PHYSIOLOGICAL ADAPTATIONS OF COPEPODS FROM THE NORTH SEA AND THE NORTH ATLANTIC TO CHANGING NUTRITIONAL CONDITIONS

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PHYSIOLOGICAL ADAPTATIONS OF COPEPODS FROM
THE NORTH SEA AND THE NORTH ATLANTIC
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UNTersuchungen zur ernährungspHysioLOGIE
Dominanter copepodenarten
der nordsee und des norDatlantiks

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Physiological responses of *Temora longicornis* females (Crustacea, Copepoda) to changing nutritional conditions in the North Sea

Publication II

Short term variation of nutritive and metabolic parameters in *Temora longicornis* females (Crustacea, Copepoda) as a response to diet shift and starvation

Publication III

Influence of physiological conditions and feeding history on digestion, metabolism and fatty acid composition of *Temora longicornis* females (Crustacea, Copepoda) under different nutritional conditions
ABSTRACT

Marine calanoid copepods constitute a major component of the pelagic food web. They are an important link between phyto-/microzooplankton and higher trophic levels. In the marine realm, copepods have to adapt to changing environmental conditions in order to efficiently use dietary components for growth and reproduction. There are numerous studies on feeding behaviour and functional responses of calanoid copepods, as well as physiological responses to changing nutritional conditions. However, no combined information is available on functional and physiological responses in copepods to changing nutritional conditions. Detailed knowledge on their feeding behaviour, their physiological responses to nutritional conditions, i.e. digestive and metabolic activities, and their functional responses, i.e. reproduction, is of major importance in order to better understand how diet affects growth and reproduction of copepods. The project “Trophic interactions in pelagic ecosystems: the role of zooplankton” at the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, in cooperation with the Marine Zoology at the University Bremen, aims therefore at comprehensively investigating feeding behaviour, physiological and functional responses of copepods to changing environmental conditions in order to obtain a more precise picture of processes influencing population dynamics. The present thesis, embedded within this project, aims at elucidating physiological adaptations of calanoid copepod species to changing nutritional conditions and how these are related to different life cycle strategies, metabolic requirements and biochemical properties, i.e. lipid and protein contents. For this purpose, species with different life strategies from the southern North Sea, i.e. Temora longicornis, Acartia clausi and Centropages typicus, and the lower St. Lawrence estuary (eastern Canada), i.e. Calanus finmarchicus and Metridia longa, were investigated during several phytoplankton blooms in 2005 and 2006. A comprehensive data set on digestive, metabolic and functional responses to changing nutritional conditions, traced via fatty acids trophic biomarkers, is presented and the data are discussed with respect to different life strategies.

The multi-voltine species with short life spans Temora longicornis, Acartia clausi and Centropages typicus were exposed to variable nutritional conditions during the phytoplankton blooms in spring 2005 (T. longicornis, A. clausi) and autumn 2006 (C. typicus). They were characterised by low lipid contents (2-9% of dry mass (DM)), high specific metabolic activities and high specific digestive potentials. Primarily proteins were accumulated during favourable feeding conditions, whereas lipid build-up followed secondarily. T. longicornis is well adapted to changing nutritional conditions by digesting different dietary components efficiently, thus enabling successful reproduction. Early in the season, when phytoplankton abundance was low, T. longicornis females fed omnivorously; the diet consisted of lipid-poor and potentially carbohydrate-rich diatoms, as well as of heterotrophic prey items like copepod eggs and nauplii. Later in the season, the diet changed towards mainly autotrophic, lipid-rich particles. According to these changes in diet, the digestive response of T. longicornis changed. At the
beginning of the time series the activities of the digestive enzymes proteinase and amylase were high, indicating feeding on protein- and carbohydrate-rich particles. When the diet changed, the digestive activity decreased, particularly amylase activity, suggesting a shift in the enzymatic pattern. At the same time lipid content increased in females indicating accumulation of dietary fatty acids, mainly diatom- and dinoflagellate-specific fatty acids such as 16:1(n-7) and 18:4(n-3). This high plasticity of digestive enzymes was confirmed in several experiments. Feeding and starvation experiments revealed that *T. longicornis* adapts its digestive activity within 24 h to changing nutritional conditions by strong reduction of digestive activities under starvation, increasing or decreasing activities under surplus food conditions, depending on their feeding history, and by diet-induced secretion of specific lipases when feeding on different diets. This high adaptive potential resulted in strong increases of specific dietary fatty acids within three days of incubation. In addition, due to the high adaptive potential egg production rates of *T. longicornis* females increased strongly within 24 h under surplus food conditions indicating that food was transformed successfully into egg material.

*Calanus finmarchicus* and *Metridia longa* are well adapted to the seasonal phytoplankton cycle in higher latitudes and generation times range between several months and more than one year. The species encountered changing nutritional conditions during the spring phytoplankton bloom in the St. Lawrence estuary in 2006. Both species were characterised by higher lipid contents (7-30% DM), lower specific metabolic activities and specific digestive potentials than the copepods from the North Sea. Proteins play a minor role as energy reserves in these species. Digestive activities were already elevated prior to the main phytoplankton bloom event, dominated by diatoms and dinoflagellates, and did not respond to changing nutritional conditions during the field campaign in both species, in spite different feeding behaviours. *C. finmarchicus* mainly fed on diatoms and dinoflagellates, whereas *M. longa* additionally fed on *Calanus* eggs or nauplii, indicated by fatty acid trophic biomarkers. Even under extreme feeding conditions, i.e. under surplus food as well as under starvation, *C. finmarchicus* showed no alterations in digestive activity. It can be suggested that these copepods do not respond to short-term changes but rather to distinct and long-lasting changes in food supply.

In conclusion, the results indicate that the adaptive digestive potential of copepods has implications on the life strategies. Higher adaptive potentials are found in copepods characterised by low energy reserves, short life spans and several generations per year, whereas copepod species with life spans of one year and more, which are well adapted to periodic food supply, are characterised by lower adaptive potentials.

The integrative approach applied in this study lead to a deeper understanding of the physiological adaptations of copepods to changing nutritional conditions and their implications for life-cycle strategies. Furthermore, it was demonstrated that the digestive response to different dietary conditions strongly depends on the feeding history and metabolic requirements of the investigated specimens.
ZUSAMMENFASSUNG


Temora longicornis, Acartia clausi und Centropages typicus sind durch kurze Lebensdauer und mehrere Generationen im Jahr charakterisiert. Während der Phytoplanktonblüten im Frühjahr 2005 (T. longicornis, A. clausi) und im Herbst 2006 (C. typicus) waren diese Arten unterschiedlichen Nahrungsbedingungen ausgesetzt. Sie zeichneten sich durch niedrige Lipid-
ZUSAMMENFASSUNG


OUTLINE OF PUBLICATIONS

The following overview outlines the five publications included in this thesis and my contributions to the respective publications.

PUBLICATION I

Kreibich T, Gentsch E, Hansen B, Hagen W, Niehoff B

Physiological responses of *Temora longicornis* females (Crustacea, Copepoda) to changing nutritional conditions in the North Sea

I was involved in the field work and performed the methodological development for the measurements of digestive enzyme activities. The measurements for digestive and metabolic enzyme activities, protein contents, dry mass, lipid contents and fatty acid composition were done by myself. I wrote the manuscript with scientific and editorial advice of B Niehoff and W Hagen. E Gentsch and B Hansen were involved in the field work, additionally E Gentsch provided important ideas to the manuscript and B Hansen contributed the egg production rate data.

The manuscript was submitted to Marine Biology.

PUBLICATION II

Kreibich T, Saborowski R, Hagen W, Niehoff B

Short term variation of nutritive and metabolic parameters in *Temora longicornis* females (Crustacea, Copepoda) as a response to diet shift and starvation

I developed the idea of this experimental work and performed the experiments. The analytical work like measurements of enzyme activities and fatty acid compositions was done by myself. R Saborowski was involved in writing the manuscript and provided important scientific ideas. W Hagen was involved by giving scientific and editorial advice. B Niehoff was involved in writing the manuscript and provided important ideas and scientific advice during the experiment.

The manuscript was submitted to Helgoland Marine Research.
PUBLICATION III

Kreibich T, Saborowski R, Hagen W, Niehoff B

Influence of physiological conditions and feeding history on digestion, metabolism and fatty acid composition of *Temora longicornis* females (Crustacea, Copepoda) under different nutritional conditions

I developed the idea of this experimental work together with B Niehoff. The experiments were performed by myself, as well as the analytical work like measurements of enzyme activities and fatty acid compositions. R Saborowski was involved in developing the method for the characterisation of lipase/esterase patterns by SDS-PAGE and gave scientific advice for writing the manuscript. W Hagen and B Niehoff were involved by giving scientific and editorial advice.

The manuscript will be submitted to Journal of Experimental and Marine Ecology.

PUBLICATION IV

Kreibich T, Plourde S, Joly P, Starr M, Auel H, Niehoff B

Feeding strategies, digestive activities and reproduction of *Calanus finmarchicus* and *Metridia longa* in the lower St. Lawrence estuary, Québec, Canada

I was involved in the field work and performed the measurements for digestive and metabolic enzyme activities, protein contents, dry mass, lipid contents and fatty acid compositions. The stable isotope data were evaluated by myself. S Plourde and P Joly were involved in the field work and provided the egg production rate data. M Starr provided the data for chlorophyll *a* and the phytoplankton composition. H Auel gave scientific advice for writing the manuscript. B Niehoff was involved in the field work and in writing the manuscript by giving scientific and editorial advice.

The manuscript will be submitted to Polar Biology.
PUBLICATION V

Gentsch E, Kreibich T, Hansen B, Hagen W, Niehoff B

Dietary shifts in the North Sea copepod *Temora longicornis* in spring 2005 – evidence from stable isotope signatures, fatty acid biomarkers and feeding experiments

I shared the field work with the first author and B Hansen. I performed all fatty acid analyses and evaluated the data, and provided important ideas to the manuscript.

The manuscript was submitted to Marine Ecology Progress Series.
1 INTRODUCTION

1.1 SCIENTIFIC BACKGROUND

Calanoid copepods are abundant in the marine plankton communities worldwide and they constitute a major component of the pelagic food web (Longhurst 1985). Herbivorous copepods are often the most important primary consumers and link phytoplankton and higher trophic levels (e.g. Rae and Rees 1947, Hickel 1975, Fransz et al. 1991). On the other hand, they are a major food source for e.g. fish larvae (Last 1978, Støttrup et al. 1986), krill (Atkinson and Snýder 1997, Lass et al. 2001) and whales (Beardsley et al. 1996), and the copepod faecal pellets, sinking from surface to deep-water layers, are fed upon by mesopelagic organisms (e.g. Fransz et al. 1991, Longhurst 1991, Ducklow et al. 2001).

Due to their key role in pelagic ecosystems calanoid copepods were subject to numerous studies during the last century, focusing on e.g. morphology, taxonomy, phylogeny, distribution, life strategies, reproduction or feeding behaviour (extended reviews by e.g. Huys and Boxshall 1991, Mauchline 1998). Copepods, like all heterotrophic organisms, rely on feeding on other organisms in order to cover their energetic demands for growth and to reproduce successfully. Studies on feeding physiology of copepods are therefore of major importance for the understanding of the adaptation to changing nutritional conditions and the functional responses to these changes. As a consequence, this information will expand our knowledge on trophic interactions which lead to a better understanding of ecosystem functioning in the marine environment.

Numerous studies focused on the influence of changing nutritional conditions on functional responses, such as egg production rate and hatching success of copepods. The results indicated strong relations to food diversity (Roman 1984, Støttrup and Jensen 1990), quality (Jones et al. 2002) and quantity (e.g. Checkley 1980). Food quality can be indicated by the content of different dietary components, such as essential fatty acids (Støttrup and Jensen 1990, Jónasdóttir 1994, Klein Breteler et al. 1999, Broglio et al. 2003), sterols (Ederington et al. 1995), proteins (Kleppel and Hazzard 2000) or essential amino acids (Kleppel et al. 1998). However, food quality is also defined by the digestibility of the diet. Digestion links the two processes of ingestion and assimilation, and is therefore a physiological key process. The energy required for different physiological processes is mainly provided through the hydrolysis of lipids and proteins, and these processes are catalysed by specific enzymes. Therefore, studies focused on the influence of external factors on digestive enzyme activities of copepods (e.g. Mayzaud and Conover 1975, Hirche 1981, Hassett and Landry 1983). However, those studies often led to controversial conclusions. Some authors suggested that digestive activity in copepods increases with increasing food supply, whereas others showed that the digestive activity decreases under surplus food or remains unaltered (e.g. Mayzaud and Conover 1975,
INTRODUCTION

Hirche 1981, Hassett and Landry 1983, Head et al. 1984, Harris et al. 1986). Hassett and Landry (1983) discuss that the activity may differ with the life cycle strategies of copepods, however, data are lacking. Moreover, most studies disregarded metabolic requirements and feeding histories of copepods, although it is possible that digestive activity is controlled by feedback mechanisms, which operate to meet the metabolic requirements of the individual (Roche-Mayzaud et al. 1991, Mayzaud et al. 1992). Hence, many questions still remain unanswered concerning physiological adaptive processes in copepods and the factors influencing these processes.

Combining detailed knowledge of the dietary composition (input), the digestive responses to the diet and the fuelling of metabolic activity, growth and reproduction (output), is the next essential step to better understand how diet affects physiological responses, growth and reproduction of copepods. The project “Trophic interactions in pelagic ecosystems: the role of zooplankton” at the Alfred Wegener Institute for Polar and Marine Research (AWI), Bremerhaven, in cooperation with the Marine Zoology at the University Bremen, aimed therefore to investigate feeding behaviour, physiological and functional responses of copepods to changing environmental conditions in detail in order to obtain a more precise picture of processes influencing population dynamics. The present thesis, embedded within this project, elucidates the physiological adaptations of calanoid copepod species to changing nutritional conditions, comparing species with different life strategies from the German Bight, southern North Sea, and the lower St. Lawrence estuary, eastern Canada.

1.2 LIFE STRATEGIES OF CALANOID COPEPODS IN DIFFERENT ENVIRONMENTS

In the following an overview is given on calanoid copepod life cycle strategies in different environments, focussing on the North Sea and the lower St. Lawrence estuary and the respective species, which were studied within this thesis.

Marine copepods inhabit areas with different environmental conditions, from tropical and temperate regions to high latitudes. These areas cover a wide range of abiotic and biotic conditions. E.g. salinity ranges between >40 in hypersaline environments and <25 in brackish waters, and temperature varies from -1.9°C to 40 °C (Lalli and Parsons 1993). Biotic conditions, like food availability and quality can differ strongly as well. Hence, species inhabiting regions with different environmental conditions show different life strategies.
1.2.1 NORTH SEA

Temperate regions like the North Sea are characterised by high seasonal variability of both abiotic and biotic factors (e.g. Gieskes and Kraay 1984, Kjørboe and Nielsen 1994, Krause et al. 1995). The southern North Sea shows high variability in salinity and temperature due to tidal currents, thermal fronts, inflow of North Atlantic water masses and fronts caused by upwelling or fronts caused by surface freshwater runoff of the rivers Weser, Elbe and Ems (Otto et al. 1990).

