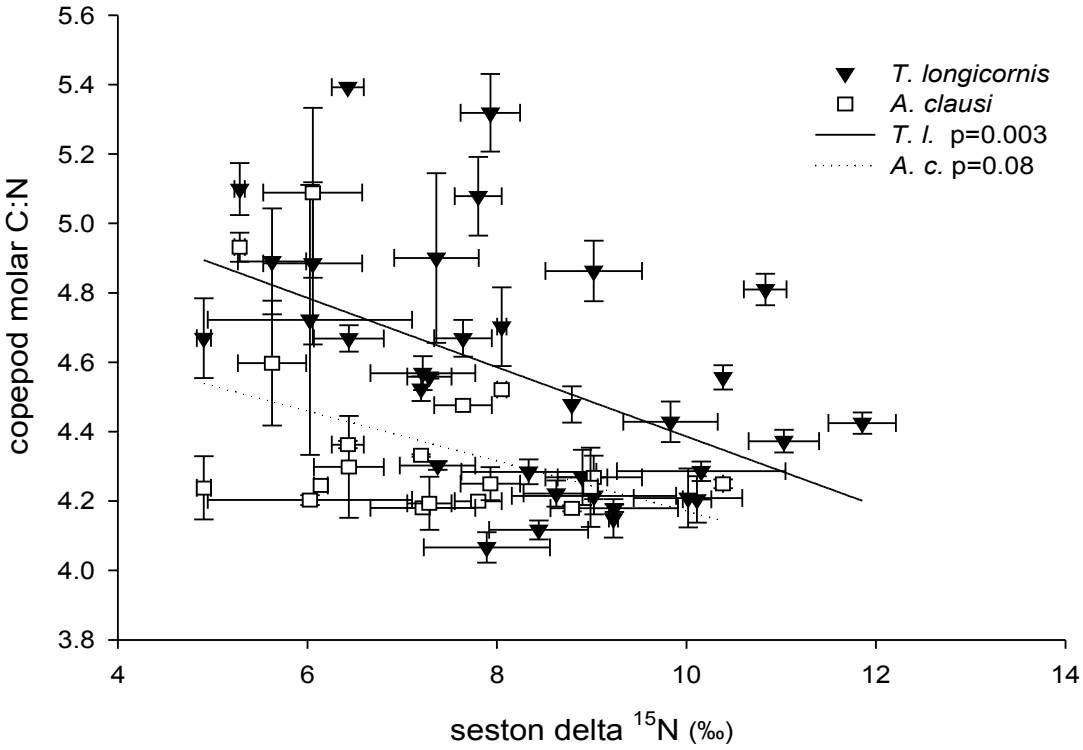


*Fig. 5.9: Correlation of seston $\delta^{15}N$ with copepod C:N (N=3, means ± SD), linear regressions for both species: T. l.-C:N = 5.4 – 0.1 * seston $\delta^{15}N$ ($r=0.49$; $r^2=0.24$, $F=10.2$, $p=0.003$); A. c.-C:N = 4.9 -0.07 * seston $\delta^{15}N$ ($r=0.41$, $r^2=0.17$, $F=3.4$, $p=0.08$).*

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

patterns. However, seasonal activity patterns of arginine (Fig. 5.10a) and alanine aminiopeptidase (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 ± SE).*

	Temora longicornis			***Acartia clausi***		
Date	**Prot (µg)**	**SE**	**Prot dm^{-1} (%)**	**Prot (µg)**	**SE**	**Prot dm^{-1} (%)**
26/02/04	6.7	± 0.6	25.3	2.5	± 0.2	31.2
08/03/04	8.0	± 0.6	26.2	3.5	± 0.2	48.5
15/04/04	11.9	± 1.1	24.4	7.1	± 0.5	59.9
24/05/04	15.6	± 0.9	32.3	5.3	± 0.3	64.3
08/07/04	11.9	± 0.7	32.3			
20/07/04	7.9	± 0.2	23.7			
06/08/04	10.6	± 0.7	26.9			
02/09/04	7.2	± 0.5	29.3			
30/09/04	6.3	± 0.4	24.6			
21/10/04	13.1	± 0.8	29.9			
11/11/04	9.7	± 1.0	30.6			
03/02/05	8.2	± 1.1	41.0			
08/03/05	7.9	± 1.1	30.6			
12/04/05	12.7	± 0.6	26.9			
10/05/05	10.7	± 0.6	25.2			
24/06/05	9.9	± 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; Tl – Temora longicornis*, dm – dry mass, ala – alanine aminopeptidase, arg – arginine aminopeptidase, est – esterase/lipase, chito – chitobiase, b-gluc – beta-glucosidase.*

	r(X,Y)	**r²**	**t**	**p**
seston δ^{15}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**
Tl 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
Tl δ^{13}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

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 $\delta^{15}N$. 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

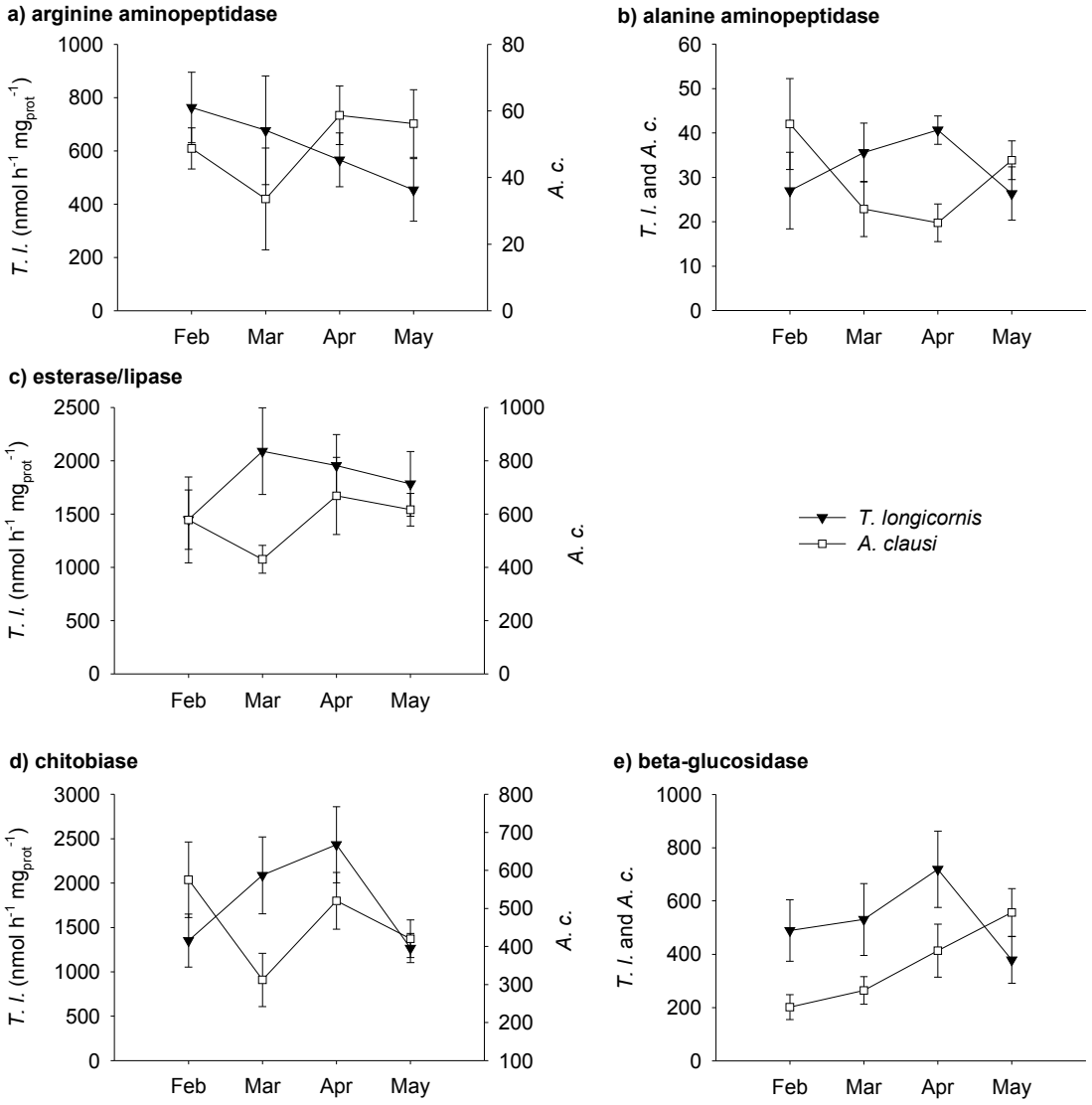


Fig. 5.10: Digestive enzymes during spring in Acartia clausi *and* Temora longicornis *(N=6, means ± SE).*

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

The copepod $\delta^{13}C$ ratio was negatively correlated with chitobiase activity and positively with arginine aminopeptidase and esterase/lipase activity. Hence, a low copepod $\delta^{13}C$ denotes high chitobiase 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).