The North Sea is characterised by two main phytoplankton blooms. The spring bloom usually takes place from the end of March until the beginning of June, while the second bloom occurs in late summer/early autumn from July until October (e.g. Fransz et al. 1991, Skogen and Moll 2000, Halsband and Hirche 2001). However, onset and magnitude of the blooms can differ regionally (Fransz et al. 1991). In recent years, changes in temperature, probably caused by global warming, influence the onset of the spring bloom in the southern North Sea as well. Wiltshire and Manly (2004) showed that the increase of the water temperature by 1.1°C off Helgoland since the 1960’s led to a delay of the spring phytoplankton bloom. This is apparently related to a warming of the autumn months, resulting in a longer persistence of copepods grazing on phytoplankton in autumn and early winter. This, in turn, may restrain the phase to build up phytoplankton biomass resulting in a delayed spring phytoplankton bloom.

In the North Sea, calanoid copepods constitute the most important portion of the zooplankton, both in terms of abundance and biomass (Hickel 1975, Fransz et al. 1991). Typical representatives are *Temora longicornis*, *Acartia clausi* and *Centropages typicus*, which were studied in the present thesis off Helgoland (Fig. 1).

**TEMORA LONGICORNIS**

The small calanoid copepod *Temora longicornis* is a temperate and neritic species with high abundances in the central and southern North Sea. It is also encountered at the Portuguese coast, in the Baltic Sea and off northern Norway (Hickel 1975, Fransz et al. 1991, Krause et al. 1995, Halsband-Lenk et al. 2002). *T. longicornis* is known as an omnivorous species feeding on phytoplankton, microzooplankton and younger copepod stages (e.g. Kleppel 1993, Dam and Lopes 2003). Reproduction usually peaks in April/May in the North Sea, closely linked to the phytoplankton spring bloom (Halsband and Hirche 2001, Arendt et al. 2005, Wesche et al. 2006). The generation times are short with 20 to 50 days, depending on the environmental temperature (Harris and Paffenhöfer 1976, McLaren 1978, Fransz et al. 1989, Klein Breteler et al. 1990); up to five generations per year are found in the North Sea (Halsband-Lenk et al. 2004). Females reproduce throughout the year, although egg production rates and the propor-
tion of spawning females are low in winter (Halsband and Hirche 2001, Wesche et al. 2006). In addition to subitaneous eggs, *T. longicornis* may produce resting eggs, which sink to the bottom (Castellani and Lucas 2003). Engel and Hirche (2004) observed hatching of *T. longicornis* nauplii from copepod eggs sampled from the sediment during their three year study in the southern North Sea, indicating that here resting eggs are produced. Due to low energy reserves and high metabolic requirements (e.g. Evjemo and Olsen 1997, Helland et al. 2003) this species can only survive short periods of starvation.

**Fig. 1:** Study area 2005 Helgoland Roads (54°11'N, 07°54'E), overview map obtained from GKSS Forschungszentrum Geestacht (http://w3k.gkss.de/kof/images/), detailed map generated with NASA World Wind, Version 1.4.0.0 (National Aeronautics and Space Administration).

**ACARTIA CLAUSI**

The neritic to oceanic species *Acartia clausi* is abundant in the North Sea as well as in other European seas, the Mediterranean and the Black Sea, inhabiting near-surface water layers (Fransz et al. 1991, Krause et al. 1995, Mauchline 1998). Also being omnivorous, this species feeds on phytoplankton, microzooplankton, copepod eggs and nauplii (Wiadnyana and Rassoulzadegan 1989, Mauchline 1998). The reproductive period lasts from February to October in the southern North Sea, with highest peaks from May to August (Halsband and Hirche 2001). The generation times are short with three to four weeks (Klein-Breteler et al. 1994), *A. clausi* produces four to six generations per year (Conover 1956, Uye 1982). Adult females are
the main over-wintering stage in *A. clausi*, with discontinued reproduction during winter (Wesche et al. 2006). The production of resting eggs is not documented in the North Sea for *A. clausi* (Halsband and Hirche 2001, Engel and Hirche 2004), but was found in other regions like the Pacific (Kasahara et al. 1974). *A. clausi* is described as a species with low energy reserves and high metabolic requirements and thus low starvation tolerance (e.g. Kattner et al. 1981, Mayzaud et al. 1992).

**CENTROPAGES TYPICUS**

*C. typicus* is a temperate, neritic species inhabiting near-surface layers and occurs from the Mediterranean Sea to the Norwegian Sea (Fransz et al. 1991 and references therein, Halsband-Lenk et al. 2001). In the North Sea, *C. typicus* is one of the dominant calanoid copepod species (Hickel 1975). This species is transported with the Atlantic current into the North Sea and most abundant during autumn and winter, however, characterised by great interannual variability (e.g. Fransz et al. 1991, Krause et al. 1995, Halsband and Hirche 2001, Halsband-Lenk et al. 2004). *C. typicus* has been described as mostly carnivorous or omnivorous feeding on phytoplankton, microzooplankton, eggs and nauplii of copepods and fish larvae (e.g. Bonnet and Carlotti 2001, Calbet et al. 2007). The reproductive cycle in the southern North Sea usually takes place in the second, warmer half of the year and ceases in winter (Halsband and Hirche 2001). Egg production rates are highest from June until September (Halsband-Lenk et al. 2004). *C. typicus* produces approximately five generations per year. No information is available on production of resting eggs or over-wintering stages in *C. typicus* (Carlotti and Harris 2007, Durbin and Kane 2007). Also *C. typicus* is not able to starve for longer than three to six days (Dagg 1977).

**1.2.2 ST. LAWRENCE ESTUARY**

The lower St. Lawrence estuary (LSLE) in Québec, Canada, a 200 km long, 20 to 40 km wide and up to 330 m deep marine estuary, is a highly dynamic physical environment, characterised by strong advective processes (e.g. Laprise and Dodson 1994, Zakardjian et al. 1999). The main characteristic is a two-layer circulation pattern, freshwater runs off from the St. Lawrence river at the surface and this is compensated by a slow advection of deep sea water into the estuary (e.g. Zakardjian et al. 1999). The deep water usually originates from the Atlantic, but is probably influenced by Arctic Water as well (Conover 1988, Plourde et al. 2002). Due to the strong advective processes in the surface layer, the phytoplankton bloom in spring does not start until mid-June (Levasseur et al. 1984). Upwelling processes periodically transport nutrient-rich cold deep water to the surface, supporting the persistence of the bloom into September (Levasseur et al. 1984, Therriault and Levasseur 1985). Dominating zoo-
plankton species inhabiting the LSLE are *Calanus finmarchicus* and *Metridia longa* (e.g. Plourde and Runge 1993, Plourde et al. 2002), which were studied off Rimouski in the lower St. Lawrence estuary in the present thesis (Fig. 2).

![Map of the study area](image)

Fig. 2: Study area 2006 in the St. Lawrence estuary off Rimouski, Québec, Canada (Rimouski Station 48°40' N, 68°35' W), maps created with NASA World Wind, Version 1.4.0.0 (National Aeronautics and Space Administration).

**CALANUS FINMARCHICUS**

*Calanus finmarchicus* is a widespread species inhabiting waters of the North Atlantic, its distribution range extends from the mid-Atlantic Shelf off the US east coast to the south of Greenland, the Norwegian Sea and the Barents Sea north of Norway. This species is transported by currents from its reproduction centres in the North Atlantic into other regions, e.g. the North Sea and the Arctic Ocean (e.g. Båmstedt 1988, Conover 1988, Krause et al. 1995 and references therein, Mauchline 1998). *C. finmarchicus* is known as a diel vertical migrating, mainly herbivorous species which, however, feeds omnivorously when phytoplankton is scarce (e.g. Marshall and Orr 1972, Mullin 1963, Båmstedt 1988, Ohman and Runge 1994, Mauchline 1998, Runge et al. 2006). This calanoid copepod is well adapted to the seasonal phytoplankton cycle in higher latitudes. It mainly overwinters in a diapause as copepodite stage V (CV) in deep layers. Prior to the spring phytoplankton bloom, the CV mouls to adult and migrate to the surface where reproduction begins (e.g. Miller et al. 1991, Diel and Tande 1992, Plourde and Runge 1993). The final gonad development and spawning of *C. finmarchicus* are usually linked to the phytoplankton bloom (e.g. Marshall and Orr 1972, Runge 1985, Diel and Tande...
1992, Hay 1995). However, low reproductive rates have been found also prior to the bloom and *C. finmarchicus* may use internal body reserves for reproduction when food is scarce (Irigoien et al. 1998, Niehoff 1998). Generation times vary greatly among locations, strongly depending on ambient temperatures. Development from egg to adult ranges between one and several months, and a complete cycle from egg to mature adult requires between 2 months and a year or longer (e.g. Marshall and Orr 1972, review in Mauchline 1998). Due to the accumulation of depot lipids, i.e. wax esters, *C. finmarchicus* can starve over prolonged periods (e.g. Miller et al. 1991, Kattner and Hagen 1995, Albers et al. 1996).

**METRIDA LONGA**

*M. longa* is one of the most common species in boreal-arctic waters. Its main distribution areas cover the Arctic Basin and the northern North Atlantic and it is found in the Norwegian Sea as well as in the St. Lawrence estuary and the North Sea (Tande and Grønvik 1983, Båmstedt and Tande 1988, Conover 1988, Krause et al. 1995, Auel and Hagen 2002, Plourde et al. 2002). This diel vertical migrating, omnivorous species feeds on phytoplankton, microzooplankton as well as on eggs and nauplii of copepods, such as *Calanus* spp. (Conover 1988, Miller et al. 1991, Plourde et al. 2002, Dalsgaard et al. 2003). As *C. finmarchicus*, *M. longa* is well adapted to the seasonal phytoplankton cycle in higher latitudes. It over-winters as physiologically active adult males and females and displays diurnal vertical migration (Tande and Grønvik 1983, Båmstedt et al. 1985, Båmstedt and Tande 1988, Blachowiak-Samolyk et al. 2006). The onset of the active reproductive cycle in *M. longa* depends on external factors. It has been reported that reproduction commences well after the onset of the spring phytoplankton bloom (Tande and Grønvik 1983), but other studies have shown that it can also start with the phytoplankton bloom (Båmstedt and Tande 1988). Due to the distribution in mainly boreal-arctic waters, where low temperatures prevail, generation times are comparably long, and *M. longa* has a one year life cycle (Grønvik and Hopkins 1984). This species accumulates storage lipids as well, however, not as extensive as *C. finmarchicus* (Lee 1975, Falk-Petersen et al. 1987, Albers et al. 1996).

**1.3 PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF COPEPODS**

Copepods from temperate and high latitudinal regions adapted to different environmental conditions in order to reproduce and grow successfully. Their life cycle strategies entail strong differences in the copepods physiology and biochemical composition. This chapter briefly lists physiological and biochemical characteristics of copepods and shortly introduces parameters which are helpful to gain a more precise view on physiological adaptive processes in copepods.
1.3.1 MAJOR ORGANIC COMPONENTS

There are three main organic macromolecules, proteins, lipids and carbohydrates, which are, beside other functions, used to cover the energetic demand in an organism. These compounds play different roles in the life strategies of copepods.

PROTEINS

Proteins are involved in almost all biological processes in living organisms. They are the most abundant organic molecules in the living cell and are used for e.g. allocation of energy, acceleration of chemical processes (enzymes), transportation of small molecules and ions, immune defence or coordination of the cell activity (hormones). Furthermore, dietary proteins provide essential amino acids to the organism, which are crucial for both, the anabolism of new proteins and the coverage of energy demanding processes. In marine zooplankton, as in other groups, proteins play an important role as energy source (e.g. Raymont and Conover 1961, Mayzaud 1976). In copepods the protein content ranges between 24 and 82% of the dry mass (DM) (Båmstedt 1986 and references therein). In general, lowest values occur in copepods from high latitudes and deep-water species from low latitudes, whereas copepods from medium latitudes contain relatively more protein (Båmstedt 1986). This is due to their life strategies. Copepods from medium latitudes, such as *Acartia tonsa*, predominantly store proteins whereas copepods from higher latitudes, such as *Calanus finmarchicus*, store lipids (review in Båmstedt 1986, Thor 2000). Proteins are easier to metabolise (Wieser 1986), but have a lower energy content with ca. 18 kJ g⁻¹ compared to ca. 39 kJ g⁻¹ for lipids (Lee et al. 2006). Thus, lipids are more suitable as energetic compounds in order to store large energy reserves on smallest volume.

LIPIDS

The term “lipids” comprises compounds of a widely varying nature as they only have in common that they solve in organic solvents (Christie 1973). Lipids fulfil a wide range of functions. They are important components of membranes, they are used as energy storages and function as hormones and antioxidants, they regulate different physiological processes or are used for buoyancy (summary in Lee et al. 2006). Lipids are grouped in two major classes, polar and neutral lipids. Polar lipids, e.g. glycerophospholipids, are the key components of biomembranes (Sargent and Whittle 1981). The phospholipid class phosphatidylcholine appears to serve also as a depot lipid in some euphausiids (e.g. Hagen et al. 1996). Neutral lipids provide energy for reproduction or for periods of starvation. Wax esters (WE), one of the neutral lipid class, are mainly used for reproduction and over-wintering (e.g. Lee et al. 1970, Lee and
Hirota 1973, Sargent and Henderson 1986, Hagen and Kattner 1998), whereas triacylglycerols (TAG) are primarily a short-term storage and utilized more rapidly than wax esters (Lee 1974, Lee and Barnes 1975, Mayzaud et al. 1998). The WE are mainly found in copepods from higher latitudes, adapted to long starving periods due to low food availability during winter, such as Calanus finmarchicus, C. hyperboreus and C. glacialis (e.g. Fraser et al. 1989a, Kattner and Krause 1989, Graeve et al. 1994). Species from temperate regions like Temora longicornis, Acartia clausi, Centropages typicus and C. hamatus in contrast, use TAG as depot lipids (e.g. publication I, II and III, Kattner et al. 1981, Fraser et al. 1989a, Brüll 2007). High amounts of TAG indicate continuous food supply (Sargent et al. 1981). The lipid content in copepods from higher latitudes reaches up to 75% of the copepods dry mass (DM), whereas in copepods inhabiting environments characterised by continuous food supply lipid content ranges between 3 and 19% DM (review in Båmstedt 1986, review in Lee et al. 2006).

CARBOHYDRATES

Carbohydrates show a wide complexity, from oligo- and polysaccharides, to amino sugars or chitin. They play, however, a minor role in energy storage in copepods and reach percentages between 0.2 and 5.1% DM only, chitin not included (Båmstedt 1986). However, a considerable proportion of organic material produced by phytoplankton such as diatoms are polysaccharides. They can contribute between 10 and 70% of organic dry matter, depending on growth phase of the algae (Haug and Myklestad 1976). Hence, in spite of low accumulation in copepods, carbohydrates may play a significant role in providing energy to the organisms.

1.3.2 METABOLIC RATES

Information on metabolic rates are obtained by e.g. measuring respiration rates in living copepods (e.g. Marshall and Orr 1958, Mayzaud 1976, Kjørboe et al. 1985, Thor 2003), measuring the activity of key metabolic enzymes in frozen samples (Vetter 1995, Salomon et al. 2000, Saborowski and Buchholz 2002). For the latter method, key enzymes such as the pyruvate kinase of the glycolysis and the citrate synthase of the carboxylic acid cycle are used as indicators for the anaerobic and aerobic potential of organisms.

Marine invertebrates differ in their metabolic rates. The metabolic activity is influenced by different factors, such as ambient temperatures, life strategies and body size. At higher temperatures the metabolic rate and, thus, the energetic demand of an organism, increase (e.g. Hirche 1984, Saborowski et al. 2002). Respiration is positively correlated with increasing dry mass (e.g. Conover and Corner 1968). Furthermore, organisms, which predominantly
accumulate proteins are characterised by higher metabolic rates, since protein synthesis is energy demanding (Thor et al. 2002).

*Temora longicornis*, *Acartia clausi* and *Centropages typicus* are known as species with high metabolic requirements and low energy reserves (e.g. Dagg 1977, Mayzaud et al. 1992, Evjemo and Olsen 1997, Helland et al. 2003, review in Gaudy and Thibault-Botha 2007). Due to their short generation times the species invest most parts of the available energy directly into growth and reproduction. In contrast, *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* characterised by a longer life span, is well adapted to the unfavourable environmental conditions in its habitats during winter and perform diapause (e.g. Tande 1982, Båmstedt and Ervik 1984, Slagstad and Tande 1990, Plourde and Runge 1993, Hirche 1996, Auel et al. 2003). During starving periods, these copepods reduce their metabolic rate in order to save energy and may even reduce not required cells of the gut epithelium (Hallberg and Hirche 1980, Thor 2003). *Metridia longa*, in contrast, does not perform diapause and feeds throughout the winter. Hence, metabolic activities are reduced to a minor extent (Båmstedt and Tande 1988). However, the food supply during winter cannot sustain the metabolic needs of *M. longa* throughout this period (Båmstedt et al. 1985), resulting in a slightly negative energy balance, at least in arctic-boreal waters (Båmstedt and Tande 1988).

### 1.3.3 DIGESTIVE ACTIVITY

Different digestive enzymes are responsible for the cleavage of dietary macromolecules, such as proteins, lipids or carbohydrates. Proteinases, also known as endopeptidases, and exopeptidases are responsible for the hydrolisation of proteins. Proteinases (E.C. 3.4.21 - 3.4.25) are involved in the hydrolisation of peptide bonds within proteins, like trypsin (e.g. García-Carreño 1992) and are the main protein-digesting group in crustaceans (Gibson and Barker 1979). They are grouped into different classes according to the composition of their active centre. These classes are characterised by e.g. different pH optima or different sites of activity like intra- or extracellular. The optimum of digestive activities in crustaceans ranges between pH 5.5 and 9.0 (García-Carreño 1992), depending on the proteinase class patterns. Serine proteinases, for example, have highest activities under alkaline pH (e.g. Fernández Gimenez et al. 2001), whereas acid aspartic proteinases show maximum at pH 3 in *Homarus gammarus* (Navarrete del Toro et al. 2006). Exopeptidases, also belonging to protein-digestive enzymes, catalyse the hydrolisation of terminal peptide bonds in proteins (e.g. Mayzaud 1986). Lipases and esterases are enzymes acting on the carboxyl ester bonds present in acylglycerols (glycerol ester hydrolases, EC 3.1.1.) (e.g. Mayzaud 1986, Díaz et al. 1999). Polysaccharides are hydrolysed by glycosidases (EC 3.2.1.), e.g. amylase (e.g. Hasler 1935b, Mayzaud 1986).
Already in the 1930's, Bond (1934) and Hasler (1935a, 1935b) identified some digestive enzymes in *Daphnia* and *Calanus* spp. Until now, there have been many digestive enzymes identified and characterised in copepods and other crustaceans, according to e.g. their kinetic properties, pH optima, molecular weight, substrate or temperature dependence in crustaceans (e.g. Manwell et al. 1967, Mayzaud and Mayzaud 1981, Mayzaud 1985, Dittrich 1990, García-Carreño 1992, García-Carreño et al. 1993, Fernández Gimenez et al. 2001, Knotz et al. 2006). Other studies focused on responses in digestive enzyme activities of copepods to different feeding conditions in the field and under laboratory conditions (e.g. Mayzaud and Conover 1975, Mayzaud and Poulet 1978, Cox 1981, Cox and Willasson 1981, Hirche 1981, Hassett and Landry 1983, Harris et al. 1986, Hassett and Landry 1988). However, despite of numerous studies investigating digestive responses in copepods, the influence of different, particularly internal, factors on these responses are barely known. Some studies proposed that digestive activities may be influenced by the copepods metabolic requirements, their feeding history and their life strategies (Tande and Slagstad 1982, Hassett and Landry 1983, Mayzaud 1986, Roche-Mayzaud et al. 1991, Mayzaud et al. 1992). But, until now, no study verified these assumptions. Thus, this thesis focused on investigating changes in digestive activities in relation to food supply, feeding history of copepods, metabolic requirements and life strategies, both in the field and in laboratory experiments.

### 1.3.4 Fatty Acids and Their Use as Biomarkers

Fatty acids, which are components of lipids, differ in their number of carbon atoms and their degree of saturation, from saturated (SFA) over monounsaturated (MUFA) to polyunsaturated fatty acids (PUFA). Organisms are able to introduce double bonds in fatty acids at different positions, depending on the desaturase types they possess. These differences enable scientists to identify the origin of fatty acids. For example, plants are the only organisms, which can synthesize (n-3) and (n-6) PUFA *de novo* in higher amounts (Dalsgaard et al. 2003 and references therein). Thus, the PUFAs 20:5(n-3) (eicosapentaenoic acid, EPA) and 22:6(n-3) (docosahexaenoic acid, DHA) and their precursors 18:2(n-6) and 18:3(n-3) are essential dietary components of heterotrophic organisms, including also, of course, the copepods (e.g. Pohl and Zurheide 1979, Sargent and Whittle 1981, Pond et al. 2002). When the animals feed on organisms, the dietary fatty acids are incorporated into the consumer’s lipids and can be detected by gas chromatography. Thus, specific dietary fatty acids serve as biomarkers, indicating feeding on specific food items, i.e. algal groups.

In general, a trophic biomarker is a compound which (i) origin is easily identified, (ii) is incorporated unselectively and unmodified in the consumer and (iii) is metabolically stable or inert. Ideally, the measurement of biomarkers should provide information on both qualitative as well as quantitative terms (e.g. Sargent et al. 1987). In marine organisms the use of specific
dietary fatty acids as trophic biomarkers is well established (e.g. Lee et al. 1971, Graeve et al. 1994), and was recently reviewed for the marine environment by Dalsgaard et al. (2003). During the last decades the fatty acid patterns of different microalgae groups, bacteria, microzooplankton or specific zooplankton, such as the calanoid copepod genus *Calanus* were identified (e.g. Kates and Volcani 1966, Ackman et al. 1968, Lee 1975, Pohl and Zurheide 1979, Sargent and Falk-Petersen 1988). Feeding on these different groups can be traced by specific fatty acids and the resulting changes in the fatty acid composition of the consumers, e.g. herbivorous, carnivorous and omnivorous zooplankton species (e.g. Lee et al. 1971, Sargent and Whittle 1981, Graeve et al. 1994, Auel et al. 2002, Stübing et al. 2003). In heterotrophic organisms, like calanoid copepods or euphausiids from polar regions, algal specific fatty acids are accumulated mainly in neutral lipids without further modification (e.g. Sargent and Henderson 1986, Kattner and Hagen 1998, Dalsgaard et al. 2003). In calanoid copepods from polar regions, the fatty acid signal integrates trophic information over a time scale of several weeks to months and, thus, reflects the major nutritional conditions and diets during that time (e.g. Graeve et al. 1994). However, for copepods from temperate regions which do not largely accumulate lipids as energy reserves like *Temora longicornis* time scales for the incorporation of dietary fatty acids is scarce. Except for the study by Veloza et al. (2006) feeding *Acartia tonsa* over five days with different algae, no information is available concerning the accumulation of dietary fatty acids in small calanoid copepod females. Therefore, a study concerning detailed information on time scales for diet-induced changes in fatty acid patterns in small calanoid copepods would enforce the use of fatty acids as trophic biomarkers, facilitating more precise descriptions of past feeding events.

1.4 Objectives

Until now, no study combined detailed information on the dietary composition in times of changing nutritional conditions in the field, including the corresponding physiological and functional responses in copepods and the influence of different life strategies on these physiological processes. However, only by combining a large variety of parameters, as listed in the previous chapters, we will gain a more detailed picture of processes involved in feeding and physiological adaptations, as well as the impact of physiological adaptations for the evolution of life strategies. As a result, this will improve our knowledge on the adaptability of different copepod species to changing environmental conditions and may help to predict changes in pelagic food webs in times of global warming.
OBJECTIVE 1

PHYSIOLOGICAL RESPONSE OF DOMINANT COPEPOD SPECIES TO CHANGING NUTRITIONAL CONDITIONS IN THE FIELD

The first objective of this thesis is to combine information on the diet (input), on physiological responses of copepods to the diet, i.e. digestive and metabolic response, and on reproduction, i.e. egg production rate (output) in order to better understand how diet affects growth and reproduction of copepods in the field. We conducted two field campaigns during spring phytoplankton blooms, one in the southern North Sea 2005 off Helgoland, and one in the lower St. Lawrence estuary 2006, eastern Canada. The copepod species investigated were characterised by different life strategies (see chapter 1.2). In the North Sea, *Temora longicornis* was investigated, and in the lower St. Lawrence estuary, the study focussed on *Calanus finmarchicus* and *Metridia longa*.

During both time series changes in biochemical and physiological parameters were documented, such as lipid and protein content, fatty acid composition, metabolic and digestive enzyme activities as well as egg production rates in copepods. Additionally, seston compositions were investigated in order to characterise potential diets and nutritional conditions by measuring carbon, nitrogen, chlorophyll a and lipid content as well as fatty acid composition. Since copepods may feed selectively (e.g. Daan et al. 1988, Breton et al. 1999, Cotonnec et al. 2001), we traced the feeding on dietary components by fatty acid biomarkers. Additionally, the study conducted in the southern North Sea, as part of the integrative research approach studying feeding and growth of dominant zooplankton organisms, benefits from the study by E. Gentsch investigating feeding behaviour of *T. longicornis*. This additional information allows to elucidating the physiological response of *T. longicornis* to its varying diet in nature in greater detail than considering solely the biochemical composition of the bulk seston.

OBJECTIVE 2

IMPLICATIONS OF THE PHYSIOLOGICAL ADAPTIVE POTENTIAL ON LIFE STRATEGIES

In order to elucidate life strategy-dependent differences in adaptive processes, the physiological responses of copepod species characterised by different life strategies to changing nutritional conditions in the field are compared. The underlying hypothesis is that species which rely on continuous food supply depend on a highly adaptive digestive system for the digestion of different dietary sources, whereas the digestive system of copepods which are able to starve for longer periods using their own body reserves, is less flexible.
The comparison is based on the results obtained during the field campaigns in the southern North Sea and the lower St. Lawrence estuary (publications I and IV). Additionally, data on the copepod *Acartia clausi* were obtained during the time series 2005 off Helgoland (Hansen 2006, Kreibich et al. unpublished data). During the autumn phytoplankton bloom 2006 in the southern North Sea, two Master theses were conducted on Helgoland embedded in the project “Trophic interactions in pelagic ecosystems: the role of zooplankton”, studying the effect of food quality on the physiology of *Centropages typicus* in the field as well as under experimental conditions (Brüll 2007, Ohlf 2007). The data are in part used in the synoptic discussion and are indicated in the respective chapters.

The adaptive digestive and metabolic potential of *T. longicornis, A. clausi, C. typicus* and *C. finmarchicus* was also investigated in experiments with surplus and without food in order to investigate physiological changes under extreme feeding conditions (publications II and III, Brüll 2007, Kreibich et al. unpublished data). Additionally, Dr. Barbara Niehoff (AWI) provided samples of *Calanus glacialis* and *C. hyperboreus* from the Arctic to study the physiological changes under experimental conditions in these species due to the onset of feeding after overwintering.

**OBJECTIVE 3**

**USE OF FATTY ACID BIOMARKERS IN SMALL CALANOID COPEPODS**

The third objective of this thesis is to obtain detailed information on time scales for diet-induced changes in fatty acid patterns in calanoid copepods which are characterised by low energy reserves. To test if and how fast specific dietary fatty acids are accumulated in the calanoid copepods *Temora longicornis* and *Centropages typicus* we conducted several feeding and starvation experiments over three days and compared the accumulation and decrease of diet-specific fatty acids in the copepods lipids (publications II and III, Brüll 2007). In addition, the influence of feeding history on the accumulation and degradation processes were investigated.
2 MATERIALS AND METHODS

This chapter summarizes briefly the methods, which I have used in this thesis. Detailed descriptions and methods applied by my co-authors are given in the respective publications.

2.1 FIELD WORK

Field work was conducted during two field campaigns, one at the Biologische Anstalt on Helgoland (BAH) in the Foundation Alfred Wegener Institute for Polar and Marine Research (AWI), Germany, and one at the Maurice-Lamontagne Institute (IML) of the Federal Government Department Fisheries and Oceans Canada in Mont-Joli, Québec, Canada, to compare species with different life cycle strategies. At the BAH, a second time series on the feeding biology of a calanoid copepod, Centropages typicus, was conducted in autumn 2006 by Pamela Ohlf (Ohlf 2007) within the research group “Trophic interactions in pelagic ecosystems”. These data are used for comparison with the species, I have studied.

ZOOPLANKTON

At the BAH in the southern North Sea, Temora longicornis females were sampled weekly from March until May 2005 at the station Helgoland Roads (54°11’N, 07°54’E) with a CalCOFI net (500 μm mesh size) which was towed for 10 min in 10 m depth at a speed of 0.3 m s\(^{-1}\) (publication I, II, III, V, Hansen 2006). Acartia clausi females were sampled sporadically, when abundant in the samples (Hansen 2006, Kreibich et al. unpublished data). Centropages typicus was caught weekly from August until October 2006 (Brüll 2007, Ohlf 2007).