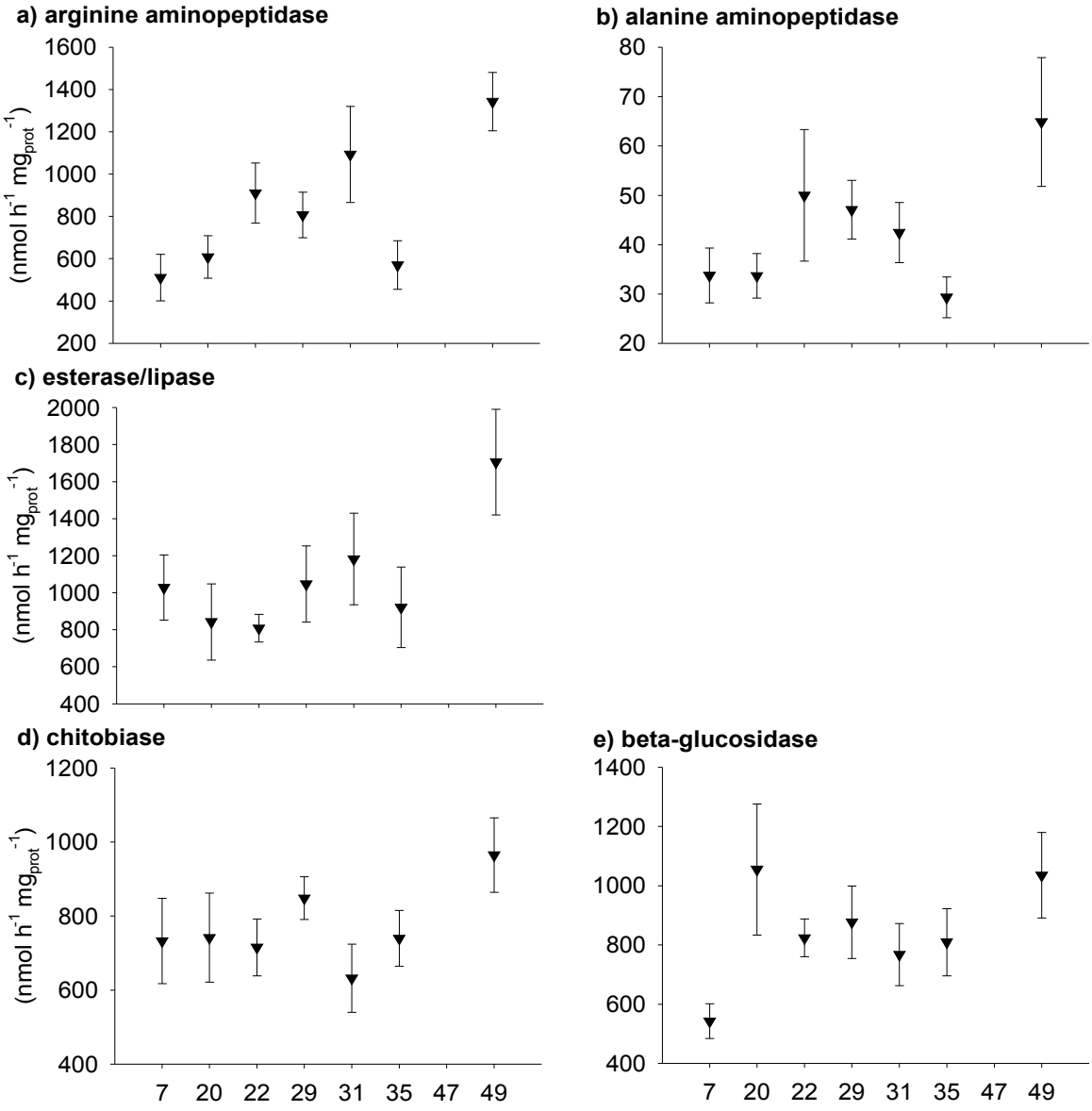


Fig. 5.11 Temora longicornis*: specific enzyme activities in the German Bight, June 2004 (N=6; means ± SE).*