Calanus finmarchicus and Metridia longa females were sampled weekly in the St. Lawrence Estuary off Rimouski, Québec, Canada, at the long-term monitoring station of the IML (48°40’ N, 68°35’ W) from May until August 2005. Here, two plankton nets (202 and 333 μm mesh size) were vertically towed from 320 to 0 m at a speed of <0.5 m s\(^{-1}\) (publication IV).

Immediately after capture, samples were transferred to buckets or jars containing surface seawater and transported to the laboratory. At least 180 healthy looking females were sorted alive under a stereo-microscope for biochemical, enzyme and stable isotope analysis. All individuals were briefly rinsed in demineralised water (aqua dem.) and transferred either to pre-weighed Zn-cartridges (carbon and nitrogen content, stable isotope analysis), to 1.5 ml reaction tubes (water soluble protein content, enzyme activities) or to pre-cleaned glass vials (dry mass, lipid analysis). The samples were immediately frozen at -80°C (T. longicornis, A. longa, A. clausi).
clausi, C. typicus) or in liquid nitrogen (C. finmarchicus, M. longa). Additional females were sorted for experiments (see chapter 2.1.2).

SESTON SAMPLES

Water samples were taken with a 10 l Niskin bottle in 3 m (BAH) and 10 m (IML) depth at the respective sampling stations and pre-filtered to remove small zooplankton such as copepod eggs and nauplii. Sub-samples (100 - 1000 ml, depending on amounts of particulate matter) were filtered on dried and pre-weighted GF/C filters (0.2 μm mesh size). Filters were briefly rinsed with aqua dem. to remove salt residues and triplicates were frozen for the analyses of the stable isotopes $\delta^{15}$N and $\delta^{13}$C (SI), C, N and lipid content and fatty acid composition.

2.1.1 FEEDING AND STARVATION EXPERIMENTS

Parallel to the time series in the North Sea and the lower St. Lawrence estuary feeding and starvation experiments were performed with T. longicornis, A. clausi and C. finmarchicus in the laboratories of the Marine Stations (chapter II, III, Hansen 2006, Brüll 2007, Kreibich et al. unpublished data). In autumn 2006 at the BAH feeding and starvation experiments were conducted with C. typicus by V. Brüll (Brüll 2007) within the research group “Trophic interactions in pelagic ecosystems”. These data are used for comparison with the other species. At the BAH, the cryptophycean Rhodomonas baltica, the bacillariophycean Thalassiosira weissflogii and the heterotrophic dinoflagellate Oxyrrhis marina were fed, or females were incubated in filtered seawater. At the IML, algae culturing was difficult due to contamination of the stock cultures. Thus, only two experiments were conducted with C. finmarchicus. In the first experiment females were fed with O. marina, or incubated in filtered seawater. In the second experiment females were fed with either Thalassiosira sp. or O. marina, or incubated under starving conditions.

ALGAE CULTIVATION

Stock solutions of R. baltica, T. weissflogii and O. marina were kindly provided by Dr. U. Tillmann, Alfred-Wegener-Institute for Polar and Marine Research, Bremerhaven.

The $\beta$-f/2 medium was prepared after Guillard (1975) in 10 and 20 l sterile flasks, medium for T. weissflogii was additionally supplemented with silicate. Sterile Nalgene or glass flasks (1 - 20 l) were used to obtain high quantities of algae. Algae cultures were cultivated under constant light and aeration at approximately 15°C. The heterotrophic dinoflagellate O. marina
was fed with *R. baltica* two or three times per week. Every day, flasks with the cultures were gently shaken to keep cells in suspension. Cell concentrations were measured with a cell counter and analyser system (CAS® Model TTC, Schärfe System GmbH) or counted in a haemocytometer. Possible contaminations in cultures were checked under a microscope at least every week and always prior to an experiment. Two to three days prior to an experiment feeding of *O. marina* with *R. baltica* was stopped in order to avoid mixed cultures during the experiment. At the beginning of the feeding experiment a defined volume of the cultures were filtered on dried GF/C filters, as seen for seston samples, and stored at -80°C for subsequent fatty acid analysis.

**EXPERIMENTS**

Several feeding and starvation experiments were carried out with healthy looking individuals over three days with *T. longicornis*, *A. clausi*, *C. typicus*. Two feeding and starvation experiments were carried out with healthy looking *C. finmarchicus* females over 12 to 18 and 7 days, respectively. In the first experiment, fed females were incubated over 12 days, and females under starvation over 18 days. The initial condition of the females was determined by means of time series sampling (chapter 2.1).

Studying the comparably small copepods from the North Sea, three 2.5 l beakers were prepared with algae suspensions or with filtered seawater (0.2 μm) for each experimental treatment. The cell density of *R. baltica* was approximately 20,000 cells ml⁻¹, of *T. weissflogii* 8,000 cells ml⁻¹ and of *O. marina* 2,000 cells ml⁻¹ to ensure surplus food conditions. About 150 females and 15 males were placed in each of the 2.5 l beakers containing approximately 2 l of algal suspension or filtered seawater. Males were required for continuous fertilization of females (Ianora and Poulet 1993). The beakers were kept in the dark in a constant temperature room under permanent aeration to keep algae in suspension. The temperature was adjusted to the water temperature in the field at the sampling day (5-8°C). Each day females were removed from the beakers and stored at -80°C for biochemical analysis, or incubated over 24h for measurements of egg production and faecal pellet production rates. On day two approximately half of the incubation water was exchanged by new medium.

Owing to the larger size of *C. finmarchicus*, for each treatment two 10 l containers were prepared with filtered seawater, and about 400 females were placed in each container. In this species, 20 to 30 females were individually incubated daily to determine egg production and faecal pellet production rates (see chapter 2.1.3). Due to low numbers of females in the samples it was not possible to take each day females from the beakers for biochemical
analysis. Every day eggs and faecal pellets were removed from the beakers and half of the water was exchanged every two days.

To study the changes in the physiology of *Calanus glacialis* and *Calanus hyperboreus* due to the onset of feeding after over-wintering, approximately 500 females of each species were caught during an expedition with RV Polarstern at the end of winter in March and April 2003. Onboard, one half of the females was kept for three weeks in pre-screened seawater and the other half in seawater inoculated with diatom cultures. Egg production rate (EPR) and faecal pellet production rate (FPR) were determined daily. In addition, female dry mass, carbon, nitrogen and lipid content were monitored (Niehoff et al. unpublished data). From these experiments, Dr. Barbara Niehoff (AWI) provided deep frozen females which allowed me to determine the activity of digestive and metabolic enzymes in these species during my thesis.

### 2.1.2 Egg Production Measurements

For measurement of *in situ* egg and faecal pellet production of *T. longicornis*, *A. clausi* and *C. typicus*, 36 to 48 females were sorted directly after capture and incubated individually in 6.3 ml cell wells with filtered sea water. Cell wells were incubated for 24 h in a constant temperature room at ambient temperature. Eggs and faecal pellets were counted every 8 h and removed from cell wells in order to avoid cannibalism of females on eggs. During feeding and starving experiments EPR and FPR were determined additionally to the *in situ* measurements on day one, two and three.

Due to their larger size, *C. finmarchicus* females were transferred to Petri dishes (45 ml volume) filled with pre-screened seawater for the starvation and feeding experiments. These females were also incubated for 24 h at ambient temperatures in the dark, however, in this species, eggs and faecal pellets were counted only once after 24h since egg cannibalism is low in the dishes (S. Plourde and P. Joly, pers. com.).

### 2.2 Analytical Work

#### 2.2.1 Dry Mass

The copepods of the species *Temora longicornis*, *Acartia clausi*, *Centropages typicus*, *Calanus finmarchicus* and *Metridia longa* were lyophilised for 24 h (Leybold-Heraeus, LYOVAC GT2). After lyophilisation dry mass was measured with a micro-balance (Sartorius, ±2 μg). During weighing procedure, samples were temporarily stored in a vacuum desiccator.
to prevent water condensation on the tissue. The lyophilised samples were stored at -80 °C for further fatty acid analysis.

2.2.2 WATER-SOLUBLE PROTEIN CONTENT

Water-soluble proteins in copepods were quantified after Bradford (1976) using a commercial protein assay (BioRad). Bovine serum albumin (BSA, BioRad) was used as standard. One ml ice-cold demineralised water was added to the deep-frozen samples kept on ice. Homogenisation was carried out in 1.5 ml reaction tubes with a micro pestle. The homogenates were centrifuged 15 min at 15,000 g (4 °C) (Heraeus Instruments, Biofuge fresco). The supernatants were transferred into new reaction tubes and kept on ice until photometrical analysis. Samples and standards (0 to 0.5 mg·ml⁻¹) were measured in triplicates. The optical density of the assay was measured at 595 nm (OD₅₉₅) after 10-15 min of incubation at room temperature (Kontron Instruments, Uvikon Spectrophotometer 941).

2.2.3 LIPID CONTENT AND FATTY ACID ANALYSIS

Twenty-four hours prior to the extraction procedure of the lipids, 1.5 or 4 ml of solvent (dichloromethane:methanol 2:1:v:v) were added to frozen copepod and phytoplankton samples, respectively. Thereafter samples were again stored at -80 °C. After adding a defined volume of the internal standard tricosanoic acid (23:0, Fluka 91470) to the samples, lipid extraction was performed with minor modifications after Folch et al. (1957) as described in Peters et al. (2006). North Sea copepods and phytoplankton filters were sonicated for 30 s at 80% of the maximum energy (Bandelin electronic, UW 2070), supernatants were transferred into centrifuge vials. New solvent was added to samples, sonication was repeated, supernatants were transferred into centrifuge vials and solvent was added to a final volume of 8 ml. Copepods from the St. Lawrence estuary were treated in a similar way, however, prior to each sonication step copepod samples were decanted into Potter-Elvehjem glass vials, filled up with solvent to a final volume of 4 ml and homogenised with a teflon pestle at 1,200 rpm for 2 min (B. Braun Biotech International, Potter S) as described in Hagen (2000). Then, supernatants were decanted into centrifuge vials and homogenisation and sonication was repeated. The final volume in the centrifuge vials was adjusted to 8 ml.

To the centrifuge vials 2 ml of ice-cooled 0.88% KCl solution were added. After vials were vigorously shaken for 30 s, samples were centrifuged at 1,500 g and 4 °C for 10 min and stored on ice. The lower lipid-containing phase was transferred into new 4 ml glass vials and evaporated with nitrogen. Thereafter, 2 ml of solvent were added. The air in vials was replaced with nitrogen and samples were stored at -80 °C.
MATERIALS AND METHODS

Sub-samples of total lipids were used for the hydrolysis of lipids in fatty acids and their conversion into methyl ester derivates (FAMEs). This procedure was carried out as described in Kattner and Fricke (1986). A defined volume of lipid extract was placed in centrifuge vials and evaporated with nitrogen. After adding 250 μl hexane and 1 ml methanol containing 3% concentrated sulphuric acid (3% H₂SO₄ MeOH) and replacing air in vials with nitrogen centrifuge vials were heated at 80°C for 4 h. Then, 2 ml H₂O and 1 ml hexane were added, samples were shaken over 30 s and centrifuged at 1,500 g and 4°C over 10 min. The upper phase was transferred afterwards into new 1.5 ml vials and evaporated with nitrogen. FAMEs were extracted again two times with 1 ml hexane. Finally, after evaporation, approximately 500 to 1000 μl hexane were added in vials, air was replaced with nitrogen and samples were stored at -80°C until measurements.

Samples were analysed with a gas chromatograph (HP 6890A), modified from Kattner and Fricke (1986) as described in Peters et al. (2006). The gas chromatograph was equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter and 0.25 μm film thickness) operating with a temperature programme. Samples were injected with a programmable temperature vaporizer injector (Gerstel®CIS3). The FAMEs and fatty alcohols were detected by flame ionisation. Identification of single fatty acids and fatty alcohols resulted from comparing their retention times with retention times obtained from the standard marinol.

2.2.4 ENZYME ANALYSIS

The digestive enzyme activities of Temora longicornis, Acartia clausi, Calanus finmarchicus, Metridia longa, C. glacialis and C. hyperboreus females were measured as activity of proteinases (endopeptidases) and amylase. For the measurement of the metabolic activity two key enzymes were measured, (1) pyruvate kinase, a component of the glycolysis, and (2) the citrate synthase, a component of the citrate cycle. All enzymatic samples and controls were measured photometrically in triplicates.

2.2.4.1 DIGESTIVE ENZYME ACTIVITIES

PROTEINASE AND AMYLASE ACTIVITIES

Total proteinase (EC 3.4.21-24) activity was determined after Saborowski et al. (2004). Originally, this method was used to determine proteinase activity in gastric fluid of the marine crab Cancer pagurus. Copepods are much smaller in size and thus provide, even if large amounts of copepods are available, relatively little biomass and enzymatic active tissue.
Moreover, one goal was to measure proteinase and amylase activity in the same sample in triplicates, and therefore this method had to be modified (Table 1).

Table 1: Modification of the methods for the measurements of proteinase and amylase activities, using different cuvettes. *substrate for proteinase: azocasein, for amylase: starch-RBB. **stopping reagent for proteinase: 8% TCA, for amylase: 1 mol l\(^{-1}\) HCl.

<table>
<thead>
<tr>
<th>cuvettes</th>
<th>proteinase activity</th>
<th>amylase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>homogenate (μl)</td>
<td>100</td>
<td>198</td>
</tr>
<tr>
<td>preincubation</td>
<td>5 min, 30°C</td>
<td>5 min, 30°C</td>
</tr>
<tr>
<td>substrate* (μl)</td>
<td>25</td>
<td>88</td>
</tr>
<tr>
<td>incubation</td>
<td>60 min, 30°C</td>
<td>60 min, 30°C</td>
</tr>
<tr>
<td>stopping reagent** (μl)</td>
<td>250</td>
<td>88</td>
</tr>
<tr>
<td>centrifugation</td>
<td>15000 g, 4°C, 15 min</td>
<td>15000 g, 4°C, 15 min</td>
</tr>
<tr>
<td>measure at 366 nm</td>
<td>measure at 600 nm</td>
<td></td>
</tr>
<tr>
<td>total volume (μl)</td>
<td>375</td>
<td>374</td>
</tr>
</tbody>
</table>

In preliminary measurements, deep-frozen copepod samples containing ten females each were homogenized with a micro pestle in 1 ml ice-cold 0.1 mol l\(^{-1}\) Tris/HCl-buffer (supplemented with 10 mmol l\(^{-1}\) CaCl\(_2\), adjusted to pH 7.0) and shortly centrifuged at 15,000 g (4°C). The supernatants were transferred into new reaction tubes and kept on ice. Proteinase and amylase activities were then measured in micro-cuvettes (700 μl volume). However, activities in copepods were low and often under limits of detection. Therefore, buffer volume was reduced to 200 μl, resulting in clear activities. Such small volume, however, allowed only two parallels of a single enzyme to be measured in one sample. To account for the small volumes of homogenised samples, substrates and stopping reagents per measurement were reduced to approximately 20 and 18% for proteinase and amylase, respectively, and ultra micro-cuvettes (50 μl volume) were used (Table 1). This enabled to perform measurements of proteinase and amylase activities in triplicates within a single sample. The final procedure for measuring proteinase activities was as follows: 20 μl of sample (20 μl of buffer for the controls) were incubated in 1.5 ml reaction tubes for 5 min at 30°C. 5 μl azocasein (1% in aqua dem., Fluka 11615) were added and incubated for 60 min at 30°C. Reactions were stopped with 50 μl of 8% TCA (trichloroacetic acid, 8% in aqua dem.) cooled on ice. Immediately after stopping the reaction, the samples were centrifuged at 15,000 g (4°C) for 15 min. Supernatants of samples and controls were then measured photometrically at 366 nm. Amylase activities were measured with the substrate starch-RBB (Loewe Biochemica GmbH, 4 mg ml\(^{-1}\) solution). Incubation, stopping of reactions and centrifugation were carried out as described for the total proteinase activities, however, with different volumes. Here, 36 μl of sample (36 μl of buffer for the controls, respectively) and 16 μl of starch were used. Reactions were stopped with 16 μl of 1
mol l\(^{-1}\) HCl. Supernatants of both samples and controls were measured photometrically at 600 nm after centrifugation.

**QUANTITATIVE LIPASE/ESTERASE ACTIVITIES**

The lipolytic activity in *Temora longicornis* was documented fluorometrically by measuring the activity of lipases/esterases (Carboxylic ester hydrolases (E.C. 3.1.1)) in individual females as described by Knotz et al. (2006). For the measurement of the lipase/esterase activity the substrate 4-Methylumbelliferyl butyrate (Fluka BioChemika, 19362) was used. Stock solution of substrate was prepared in ethylene glycol monomethylether (5 mmol l\(^{-1}\)). Standard curves were prepared with 4-Methylumbelliferone (Sigma, M1381) in ethylene glycol monomethylether, as well. All samples and controls were measured in triplicates. Deep-frozen individuals were homogenized in 200 µl ice-cold 0.1 mol l\(^{-1}\) Tris/HCl-buffer (adjusted to pH 7.0) with a micro pestle and centrifuged for 10 min at 15,000 g (4°C). Thereafter, the supernatants were transferred into new microtubes and kept on ice until measurements. Assays were run at 25°C with 10, 20, 30 or 40 µl of sample and 10 µl substrate (5 mmol l\(^{-1}\)) ad 500 µl Tris/HCl-buffer. Substrate concentrations in the assays were 100 µmol l\(^{-1}\). Fluorescence was measured after 30 min of incubation at 25°C at 360 nm (excitation) and 450 nm (emission) with a Kontron SFM 25 device. Controls were run in triplicates. The rate of autolysis was tested and subtracted from the assay-results.

**QUALITATIVE LIPASE/ESTERASE ACTIVITIES**

For the electrophoretic separation of the proteins a discontinuous sodiumdodecylsulfate-polyacrylamideggelectrophoresis (SDS-PAGE) was applied after Laemmli (1970). In order to obtain enough enzymatic activity of small copepods on gels, the homogenisation procedure had to be adapted. The method developed by Laemmli has been applied successfully on decapods in the past. Originally, gastric fluid or homogenates of whole hepatopancreas of decapods were transferred into reaction tubes, centrifuged and aliquots with the supernatants were frozen at -80°C (García-Carreño et al. 1993, Saborowski et al. 2004). Enzyme extracts were then diluted 1:1 v/v in sample buffer and applied on gels. However, in order to obtain clear enzymatic bands, single copepods had to be homogenised in the smallest volume possible. Therefore, individual females were homogenised with a micro pestle in 15 µl demineralised water and 15 µl sample buffer (25% stacking gel buffer (0.5 mol l\(^{-1}\) Tris/HCl, adjusted to pH 6.8), 20% bromphenol blue, 30% glycerine, 4% sodium dodecyl sulfate, 21% demineralised water) and centrifuged for 10 min at 15,000 g and 4°C. Supernatants were transferred into new microtubes and kept on ice until applying 10 µl to mini-gels (8 cm x 10 cm x 0.75 mm). By homogenisation of copepods in demineralised water and sample buffer, the
enzyme concentration in samples increases compared to the method described by Garcia-Carreño et al. (1993). Therefore, it was possible to run two gels with sub-samples of single individuals. As molecular marker the SigmaMarker Wide Molecular Weight Range (Sigma, M4038) was used. The mini-gels consisted of a stacking gel (0.5 mol l⁻¹ Tris/HCl, adjusted to pH 6.8; T=4% total acryl amide and bisacryl amide concentration and C=2.6% cross linker concentration), and a separation gel (1.5 mol l⁻¹ Tris/HCl buffer, adjusted to pH 8.8; T=12.3% and C=2.6%) (Laemmli 1970, Schägger and von Jagow 1987). A 0.025 mol l⁻¹ Tris/HCl buffer with 0.192 mol l⁻¹ glycine and 0.1% SDS, adjusted to pH 8.3, was used as electrode buffer. Two gels with the sub-samples were run at the same time in a cooled mini vertical electrophoresis unit (Hoefer, SE 250 Mighty Small II) for approximately 90 min at maximum 300 V, 15 mA and approximately 2°C. After the run, lipolytic active bands on the gels were made visible as described in Díaz et al. (1999). Gels were gently washed with demineralised water and incubated in a washing solution 2.5% Triton X 100® for 30 min at room temperature. Afterwards the gels were washed briefly in 50 mmol l⁻¹ phosphate buffer (pH 7.0) and subsequently incubated 5 to 10 min in a 100 μmol l⁻¹ MUF-butyrate solution (dilated in 50 mmol l⁻¹ phosphate buffer, pH 7.0). Then images were taken with the Molecular Imager ChemiDoc XRS System (Bio-Rad) after short UV illumination. Images were analysed with the Quantity One 1-D Analysis Software (Bio-Rad).

Gels were then washed briefly with aqua dem. for subsequent protein silver staining. Additional protein staining was required to make visible molecular marker bands on the gel in order to calculate molecular weights of identified lipases/esterases bands. The PlusOne Silver Staining Kit (GE Healthcare, 17-1150-01) was used. During all steps, gels were gently shaken on a staining tray. Gels were soaked in fixing solution (75 ml ethanol, 25 ml glacial acetic acid, ad 250 ml aqua dem.) for 60 min. After removing fixing solution, sensitising solution (75 ml ethanol, 10 ml sodium thiosulfate (5% w/v), 17 g sodium acetate, 1.25 ml glutardialdehyde (25% w/v), ad 250 ml aqua dem.) was added and gels were sensitised over 60 min. Then, gels were washed four times over 15 min with aqua dem. and placed in silver solution over 60 min (25 ml silver nitrate solution (2.5% w/v), ad 250 ml aqua dem., 0.1 formaldehyde (37% w/v)). Thereafter, gels were washed four times with aqua dem. for 1 min to remove silver solution, and developed over four to six min (developing solution: 6.25 g sodium carbonate, ad 250 ml aqua dem., 0.2 ml formaldehyde (37% w/v)). Then, gels were transferred immediately into stop solution (3.65 g EDTA-Na₂•2H₂O, ad 250 ml aqua dem.) and kept therein for 60 min. Gels were soaked in preserving solution (75 ml ethanol, 11.5 ml glycerol (87% w/w), ad 250 ml aqua dem.) over 60 min. Thereafter, images of silver stained gels were taken as described above for lipase gels, but with transmission light.
2.2.4.2 METABOLIC ENZYME ACTIVITIES

Homogenisation of copepod samples was performed as described in Saborowski and Buchholz (2002), except that 200 μl buffer were used instead of 1 ml, since metabolic activities of copepods were low and near limits of detection when using 1 ml buffer. Using 200 μl higher metabolic activities were achieved and facilitated to measure both metabolic enzyme activities within one sample in triplicates. Deep-frozen samples were homogenized in ice-cold 50 mmol l⁻¹ Tris/HCl-buffer (adjusted to pH 7.5) with a micro pestle and shortly centrifuged at 15,000 g (4°C). The supernatants were transferred into new reaction tubes and kept on ice until measurements.

CITRATE SYNTHASE ACTIVITY

Citrate synthase activity (CS, EC 4.1.3.7) was determined with modifications after Stitt (1984) as described in Saborowski and Buchholz (2002). For the activity measurements, 20 μl DTNB (5,5’-Dithiobis(2-nitrobenzoic acid), 6 mmol l⁻¹ in buffer) (Sigma Aldrich), 20 μl Acetyl-CoA (Acetyl-Coenzyme A trilithium salt, 6 mmol l⁻¹, Roche diagnostics) and 20 μl sample were placed in a cuvette and blended with 520 μl 50 mmol l⁻¹ Tris/HCl-buffer (supplemented with 100 mmol l⁻¹ KCl and 1 mmol l⁻¹ EDTA, adjusted to pH 7.5). After 5 min of incubation at 25°C, 20 μl oxalacetic acid (12 mmol l⁻¹, Sigma Aldrich) were added to start the reaction. The increase in the absorbance was measured continuously for 180 s (25°C) at 405 nm.

PYRUVATE KINASE ACTIVITY

Pyruvate kinase activity (PK, EC 2.7.1.40) was determined after Saborowski and Buchholz (2002). For the pyruvate kinase activity measurements, 20 μl NADH (β-NADH reduced disodium salt hydrate, 7 mmol l⁻¹, Sigma Aldrich), 20 μl PEP (Phospho(enol)pyruvic acid (tricyclo-hexylammonium) salt, 16 mmol l⁻¹, Sigma Aldrich), 20 μl LDH (Lactate dehydrogenase, 10 U, Roche Diagnostics) and 20 μl sample were placed in a cuvette and mixed with 500 μl 50 mmol l⁻¹ Tris/HCl-buffer (supplemented with 60 mmol l⁻¹ KCl and 4 mmol l⁻¹ MgSO₄, adjusted to pH 7.5). After 5 min of incubation at 25°C, 20 μl ADP (Adenosine diphosphate, 17 mmol l⁻¹, Roche Diagnostics) were added to start the reaction. The decrease in absorbance was measured continuously for 180 s (25°C) at 340 nm.

2.2.5 STATISTICS

An arc sine square root transformation was performed for statistical operations that require normal distribution. Data sets were tested for normal distribution using the Kolmogorov-
Smirnov or Shapiro-Wilks test and for variance homogeneity with the Levene’s test. For normally distributed data showing variance homogeneity an ANOVA was performed followed by the Holm-Sidak post-hoc test or the Tukey HSD post-hoc test. A Kruskal-Wallis ANOVA was used for not normally distributed data sets, followed by a Tukey post hoc test. In case of unequal treatment group sizes a Dunn’s post hoc test was performed. A Pearson’s correlation for normally distributed data or Spearman’s rank correlation for not normally distributed data were used. The data were analysed with the programme GraphPad Prism version 3.00 (GraphPad Software, San Diego, California) or SigmaStat 3.5 (Systat Software, Inc.).
3 RESULTS AND SYNOPTIC DISCUSSION

This thesis presents a comparative study on the physiological adaptations of dominant copepod species from the southern North Sea and from the lower St. Lawrence estuary to changing nutritional conditions in the field as well as under experimental conditions. In the following chapter, biochemical and physiological parameters of the copepods are compared to elucidate differences in the copepods’ life strategies and their physiological adaptive potential (section 3.1). Emphasis is also placed on fatty acid biomarkers and their applicability in calanoid copepod species from the North Sea (section 3.2).

3.1 IMPLICATIONS OF THE PHYSIOLOGICAL ADAPTIVE POTENTIAL ON LIFE STRATEGIES

This chapter aims at comparing biochemical, metabolic and digestive characteristics of copepods with different life strategies from the North Sea, i.e. *Temora longicornis*, *Acartia clausi* and *Centropages typicus*, and from the lower St. Lawrence estuary, i.e. *Calanus finmarchicus* and *Metridia longa*, and their physiological response to changing nutritional conditions.

3.1.1 BIOCHEMICAL AND METABOLIC CHARACTERISTICS OF COPEPODS

Copepods with different life strategies display differences in their biochemical composition, such as lipid and protein content (e.g. Båmstedt 1986, Lee et al. 2006), and in their physiological properties like metabolic activities and biomass turnover rates (e.g. Dagg 1977, Båmstedt 1986, Mayzaud et al. 1992, Thor 2000). These differences may be related to the copepods’ adaptive potential to changing nutritional conditions. However, detailed comparative studies on species with different life cycle strategies and feeding behaviour have not been carried out yet. Therefore, the following discussion aims at (i) comparing biochemical and physiological characteristics of five copepod species and (ii) relating these characteristics to life cycle strategies.

The data were obtained during a spring (*T. longicornis*, *A. clausi*, publications I and IV, Hansen 2006, Kreibich et al. unpublished data) and during an autumn phytoplankton bloom (*C. typicus*, Brüll 2007, Ohlf 2007) in the southern North Sea and a spring bloom in the lower St. Lawrence estuary (*C. finmarchicus*, *M. longa*, publication IV).
3.1.1.1 LIPID AND PROTEIN CONTENTS OF COPEPODS

The copepod species differed strongly in size. Thus, lipid and protein content are presented as % of dry mass (% DM) in order to allow direct comparison irrespective of size (Fig. 3). The temperate/boreal species *T. longicornis* and *C. typicus* are characterised by low lipid contents, whereas the North Atlantic *C. finmarchicus* and the Arctic *M. longa* show higher contents (Fig. 3). Lipid content in *A. clausi* was not determined in this thesis, but, according to Kattner et al. (1981), this species is characterised by similar lipid contents like *T. longicornis*. Since lipids have a much higher mass-specific energetic content than proteins (e.g. Lee et al. 2006 and references therein), lipids are very efficient energy depots. Copepods like *C. finmarchicus* and *M. longa* which are exposed to food scarcity over several months accumulate large amounts of lipids in order to cover their energetic demands. During the field campaign 2006 in the lower St. Lawrence estuary, *C. finmarchicus* and *M. longa* accordingly reached lipid contents of up to 30% DM whereas the lipid content of copepods sampled off Helgoland did not exceed 9% DM (publications I and IV, Brüll 2007, Ohlf 2007).