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

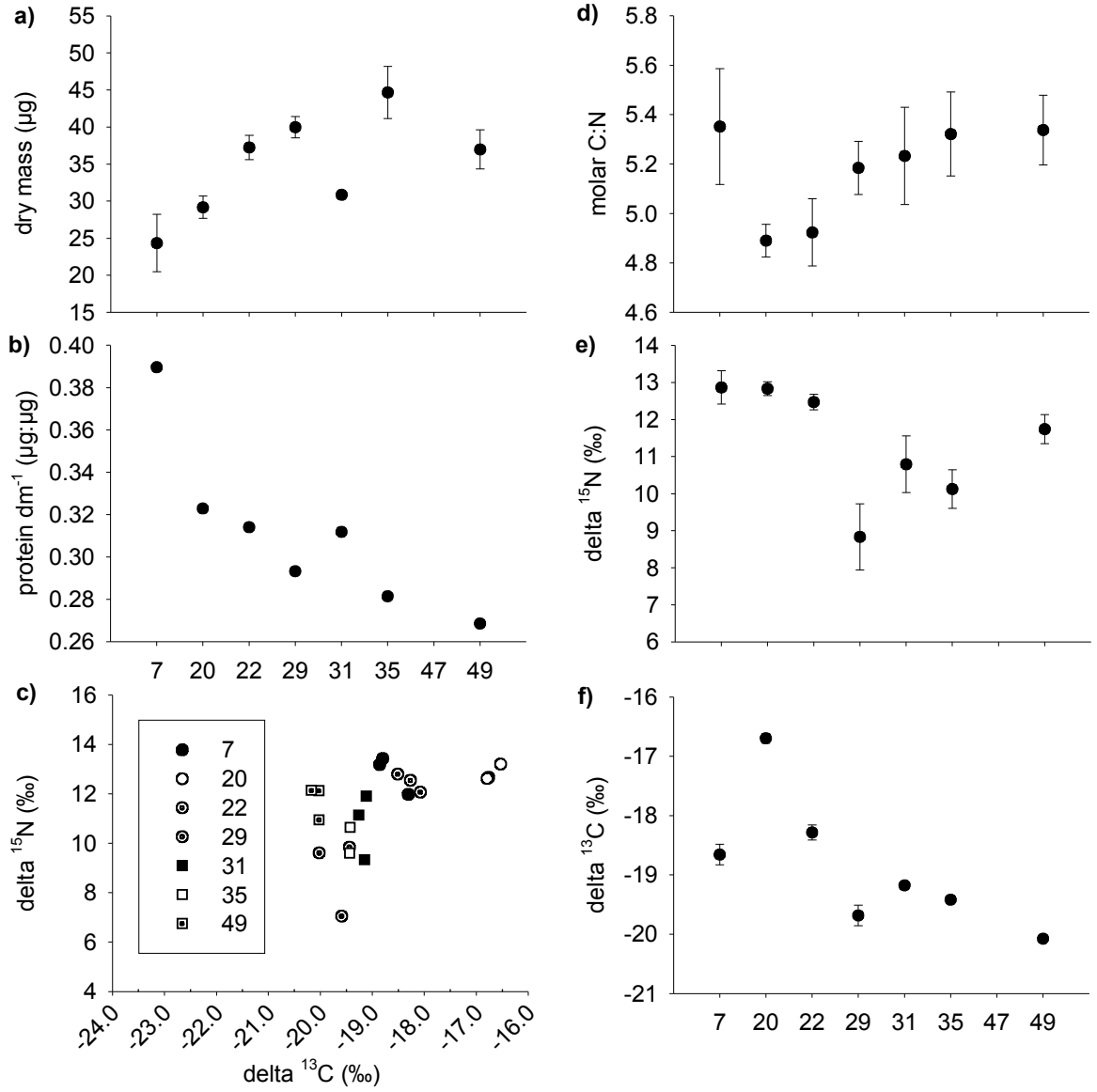


Fig. 5.12 Biochemical and stable isotope data of T. longicornis in the southern North Sea: a) dry mass, b) protein proportion of dry mass, c) copeod $\delta^{15}N$ versus $\delta^{13}C$:, d) molar C:N, e) $\delta^{15}N$, f) $\delta^{13}C$ (means ± SE).

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 $\delta^{13}C$ ($p<0.05$).