Typically, the energy reserves of copepod species from higher latitudes are accumulated and stored as wax esters (WE). This lipid class is commonly found in e.g. *C. finmarchicus*, *C. glacialis*, *C. hyperboreus* and *M. longa* from the Arctic, as well as in e.g. *Calanoides acutus*, *Rhincalanus gigas*, *M. gerlachei* and *Euchaeta antarctica* from the Antarctic region (e.g. Lee et al. 1970, 1971, Hagen 1988, Sargent and Falk-Petersen 1988, Kattner and Hagen 1995, Albers et al. 1996, Hagen and Schnack-Schiel 1996). Within this thesis, WE content was not quantified. However, fatty alcohols were detected in samples of *C. finmarchicus* and *M. longa*, which indicate the presence of WE (publication IV). This lipid class consists of simple esters of long-chain primary alcohols and long-chain fatty acids (e.g. Lee et al. 2006).

In contrast, copepods with low energy reserves like *T. longicornis*, *A. clausi*, *C. typicus* and *C. hamatus* show low WE contents or even lack this lipid class (e.g. Kattner et al. 1981). This was confirmed in our studies by the lack of fatty alcohols in the female copepods (publications I, II and III, Brüll 2007, Ohlf 2007). In these species mainly triacylglycerols (TAG) act as lipid energy stores which are accumulated when food availability is high like during phytoplankton blooms (Kattner et al. 1981). Hence, the increasing lipid content from 2% to 9% DM of *T. longicornis* females during the spring bloom 2005 off Helgoland points to the accumulation of TAG (publication I).

In calanoid copepods which are characterised by lower lipid contents and low starvation tolerance, proteins play a major role as energetic compounds, since proteins are easier to metabolise and, thus, quickly provide energy to the organism (e.g. Wieser 1986, Schmidt-Nielsen 1997). Accordingly, *Acartia tonsa*, also known to be unable to starve over prolonged
RESULTS AND SYNOPTIC DISCUSSION

periods (Dagg 1977), maximises protein ingestion by selective feeding behaviour (Houde and Roman 1987, Cowles et al. 1988). In general, copepods from temperate regions living in the surface layer are characterised by higher protein contents than species from deeper layers or higher latitudes (reviewed by Båmstedt 1986). This is in accordance with the present results showing higher water-soluble protein content in *T. longicornis* (25% DM) and *A. clausi* (39% DM) compared to *C. finmarchicus* and *M. longa* (both 14% DM) (Fig. 3 b). Yet, females of *C. typicus* were characterised by a low and constant protein content of 15% DM during the autumn field campaign 2006 (Fig. 3, Ohlf 2007). During this time *C. typicus* females apparently invested the available energy directly into reproductive processes (see chapter 3.1.1.2) and, thus, did not accumulate internal energy stores. In contrast, water-soluble protein content varied strongly in *T. longicornis* and *A. clausi* during the spring phytoplankton bloom in the North Sea (15-60% DM) (Fig. 3, publication I, Kreibich et al. unpublished data). During this time seston composition changed from primarily detrital to primarily autotrophic which was indicated by increasing chlorophyll *a* and lipid contents and both decreasing C:N ratio and the percentage of the fatty acid 18:0 (publication I). Levels of saturated FAs are usually elevated in detritus, since they are more stable to biological and chemical remineralisation processes than unsaturated fatty acids (e.g. Kattner et al. 1983). In addition, food availability increased during the time series indicated by increasing phytoplankton standing stock (publication V). The good nutritional conditions entailed the accumulation of storage products during the second half of the time series. Predominantly proteins were stored in *T. longicornis*, increasing the concentration from 15 to 40% DM, whereas the lipid contents reached only to 9% DM at maximum.

![Fig. 3: Lipid content (a) and water-soluble protein content (b) in % of dry mass (DM) of *T. longicornis*, *A. clausi*, *C. typicus*, *C. finmarchicus* and *M. longa* (lipid content: each data point n=1; protein content: each data point n=3). Protein contents were calculated per mean value dry mass of the corresponding sampling date. Lipid content of *A. clausi* was not determined.](image-url)
KEY RESULTS

- *Temora longicornis* and *Centropages typicus*, both species with short generation times producing up to five generations per year, are characterised by low potentials to store lipids. The protein contents in *T. longicornis* and *Acartia clausi*, also a multi-voltine species, vary greatly (15-60% of dry mass) and both species are strongly influenced by the nutritive conditions, which they encounter in their habitats. In *T. longicornis* primarily proteins are accumulated when feeding conditions are favourable while lipid accumulation follows secondarily. *C. typicus* females are characterised by low and constant protein contents and apparently invest the available energy strait into reproductive processes.

- *Calanus finmarchicus* and *Metridia longa* are well adapted to the seasonal phytoplankton cycle in higher latitudes and they are characterised by long generation times. Both species strongly accumulate lipids under good feeding conditions, indicated by the strong increase of storage lipids from 7 to 30% of dry mass. Protein contents remained low and constant throughout phytoplankton bloom indicating, that proteins play only a minor role as energy reserves in both species.

3.1.1.2 METABOLIC ACTIVITY OF COPEPODS

The species from the North Sea and the lower St. Lawrence estuary were characterised by differences in their lipid and protein content, as well as in the accumulation of these macromolecules as energy reserves during favourable feeding conditions (see section 3.1.1.1). Thus, the question arises whether the accumulation of different macromolecules may have an effect on the metabolic activity of copepods. Information on the metabolic activity of an organism can be obtained by measuring respiration rates (e.g. Corner 1961, Conover and Corner 1968) or indirectly by analysing the activities of metabolic key enzyme systems such as the mitochondrial respiratory electron transport systems (ETS) or single enzymes, like pyruvate kinase or citrate synthase (e.g. Owens and King 1975, Stitt 1984, Vetter and Buchholz 1997). In this thesis the latter enzymes, i.e. pyruvate kinase (PK) and citrate synthase (CS) were investigated. PK activity displays the anaerobic metabolic potential (e.g. Thuesen and Childress 1993) whereas CS activity is an indicator for the aerobic potential of an organism, closely correlating with its oxygen consumption rates (Childress and Somero 1979). Metabolic activity corresponds to the size of an organism, and thus as in other taxa, respiration rate and dry mass in copepods correlate significantly (e.g. Conover and Corner 1968). Since the copepod species investigated in this thesis differ strongly in size, the enzyme activities are presented as activity per unit dry mass (specific activities, units mg\textsubscript{DM}\textsuperscript{-1}) in order to correct for differences in body mass (Figs. 4 a and b).
3.1.1.2.1 DIFFERENCES IN SPECIFIC METABOLIC ACTIVITIES

Specific PK and CS activities of *Acartia clausi*, *Temora longicornis* and *Centropages typicus* were in general higher than those of *Calanus finmarchicus* and *Metridia longa*. These results are consistent with other studies which show that small calanoid copepods like *A. clausi* and *A. tonsa* are characterised by high metabolic activities and high biomass turnover rates (e.g. Heinle 1966 cited in Conover and Corner 1968, Båmstedt 1986, Mayzaud et al. 1992). However, metabolic activities may be influenced by other factors including accumulation of storage products, the amount of lipid accumulated, allometric constraints, environmental temperatures, and the reproductive activity.

Moreover, different traits of energy accumulation may influence the metabolic activities as well. As suggested by Thor (2000), who studied *A. tonsa* copepodite stage V (CV) and *C.*
**Results and Synoptic Discussion**

finmarchicus CV, the deposition of proteins causes higher respiration rates than the deposition of lipids. This may also explain elevated metabolic activities in A. clausi, T. longicornis and C. typicus, and lower activities in C. finmarchicus and M. longa because the former species primarily accumulate proteins under good feeding conditions while the latter species predominantly store lipids (see section 3.1.1.1).

The different lipid contents of copepods may affect the specific metabolic activities as well. Storage lipids can be accumulated in oil sacs which can occupy large parts of the body cavity as in many copepod species from higher latitudes (e.g. Calanus spp.) (Lee et al. 2006 and references therein). The accumulated lipids in these oil sacs contribute to an increase of total dry mass, but not to an increase of metabolically active tissue. Thus, high lipid contents consequently reduce the specific metabolic activity, i.e. the activity per unit body mass decreases. In order to test whether differences in lipid content affect the specific metabolic activity of the presented species, lipid contents were subtracted from dry mass and specific metabolic activities were calculated. The results show, however, that the differences in lipid contents had only minor effects on the specific metabolic activities of copepods (Figs. 4 c and d).

For vertebrate animals it is established that the specific oxygen consumption rate per unit body mass decreases when the total body mass increases (e.g. Schmidt-Nielsen 1997). This effect is denoted as “allometric scaling” and, thus, it seems possible that differences between the copepod species can be ascribed to different body masses rather than to differences in the type of storage compounds.

Furthermore, the ambient temperature has a direct effect on the CS activity since higher temperatures cause higher respiration rates in organisms (e.g. Mauchline 1998 and references therein). During the field campaigns temperature ranged between 1 and 8°C (time series lower St. Lawrence estuary, C. finmarchicus and M. longa), 3 and 10°C (spring phytoplankton bloom North Sea, T. longicornis and A. clausi) and between 14 and 20°C (autumn phytoplankton bloom North Sea, C. typicus) (publications I and IV, Hansen 2006, Ohlf 2007). All measurements were performed according to the same protocol with identical parameters (e.g. temperature, pH, incubation times), thus, differences due to methodological discrepancies can be excluded.
RESULTS AND SYNOPTIC DISCUSSION

Fig. 5: Egg production rate (EPR, mean value, n=36-48) and citrate synthase (CS, units·10⁻² ind⁻¹, mean value, n=3) activity and specific CS activity (units mg DM⁻¹, mean value, n=3) of *T. longicornis*, *A. clausi*, *C. typicus*, *C. finmarchicus* and *M. longa* during the time series.

Finally, the differences observed in specific metabolic activity may originate from differences in the copepods’ reproductive activity. In times of higher energy demand, e.g. during reproduction, the metabolic activity should increase to provide additional energy in terms of ATP to cover the demand of the female, as shown for *T. longicornis* during the spring bloom 2005 (publication I). However, in all five copepod species EPR increased during the field campaigns as CS activity did (Fig. 5). The maximum EPRs were measured for *C. finmarchicus* (76 eggs female⁻¹ d⁻¹) and *C. typicus* (65 eggs female⁻¹ d⁻¹). However, when comparing the different species according to their specific CS activity, highest activity did not correspond with
highest EPR. The species with highest specific CS activity, *A. clausi* (Figs. 4 and 5), produced almost 25 eggs female\(^{-1}\) d\(^{-1}\) while *C. finmarchicus*, which produced at maximum 80 eggs female\(^{-1}\) d\(^{-1}\) showed lowest CS activities, mostly below 2 units mg\(_{DM}\)^{-1}. It can therefore be suggested that denoted differences in the specific metabolic activity do not originate from differences in reproductive activity. When interpreting the data, it should be considered that the data set for *A. clausi* comprises only a small number of values, compared to the other species.

In summary, the copepods from the North Sea reached in general higher specific metabolic activities than the copepods from the St. Lawrence estuary. These differences do not originate from differences in reproductive activities. Highest EPR but lowest specific CS activities were observed in *C. finmarchicus*, whereas *A. clausi* showed highest specific CS activities but lowest EPR. These results suggest that mainly differences in the accumulation of energetic compounds as well as differences in biomass turnover rates influence the specific metabolic activities of the copepods.

### 3.1.1.2.2 RESPONSE TO CHANGING NUTRITIONAL CONDITIONS

During our field campaigns, all copepods species showed an increase in metabolic activities and egg production rates under favourable conditions (publications I and IV, Ohlf 2007, see section 3.1.2). Nevertheless, we can hypothesize that, due to differences in metabolic requirements, biomass turnover rates and energy reserves, *Temora longicornis*, *Acartia clausi* and *Centropages typicus* may react differently from *Calanus finmarchicus* and *Metridia longa* to changing nutritional conditions, particularly to starvation. In order to test this hypothesis, several feeding and starvation experiments were conducted with *T. longicornis* (publications II and III), *A. clausi* (Hansen 2006, Kreibich et al. unpublished data), *C. typicus* (Brüll 2007) and *C. finmarchicus* (Kreibich and Niehoff unpublished data). In addition, feeding and starvation experiments were performed with *C. glacialis* and *C. hyperboreus*, which were sampled in the Arctic at the end of diapause, in order to investigate the metabolic responses of these species to changing nutritional conditions (Niehoff et al. unpublished data).

Previous studies have shown that the respiration rate of copepods decreases considerably during starvation (e.g. Ikeda 1977, Abou Debs 1984), e.g. in *Acartia tonsa* by approximately 50% within 10-20 h (Mayzaud 1976, Kierboe et al. 1985). Simultaneously, the activity of the electron transport system (ETS) decreased in *A. tonsa*, however, more slowly than respiration rates did (Båmstedt 1980).
Metabolic activities of *T. longicornis*, *A. clausi* and *C. typicus* decreased strongly under starvation (Fig. 6, publications II and III, Brüll 2007, Kreibich et al. unpublished data). Citrate synthase activity declined within 24 hours, similar to the findings of Clarke and Walsh (1993) for *T. longicornis* and *A. tonsa*. This strong reduction of the capacity of aerobic energy generation in the copepods from the North Sea must be attributed to scarcity of dietary substrates and to low energy reserves. Fast reduction of the metabolic activity down to basal metabolic rates prolongs the survival time under starvation (e.g. Tsuda 1994), which amounts to only a few days in these species (e.g. Dagg 1977). The degree of CS activity reduction differed in *T. longicornis*, *A. clausi* and *C. typicus* and ranged between 10% (*T. longicornis*, *C. typicus*) and 30% after 24 h (*A. clausi*). These values fall within the range of a previous study (-15% in *T. longicornis* and -40% in *A. tonsa*, Clarke and Walsh 1993). The degree of CS reduction depends on the initial activity of copepods from the field, i.e. highest initial activities result in strongest decreases. Thus, *T. longicornis* and *C. typicus* females, which showed specific CS activities of approximately 2.5 units mgDM$^{-1}$, were characterised by a lower decrease in % of the initial activity than *A. clausi* females, which had higher specific activities of approximately 3.5 units mgDM$^{-1}$ (Brüll 2007, Kreibich et al. unpublished data).