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 $\delta^{15}N$ 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 $\delta^{15}N$ 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

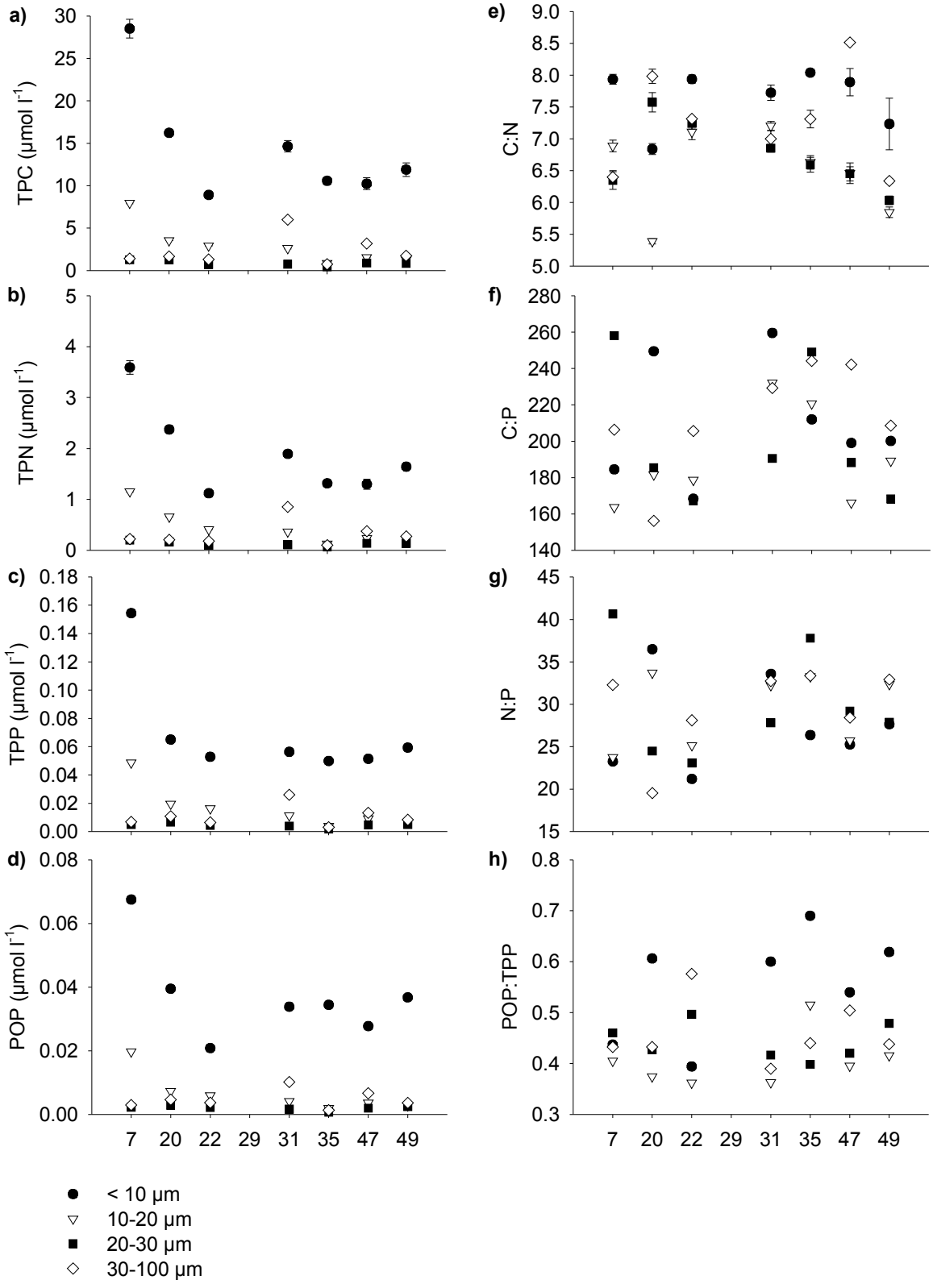


Fig. 5.13 Seston quantity and quality parameters at GLOBEC stations in the North Sea, June, 2004 (N=3; means ± SE or average ratios).

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 $\delta^{15}N$ range of 6 ‰ (Adams and Sterner 2000). Starving *Daphnia* showed

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 $\delta^{13}C$. 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 $\delta^{13}C$ dropped more dramatically than in 2004. But *T. longicornis* fed on a similar food source as in spring 2004 in respect to $\delta^{13}C$. In the summer months, in autumn, and in winter, copepods exhibited a $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 ($\delta^{13}C$ ranging from -15 to -19) compared to most particulate matter that is rather ^{13}C depleted ($\delta^{13}C$ ranging from -21 to -25) (Fry and Wainright 1991). *T. longicornis* $\delta^{13}C$ was only below -20 ‰, when being on very high $\delta^{15}N$ > 12 ‰ at the same time. However, marine microalgal $\delta^{13}C$ 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 $\delta^{13}C$ (Bouillon et al. 2000) and an inferior food source for copepods (Mayzaud et al. 1998). High seston C:N together with low seston $\delta^{13}C$ 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 $\delta^{15}N$, riverine phytoplankton is depleted of ^{13}C compared to

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 chitobiase activity was correlated to a high body C proportion and a rather depleted body $\delta^{13}C$. The seston $\delta^{13}C$ was inversely correlated to egg production in *T. longicornis* and other copepods. Further, as stated above, nitrogen limited growth decreases the phytoplankton $\delta^{15}N$ and leads to a high body C:N of cladocerans (Adams and Sterner 2000). Accordingly, we found a negative correlation between seston $\delta^{15}N$ and copepod C:N. The negative correlation of arginine aminopeptidase and esterase/lipase with copepod C:N and the positive correlation to seston $\delta^{15}N$ 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 $\delta^{15}N$ 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

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 $\delta^{13}C$ below -20 ‰ and if, then in combination with a high $\delta^{15}N$. 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 $\delta^{15}N$ 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 $\delta^{15}N$ and $\delta^{13}C$ were found implying a more herbivorous food source than at the other stations.

Conclusions

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

copepod species. At certain times such as spring, the seston $\delta^{15}N$ pattern may be used to predict copepod egg production with a time shift of about 10-15 days. Further studies of seston-zooplankton-$\delta^{15}N$ 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

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 timeas 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 CO_2. 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)

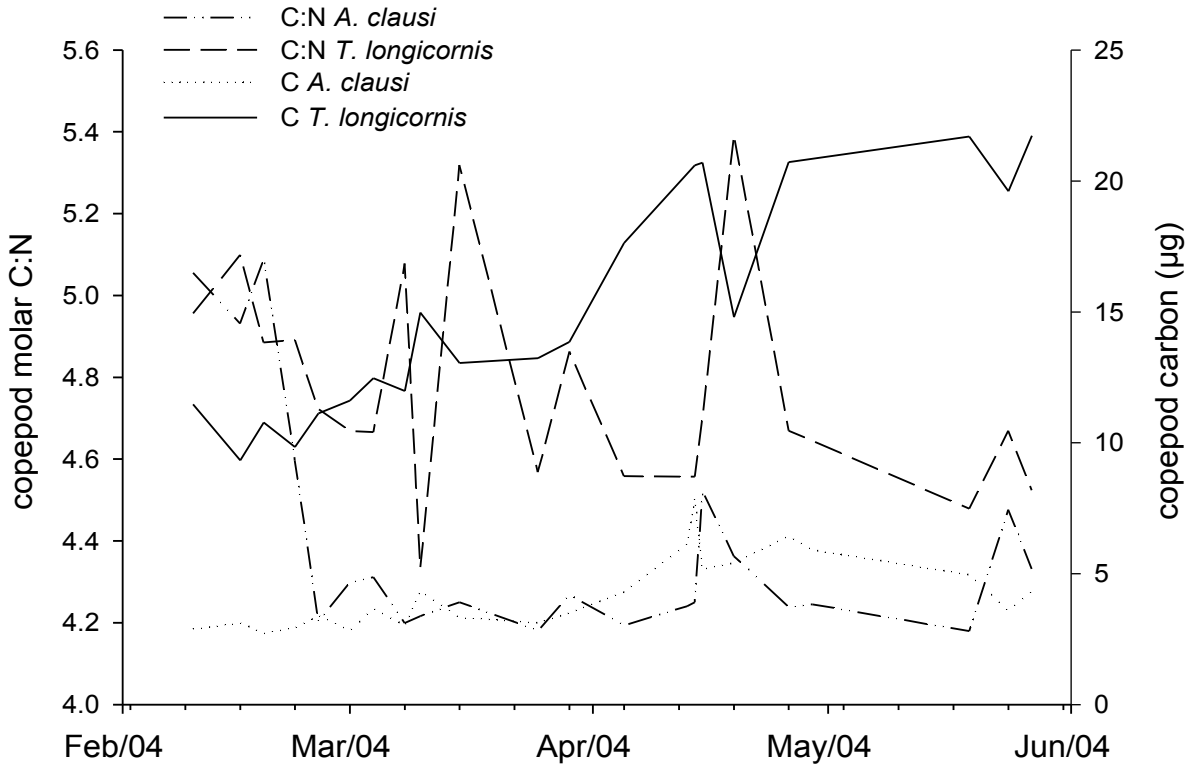


Fig. 6.1: Body C:N variability of A. clausi *and* T. longicornis *during spring 2004 and absolute carbon contents that were taken to determine the average stoichiometric ratio in both species during spring.*