The decreasing PK activity in *T. longicornis*, *A. clausi* and *C. typicus* levelled out after 24-48 h of starvation, suggesting that the basal activity was reached (Fig. 6 b). This activity seems to display the minimum activity needed in order to sustain vital physiological processes during starvation. *A. clausi* and *T. longicornis* obtain the energy for basal physiological processes mainly from endogenous proteins (Mayzaud 1976, Helland et al. 2003). However, strong losses of lipids have been reported as well. For example, starving *T. longicornis* had lost approximately 50% of total lipids after 64 h (Evjemo 2001 cited in Helland et al. 2003). In this thesis, strong losses of lipids as well as reduction of protein content in starving *T. longicornis* were observed. It seems likely that those storage products are utilised most rapidly which form the highest share in the copepods (publication III). Starving *T. longicornis* females with low initial lipid contents catabolised both endogenous proteins and lipids, which was reflected in the overall decrease in dry mass by 28% within three days. In contrast, females with high initial lipid contents in the field showed a decrease of 28% solely in lipids after three days of starvation, while the protein content remained unaltered. It seems likely that primarily lipids are used to fuel basal physiological processes during starvation when copepods accumulated lipid reserves prior to starvation. In times of good feeding conditions *T. longicornis* store triacylglycerols (TAG) (see section 1.3.1) as energy reserves, albeit not as extensively as copepods from polar regions. TAG are primarily short-term reserves (see section 1.3.1) and, thus, are utilised rapidly upon starvation. After the depletion of the lipid reserves, starving copepods catabolise progressively endogenous proteins.
RESULTS AND SYNOPTIC DISCUSSION

Fig. 6: Citrate synthase (CS) (a) and pyruvate kinase activities (PK) (b) in % of initial activities of *T. longicornis*, *A. clausi*, *C. typicus* and *C. finmarchicus* under starvation over several days (mean values, *n*=3, except for *T. longicornis*: mean value of three experiments with *n*=3 each).

Starvation had also an influence on the EPR of *T. longicornis*, *A. clausi* and *C. typicus*, (publications II and III, Hansen 2006, Brüll 2007). In *T. longicornis*, EPR decreased to <15 eggs female⁻¹d⁻¹ within 24 h, independent of the initial rate, which ranged between 25 and 38 eggs female⁻¹d⁻¹. In *A. clausi* strongest decrease also occurred during the first 24 h, from approximately 6 eggs female⁻¹d⁻¹ to 2 eggs female⁻¹d⁻¹. However, the low initial EPR in *A. clausi* may indicate that these females were already food limited in the field. The EPR in *C. typicus* females was reduced from 45 to 15 eggs female⁻¹d⁻¹ within 48 hours. This shows that the species are unable to sustain higher EPR in periods of food scarcity, but continue to release low numbers of eggs. Here, internal body reserves support the release of eggs and it is suggested that the haemolymph may provide internal nutrients as a short-term nutrient pool (see review in Niehoff 2007).

When feeding *T. longicornis* with *Rhodomonas baltica*, *Thalassiosira weissflogii* or *Oxyrrhis marina ad libitum* for three days, no significant effects on metabolic activities of females were detected, indicating that females maintained metabolic capacities similar to those of females from the field (publications II and III) even though, the EPR increased within 24 h. Unaltered metabolic activities and simultaneously increasing EPR may indicate that the quality of the algae was higher than that of the food the females encountered in the field, i.e. that the females in the experiments had to invest proportionally less energy for the production of the same quantity of eggs. This could be achieved when e.g. diets provide biochemical components, which can be allocated to oocyte maturation without biochemical modification. The increasing EPR within 24 h shows that dietary components were rapidly digested and efficiently transformed into egg material and used to fuel metabolic processes. The conversion of diet
into egg material takes place within 24 h in *T. longicornis*, but shows high interspecific variability in copepods, ranging between 6 and up to 90 h (Smith and Hall 1980, Tester and Turner 1990). Highest EPR increase from 38 to 82 eggs female\(^{-1}\cdot d^{-1}\) within 24 h was observed when *T. longicornis* females fed on the heterotrophic dinoflagellate *O. marina*. This dinoflagellate provides high amounts of essential fatty acids, such as 20:5(\textit{n}-3) (eicosapentaenoic acid, EPA) and 22:6(\textit{n}-3) (docosahexaenoic acid, DHA), which play an important role during reproduction, hatching success and development of copepods (e.g. Støttrup and Jensen 1990, Jónasdóttir 1994, Klein Breteler et al. 1999, Hazzard and Kleppel 2003, Thor et al. 2007).

In contrast to *T. longicornis*, metabolic activities of *C. typicus* changed, when females were feeding on *R. baltica*, *T. weissflogii* and *O. marina* over three days (Brüll 2007). Metabolic activities decreased when fed with *R. baltica*, but increased or remained constant when females were fed with *T. weissflogii*. *O. marina* induced an increase in metabolic activity as well. This was also reflected in the EPR. Females produced only few eggs d\(^{-1}\) when fed with *R. baltica*, whereas EPR remained constant or increased when feeding on *T. weissflogii* and *O. marina*. *O. marina* as diet enabled *C. typicus* to increase EPR to a maximum of 111 eggs female\(^{-1}\cdot d^{-1}\) (Brüll 2007), which confirms that *O. marina* is a high quality food for copepods (Klein Breteler et al. 1999, Veloza et al. 2006). In contrast, *R. baltica* seems to be of minor quality for *C. typicus*, contradicting earlier findings on *T. longicornis*, *Acartia* spp. and *Pseudocalanus elongatus* (e.g. Jónasdóttir 1994, Jónasdóttir and Kiørboe 1996, Koski et al. 1998, Tang et al. 2001, Klein Breteler et al. 2004).

During the first 24 h of the starvation experiment over 12 days, metabolic enzyme activities in *C. finmarchicus* females decreased by approximately 15% (CS) and 20% (PK) of the initial activities. This decrease was similar to *T. longicornis*, *A. clausi* and *C. typicus* (Fig. 6). Thereafter, in contrast to the other species, metabolic activities decreased more slowly but constantly to approximately 55% of the initial activities on day 12. Preliminary results of a second starvation experiment also indicate that CS activity in *C. finmarchicus* females decreases by almost 50% after 7 days of starvation (Kreibich et al. unpublished data). This shows that *C. finmarchicus* also reduces its metabolic activity upon starvation to save energy. During starvation, *C. finmarchicus* catabolises lipids as well as proteins to provide energy to the organism. In a starvation experiment with *C. finmarchicus* CV over 56 days Mayzaud (1976) showed that this species alternates between periods of protein-dominant catabolism and lipid-dominant catabolism. The neutral lipid class used primarily as energy reserve during starvation in copepods can change from triacylglycerols to wax esters, depending on the duration of starvation. Triacylglycerols are primarily catabolised at the beginning of a starvation period, whereas wax esters are utilised when starvation continues (Lee and Barnes 1975).
During the starvation experiments with *C. finmarchicus* females for 12 and 7 days, EPR decreased from 4 to <1 eggs female\(^{-1}\) d\(^{-1}\) and from 77 to <20 eggs female\(^{-1}\) d\(^{-1}\), respectively. This corroborates earlier findings that *C. finmarchicus* females depend on external food supply in order to reproduce at high rates (e.g. Plourde and Runge 1993, Ohman and Runge 1994, Hirche et al. 1997, Plourde et al. 2001). The differences between the two experiments may be related to females’ nutritional status, which may influence the capacity to fuel reproductive processes during starvation, as suggested by Niehoff (2007). Nevertheless, the high EPR measured during the second experiment is remarkable, since it was shown that *C. finmarchicus* can only compensate periods of food scarcity for up to two days (Niehoff 2000). However, it should be considered that in our experiments females were incubated in large beakers without egg separators. Although eggs and faecal pellets were removed every day, it cannot be excluded that females were feeding on their own eggs and nauplii as *C. finmarchicus* does when food availability is low (Basedow and Tande 2006). Thus, cannibalism likely has fuelled the production of new eggs.

Metabolic activities of *C. finmarchicus* fed with high concentrations of *O. marina* for 12 days increased continuously from 20·10\(^{-2}\) units ind\(^{-1}\) on day 1 to 70·10\(^{-2}\) units ind\(^{-1}\) on day 12 (Kreibich and Niehoff unpublished data). Also EPR increased continuously from 4 eggs female\(^{-1}\) d\(^{-1}\) on day 1 to 37 eggs female\(^{-1}\) d\(^{-1}\) at the end of the experiment. This shows that *C. finmarchicus* was able to convert the offered food into egg material. In the second experiment, *C. finmarchicus* females were fed with either *Thalassiosira* sp. or *O. marina*. In contrast to the first experiment, preliminary results indicate that the metabolic activity remained constant in females during this experiment (approximately 90·10\(^{-2}\) units ind\(^{-1}\)). EPR reached similar values in both treatment groups and ranged between 60 and 70 eggs female\(^{-1}\) d\(^{-1}\), compared to 77 eggs female\(^{-1}\) d\(^{-1}\) in the field. Thus, the quality of *O. marina* and *T. weissflogii* as food were not sufficient in order to induce higher EPR, in contrast to *T. longicornis* and *C. typicus*.

The influence of the physiological state of copepods on the metabolic response to food scarcity and supply has also been in the focus of a study by Niehoff et al. (unpublished data), which investigated the metabolic response of *C. glacialis* and *C. hyperboreus* females to starvation at the end of the over-wintering period. The data show that continued starvation over three weeks does not influence the metabolic enzyme activity of the females. Starving *C. glacialis* did not start to produce eggs, and egg production of starving *C. hyperboreus* was not different from that of feeding females. Under surplus food conditions, the metabolic activity in *C. glacialis* females responded with a delay of 5 days to the initiation of feeding. This indicates that the copepods require some time to increase their metabolism at the end of diapause in presence of food. Concomitantly with the enzyme activity, EPR increased from <1 to 25 eggs female\(^{-1}\) d\(^{-1}\). Starving and fed *C. hyperboreus* females, in contrast, did not differ in their metabolic activities, thus, food supply does not induce an increase of metabolic activity in this
species at the end of the over-wintering period. The reproduction of this species is entirely fuelled by internal energy reserves (e.g. Hirche and Niehoff 1996, Niehoff 2007 and references therein).

**KEY RESULTS**

- *Temora longicornis, Acartia clausi* and *Centropages typicus* were characterised by higher metabolic activities than *Calanus finmarchicus* and *Metridia longa* due to differences in macromolecules used as energy storage and biomass turnover rates.

- *T. longicornis, A. clausi* and *C. typicus* showed distinct declines of metabolic activities within 24 h of starvation. The degree of CS activity reduction differed in dependence of the initial metabolic activities. Highest reduction in % of the initial activity was observed in copepods with highest initial activities. EPR decreased noticeably within 24 h in these species.

- In *T. longicornis*, the energy required for basal physiological processes during starvation is provided by catabolism of proteins and lipids. It seems likely that primarily lipids are used to fuel basal physiological processes during starvation. After the depletion of the lipid reserves, starving copepods catabolise endogenous proteins.

- The response of metabolic activities of *T. longicornis* and *C. typicus* differed under surplus food conditions. In *T. longicornis*, the metabolic activity remained unaltered independent of the diet. In *C. typicus*, in contrast, activities increased or decreased, depending on the diet. In general, EPR increased in both species under surplus food conditions within 24 h. However, the maximum was strongly influenced by the quality of the diet. Feeding on *R. baltica* even reduced EPR indicating that *R. baltica* is a low-quality diet for *C. typicus*.

- In times of food scarcity metabolic activity in *C. finmarchicus* decreases more slowly than in *T. longicornis, A. clausi* and *C. typicus*. A fast reduction of EPR was observed within 24 h.

- At surplus food, metabolic activities of *C. finmarchicus* increased continuously when females were characterised by low activities in the field, as did the EPR. Metabolic activities remained unaltered when initial activities were high. Here, surplus food conditions did not induce an increase of EPR, in spite of good food quality.

- Starvation over three weeks did not influence metabolic activities of *C. glacialis* and *C. hyperboreus*, which were sampled at the end of their diapause. Under surplus food conditions, metabolic activity in *C. glacialis* females increased after a time lag of 5 days, as did
EPR. In *C. hyperboreus*, in contrast, food supply did not induce an increase of the metabolic activity or in EPR.

### 3.1.2 Digestive Activity of Copepods

Digestion is the physiological key-process linking ingestion and assimilation in heterotrophic organisms. It provides the organism with energy and essential compounds for metabolic processes. In contrast to tropical areas, where food supply is low but constant, copepods in temperate and higher latitudes are exposed to a high seasonal variability in food supply (e.g. Kiørboe and Nielsen 1994, Siegel et al. 2002). Theoretically, they have to adapt to these changing conditions on a physiological level, i.e. to save energy upon starvation and to utilize the food efficiently in times of high food availability for e.g. growth and reproduction or accumulation of energy stores. However, the influence of different internal factors on physiological processes involved in adaptation to changing nutritional conditions is still unclear.

The physiological processes during digestion were subject to numerous studies focussing on the influence of internal and external factors on digestive activities of calanoid copepods. However, the results were controversial and so were the conclusions concerning adaptive processes. Several authors describe positive correlations between digestive enzyme activities of either bulk zooplankton extracts or adults of single copepod species with potential food supply (e.g. Boucher and Samain 1974, Mayzaud and Conover 1975, Mayzaud and Poulet 1978, Head and Conover 1983). It has also been shown that digestive enzyme activities decrease in starving organisms (e.g. Boucher and Samain 1974, Cox and Willason 1981, publications II and III). These results suggest that digestive enzyme levels are controlled by concentration and biochemical composition of the diet. In other studies, in contrast, digestive activities did not increase with increasing food supply, or were even negatively correlated with food concentration (Hassett and Landry 1983, Head et al. 1984, Hassett and Landry 1988). It was therefore suggested that digestive enzyme activities are not related to instantaneous digestion rates (Head et al. 1984), and that the maintenance of high levels of digestive enzyme activities during periods of low food availability might occur in anticipation of future feeding opportunities (Hassett and Landry 1983).

Studies on digestive enzyme activity, however, are difficult to compare. First, the activities of different enzymes, such as trypsin, amylase, laminarinase, maltase, cellobiase, acidic and alkaline proteases were measured. Since every single enzyme has its own characteristics e.g. acting on specific substrates or being active extracellularly or intracellularly, it is difficult to derive general patterns of digestive responses to changing nutritional conditions on the basis
of single enzyme activities. In addition, the studies focused on species with different life strategies, e.g. non-migrating copepods with low energy reserves, such as *Temora longicornis* and *Acartia clausi*, and on migrating copepods with high energy reserves performing diapause, such as *Calanus finmarchicus* and *C. pacificus*. As proposed by some authors, nutrition and digestive activities may be influenced by the copepods’ life strategies (e.g. Hassett and Landry 1983, publications I and IV), by the developmental stage due to food selection (Hirche 1981), by the daytime due to feeding periodicity (e.g. Båmstedt 1988) as well as by the copepods’ metabolic requirements (Mayzaud 1986, Roche-Mayzaud et al. 1991). Additionally, the response of the digestive activity seems to be influenced by the copepods’ feeding history as well (Tande and Slagstad 1982, Mayzaud et al. 1992, publications II and III). Thus, it appears that digestive responses of copepods to changing nutritional conditions depend on several factors, which should be considered when interpreting adaptive physiological processes. A main focus of this thesis is therefore to elucidate the influence of different metabolic requirements and life strategies on physiological adaptive potentials of copepods in times of variable nutritional conditions. Therefore, the information on the biochemical and metabolic characteristics of the different species studied in this thesis (see section 3.1.1) are combined with data on feeding and digestive enzyme activity in order to better understand adaptive digestive processes.