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 $\delta^{13}C$ 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 $\delta^{13}C$. 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

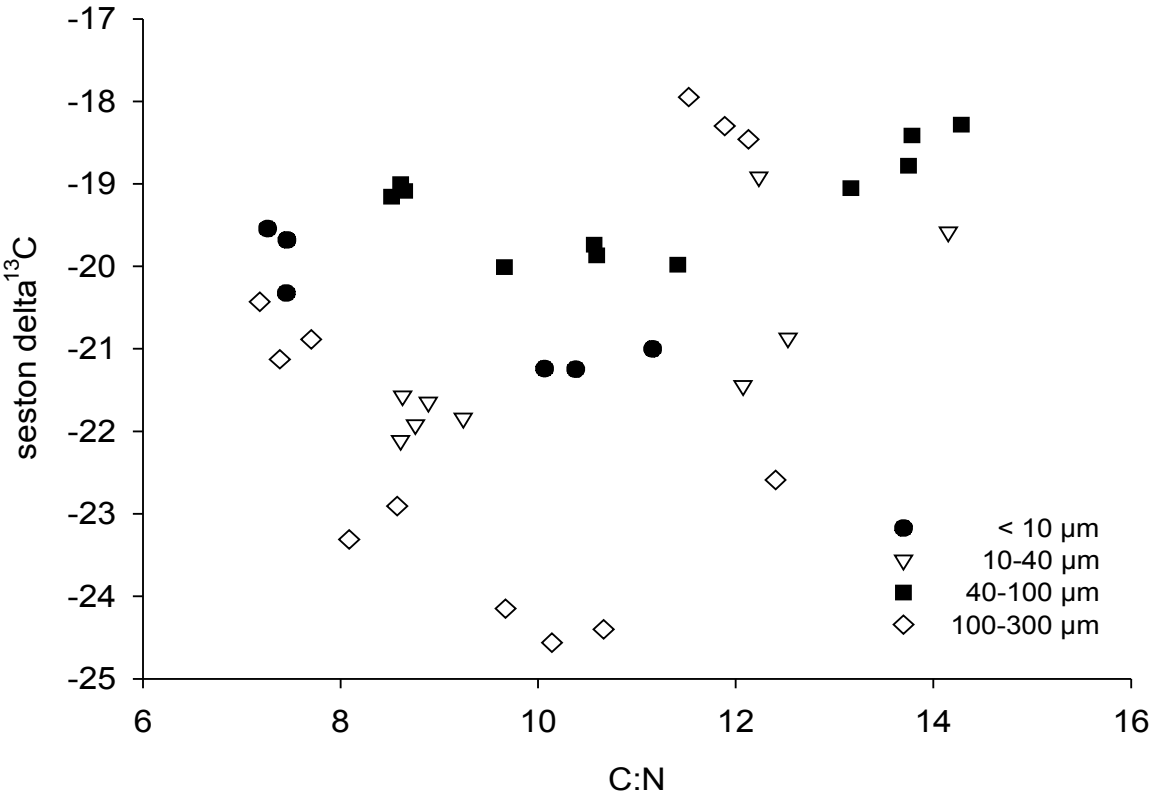


Fig. 6.2: Seston $\delta^{13}C$ versus seston C:N in size classes.

C compared to other nutrients (Katechakis et al. 2002; Darchambeau 2005). In the present study a positive correlation between *T. longicornis* C:N and chitobiase activity was found and a negative correlation to arginine aminopeptidase activity. Chitobiase is a carbohydrase that digests chitobiose, 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 chitobiase 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

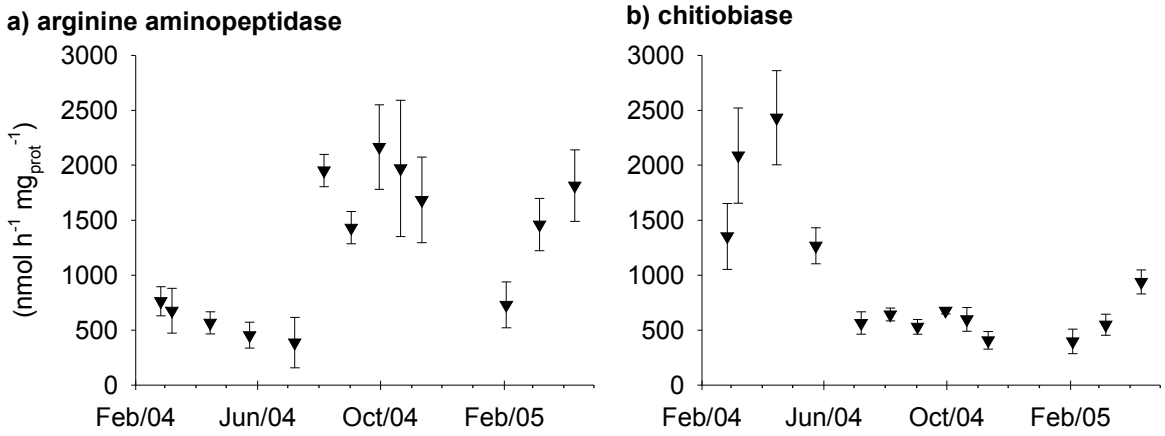


Fig. 6.3: Specific a) arginine aminopeptidase and b) chitobiase activity of T. longicornis during the year.

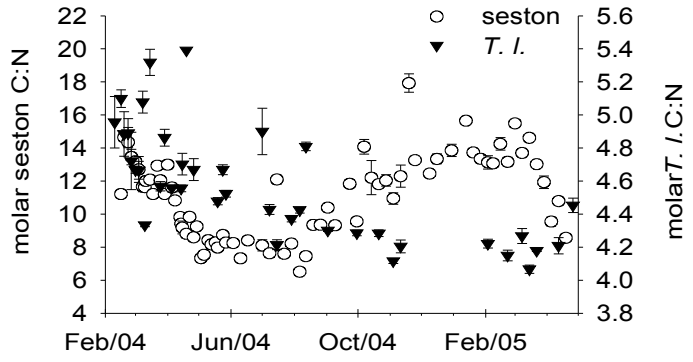


Fig. 6.4: Seston and T. longicornis molar C:N during the year.

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 chitobiase 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 $\delta^{15}N$ 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

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

change upon short-term starvation in two *Acartia* species (chapter 3). Chitobiase 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 chitobiase to arginine aminopeptidase (or also esterase/lipase and beta-glcuosidase) ratio may, therefore, indicate a food limiting situation. High ratios of chitobiase 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 $\delta^{13}C$ 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 µm 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 chitobiase may be good indicators of the elemental assimilation of N and C, respectively, although it remains to be seen how this works mechanistically. Despite

the selective feeding of the copepods, bulk seston C:N or also $\delta^{13}C$ 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 theses 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. Chitobiase 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

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 (µmol µmol^{-1} day^{-1}) and then were only slightly higher. However, C uptake reached values of up to 2.5:1 (µmol µmol^{-1} 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 $\delta^{15}N$ signal, but they utilised different carbon sources within the seston as displayed by $\delta^{13}C$. Over the year seston $\delta^{13}C$ 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 $\delta^{13}C$ values in *T. longicornis* coincided with highest $\delta^{15}N$ values. Chitobiase activity of *T. longicornis* was positively correlated to body C:N and negatively to body $\delta^{13}C$, while arginine aminopeptidase, esterase/lipase and beta-glucosidase were negatively correlated to body C:N and positively to body $\delta^{13}C$. Therefore, the two enzyme types denote assimilation of C and N, respectively. A high relative body N content indicates good fitness. A low body $\delta^{13}C$ 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 inter-spezifische 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

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 (µmol µmol^{-1} Tag^{-1}). Jedoch ereichte die C-Aufnahmerate Werte von bis zu 2.5:1 (µmol µmol^{-1} 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 (Kapitlel 4).

Aus den δ^{15}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 δ^{13}C-Signaturen. Über das Jahr war Seston δ^{13}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 δ^{13}C-Werte in *T. longicornis* traten zusammen mit den höchsten δ^{15}N-Werten auf. Die Chitobiaseaktivtiät von *T. longicornis* war positiv korreliert mit dem Körper C:N-Verhältnis und negativ mit dem Körper δ^{13}C, während Arginin-Aminopeptidase, Esterase/Lipase und Beta-Glucosidase positiv mit dem Körper C:N-Verhältnis und negativ mit dem δ^{13}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 δ^{13}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 Analysentypen. 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.

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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 Zusammen-arbeit 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 Elektrobefischung 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-Insituts 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 Wilthire provided Date on phytoplankton, temperature and salinity.

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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