### 3.1.2.1 Differences in Digestive Potentials due to Metabolic Requirements

Via digestion, dietary energetic components are made available to the organism, and higher metabolic activities are concomitant with elevated energy demands of organisms. Thus, the higher energy demand of the species sampled in the North Sea, *Temora longicornis* and *Acartia clausi*, is possibly covered by means of higher specific digestive potentials as compared to those of the species from the lower St. Lawrence estuary, *Calanus finmarchicus* and *Metridia longa*.

Our data show that indeed *T. longicornis* and *A. clausi* reached higher specific proteinase and amylase activities, and, thus, have higher digestive potentials (Figs. 7a and b). To account for differences in lipid content among the species, in addition specific digestive activities were calculated based on dry mass without lipids. The results show, however, that specific digestive activities in the copepods from the North Sea, i.e. *T. longicornis*, were still higher than in *C. finmarchicus* and *M. longa* (Figs. 7 c and d). This indicates that lipid content is not a major factor for explaining the differences in specific digestive potentials. It should be noticed that higher proteinase and amylase activity does not necessarily result in increasing EPR in copepod species and, thus, intraspecific differences in energy demand cannot be deduced from digestive activities.
3.1.2.2 DIGESTIVE RESPONSE TO VARIABLE NUTRITIONAL CONDITIONS

During the phytoplankton blooms in the southern North Sea and the lower St. Lawrence estuary Temora longicornis, Acartia clausi, Calanus finmarchicus and Metridia longa were exposed to variable nutritional conditions (publications I, IV and V, Hansen 2006).

T. longicornis reacted fast to changing nutritional conditions, reflected in the digestive activity (publication I). At the beginning of the time series, T. longicornis fed omnivorously. The diet consisted of lipid-poor and potentially carbohydrate-rich diatoms, as well as of eggs and nauplii (publications I and V). During this time, proteinase and amylase activities were high, indicating feeding on protein- and carbohydrate-rich items. Later in the season the diet changed towards mainly autotrophic, lipid-rich particles. When the diet changed, the digestive
activity decreased, particularly amylase activity, suggesting a shift in the enzymatic pattern. Proteinase activity recovered fast to initial activities, indicating that protein-rich components were still available during the second period of the field campaign. Amylase activity increased gradually, suggesting that polysaccharides became again increasingly available. During this second period, the lipid content of females increased and specific dietary fatty acids, mainly those for diatoms and dinoflagellates were accumulated in females’ lipids (publications I and V). These fatty acids, which cannot be synthesised de novo by the copepods or only in small amounts (e.g. Fraser et al. 1989b, Klein Breteler et al. 2004), are primarily incorporated into storage lipids of copepods, as shown for e.g. T. longicornis (Peters et al. 2007).

The results of T. longicornis show that females are able to react fast to changing nutritional conditions. This is corroborated by the results on feeding and starving experiments, conducted parallel to the field campaign 2005 in the southern North Sea (publications II and III). The response of the females to changing nutritional conditions did not follow a predetermined way and, thus, confirms the suggestions made by some authors that the response depends on the copepods’ feeding history and metabolic requirements (e.g. Tande and Slagstad 1982, Mayzaud 1986, Roche-Mayzaud et al. 1991, Mayzaud et al. 1992). Proteinase activity of copepods increased to approximately 170% within 24 h by feeding on Thalassiosira weissflogii and Oxyrrhis marina, when initial activities were low in the field (10·10^{-3} \ \mu E_{366} min^{-1} ind^{-1}). In contrast, high initial activities (21·10^{-3} \ \mu E_{366} min^{-1} ind^{-1}) resulted in slightly decreasing or constant activities when females were feeding on T. weissflogii, O. marina or Rhodomonas baltica. The decrease in proteinase activity might be explained by heterogeneous diets females encounter in the field compared to the homogeneous monocultures during the feeding experiments. In periods of heterogeneous dietary composition copepods provide a heterogeneous set of enzymes. Feeding on a monoculture may then result in a loss of the digestive heterogeneity and, thus, in a reduction of the overall digestive activity since only a small spectrum of enzymes is provided for the digestion of this specific diet. Since total proteinase activities were measured, it is not possible to discriminate between different proteinase classes as each class is characterised by a specific pH-optimum (e.g. García-Carreño 1992). In the present study, all measurements were conducted at pH 7, thus, differences in proteinase patterns may result in different total proteinase activities. Despite different feeding histories and different digestive activities in the field, T. longicornis females reached similar proteolytic activities in the experiments, when feeding on T. weissflogii. This may indicate that the digestive system adapted similarly to this monoculture by secreting similar proteinases (publication III). However, whether and to what degree proteinase patterns change due to the copepods diet certainly needs further studies. However, a first evidence for diet-specific enzyme secretion is that the lipase patterns of copepods, as made visible by means of SDS-PAGE (sodium dodecyl sulfate-polyacrylamid gel electrophoresis), changed with the diet (publication III) and, thus, similar mechanisms are possible for the proteinases.
Some experimental studies showed that the activities of digestive activities in starving animals are characterised by an initial period of stability or a small increase followed by a decrease (Boucher and Samain 1974), whereas other authors observed that the activity decreases in absence of food without an initial period of stability (Cox and Willason 1981). Mayzaud and Mayzaud (1985) showed for *Acartia clausi* that the response of the digestive activity under starving conditions depends on the investigated enzymes. In this thesis, both scenarios were observed in *T. longicornis* females by measuring the same enzyme. The response in digestive activities of copepods to starvation was strongly influenced by the initial activities that females had in the field (publication III). *T. longicornis* and *A. clausi* showed a decrease in proteinase activity within 24 to 48 h of starvation (Fig. 8) and after three days activities ranged between approximately 20 and 50% of the initial activity. Prior to the experiments, proteinase activities of *T. longicornis* females differed strongly, and ranged between 10·10^{-3} and 21·10^{-3} dE_{366} min^{-1} ind^{-1}. In females, which had highest activities in the field, activity decreased strongly to 40% of the initial activity within 24 h. In contrast, in females characterised by lower digestive activities in the field, proteinase activities remained constant during the first 24 h and decreased thereafter. The results confirm the suggestion made by Mayzaud (1986) that greater metabolic requirements and higher food levels before starvation will have greater effects on the digestive response of copepods. In addition, the observations made by Mayzaud and Mayzaud (1985), that the response depends on the investigated enzyme, cannot be supported for *T. longicornis*, since different responses of the same enzymes to changing nutritional conditions were observed.

In *T. longicornis*, proteinase and amylase activity were not correlated during our time series, indicating that the expression of these two enzymes is controlled by different factors, e.g. specific substrate availability or demand (Fig. 9). In contrast, amylase and proteinase activities were significantly correlated in *A. clausi* during the same time series (R^2=0.786). This may indicate that the ratio of both substrates, i.e. proteins and carbohydrates, was similar in the diet of *A. clausi* females. The results on *T. longicornis* and *A. clausi* may indicate that the species fed on different components of the seston. However, it should be considered that *A. clausi* was sampled sporadically during the time series and, thus, comparison of the two species’ feeding behaviours is limited.
Digestive enzyme activities of *C. finmarchicus* and *M. longa* responded in a completely different way to changing nutritional conditions in the field than those of *T. longicornis*. At the beginning of the time series (end of May), food-limited conditions prevailed, as indicated by low phytoplankton and protist abundances and low chlorophyll a content (publication IV). At the beginning of July, the main phytoplankton bloom developed, dominated by diatoms and dinoflagellates. Both copepod species fed on these components of the seston, indicated by strong accumulations of typical diatom and dinoflagellate fatty acids, 16:1(n-7), EPA, 18:4(n-3).
and DHA. *M. longa* additionally fed carnivorous, probably on *Calanus* spp. eggs and nauplii as levels of typical fatty acid biomarkers for *Calanus* spp., 22:1(n-11) and 20:1(n-9), were elevated in this species (e.g. Sargent and Henderson 1986, Kattner and Hagen 1995). Although it cannot be excluded that *M. longa* may synthesize these fatty acids *de novo* as well, the occurrence of WE in *Calanus* eggs suggests that *M. longa* was feeding on eggs during the field campaign (Ohman and Runge 1994). This is corroborated by the higher enrichment of the $\delta^{15}$N isotope in *M. longa* by approximately 1.5‰ compared to *C. finmarchicus* (publication IV). Despite of the changing nutritional conditions and the different life strategies (see 1.2.2) digestive activities of both species did not reveal a clear response to food concentration and quality. Furthermore, it is remarkable that digestive enzyme activities in both species were already high prior to the phytoplankton bloom, when food-limiting conditions prevailed. Hassett and Landry (1983, 1990) have shown for *Calanus pacificus* that digestive enzyme activities, once developed, remained high even during periods, when feeding rates were declining. The authors suggested that it might be energetically advantageous for copepods, which encounter food on a periodic basis, to maintain high levels of digestive enzyme activities in order to be capable to rapidly exploiting high concentrations of food when encountered. These copepods might accumulate digestive enzymes in enzyme-storing cells (B-cells) during starvation, which are released when food is available. In contrast, copepods, which encounter continuous food supply, continuously synthesize digestive enzymes without accumulation during starvation. Thus, it seems plausible that digestive activities in *C. finmarchicus* and *M. longa* reached their maximum level prior to the time series and activities remained high independent of nutritional conditions. This is corroborated by the feeding and starvation experiments conducted with *C. finmarchicus* parallel to the field campaign in the lower St. Lawrence estuary. Preliminary results on the digestive response of *C. finmarchicus* females under surplus food conditions feeding on *O. marina* or *T. weissflogii* over several days (7 and 18 days) did not reveal changes in the digestive activities, despite of differences in EPR and metabolic activities (chapter 3.1.1.2). Also, no alterations in proteinase activity during the starvation experiments were observed, irrespective of the copepods’ metabolic activities in the field (Fig. 8). These results strongly support that digestive activity of *C. finmarchicus* females from the St. Lawrence estuary do not respond to changing nutritional conditions on time scales of days, as suggested for other species (e.g. Mayzaud and Poulet 1978, publications I, II and III), but that changes can be observed only on longer time scales, such as from winter to spring. During over-wintering, *C. finmarchicus* reduces its gut epithelium cells in order to save energy (Hallberg and Hirche 1980). Hirche (1979) showed that towards the end of the winter, digestive enzyme activities of *Calanus* spp. CV slightly increases, which indicates differentiation of the mid-gut epithelium. This is in accordance with the results on digestive enzyme activities of *C. glacialis* and *C. hyperboreus* females investigated in this thesis (Niehoff and Kreibich, unpublished data). Both species were captured at the end of the diapause. Despite of prolonged starvation in the experiments over three weeks females increased their
proteinase activity slightly. Under surplus food conditions with *T. weissflogii* as diet proteinase activities of *C. glacialis* and *C. hyperboreus* were additionally induced after a time lag of approximately 10 to 20 days. Proteinase activity differed between the species, and was more than twice as high in *C. glacialis* as in *C. hyperboreus* after three weeks of incubation. Thus, *C. glacialis* females were able to respond to food supply and utilised ingested food to build up body mass and to reproduce, whereas *C. hyperboreus* females were not capable to use food for reproduction (Niehoff et al. unpublished data).

Surprisingly, proteinase and amylase activities in *C. finmarchicus* and *M. longa* in the present study correlated without any variation during the field campaign ($R^2=1$; Fig. 9). Until now, to my knowledge, no study observed such strong correlations between two digestive enzymes or enzyme groups in copepods (e.g. Hirche 1981, Båmstedt and Ervik 1984, Båmstedt 1988, publication I). Hirche (1981) found correlations with low variation between trypsin and amylase in *C. finmarchicus* CIII/IV, CV and males ($R^2=0.85$, $0.93$ and $0.75$, respectively). In females, however, trypsin and amylase activity were not correlated with each other. Hirche (1981) suggested that this might be due to feeding selectively on different food items. During our field campaign in the lower St. Lawrence estuary, *C. finmarchicus* and *M. longa* were exposed to strong changes in nutritional conditions. Selective feeding on the same diet with identical biochemical characteristics seems very unlikely. As it stands, our results suggest that proteinase and amylase activity in *C. finmarchicus* and *M. longa* females in the lower St. Lawrence estuary were triggered by a single factor, e.g. enzyme secretion initiated at the occurrence of food items (Hassett and Landry 1983) or genetic regulation (Lovett and Felder 1990). It is, however, possible that a methodological peculiarity might have caused the strong correlation in the samples although there is no indication yet of any artefact. The measurements were performed according to the same protocol and with the same equipment and chemicals as we used in the other copepod species. However, a correlation without variations between the activities of two enzymes is unlikely, since a certain imprecision in measurements cannot be avoided. Additional studies would thus be essential to confirm the present results and to better understand digestive enzyme activities in *C. finmarchicus* and *M. longa*. Digestive activities should be measured in both species from the lower St. Lawrence estuary and other regions during the same season, in order to clarify if population-specific differences are responsible for the observed discrepancy.
KEY RESULTS AND CONCLUSIONS

- *Temora longicornis* and *Acartia clausi* were characterised by higher specific proteinase and amylase activities than *Calanus finmarchicus* and *Metridia longa*.

- In *T. longicornis* and *A. clausi* proteinase and amylase activities are triggered by different factors, e.g. substrate availability.

- *T. longicornis* adapts fast to changing nutritional conditions in the field as well as in experiments. The response of *T. longicornis*’ digestive enzymes to starvation or food supply depends on the initial enzyme activity copepods exhibited in the field, i.e. feeding history. In general, changes in digestive activities can be detected within 24 h. Females of *T. longicornis* express specific diet-induced lipases when feeding on different diets.

- *C. finmarchicus* and *M. longa*, exhibited different feeding behaviours, i.e. *C. finmarchicus* feeding herbivorously and *M. longa* omnivorously, as indicated by fatty acids trophic biomarkers and stable isotope signatures. Both species did not respond to changing nutritional conditions by adapting their digestive activities. Under starvation as well as under surplus food conditions *C. finmarchicus* showed no alterations in proteinase activity.

- *C. glacialis* and *C. hyperboreus* slightly increased digestive enzyme activities under starvation at the end of the diapause, indicating differentiation of the mid-gut epithelium. Under surplus food conditions, the proteinase activity of *C. glacialis* was additionally induced after a time lag. This indicates that females were able to respond to food supply. Proteolytic activity in *C. hyperboreus* increased only slightly.

- In conclusion, the results indicate that the digestive adaptive potential has implications on the copepods’ life strategies, at least in the species presented here. Higher adaptive potentials are found in copepods characterised by low energy reserves, short life spans and several generations per year, whereas lower adaptive potentials are found in copepod species with life spans of one year and more, and which are well adapted to periodic food supply.
3.1.3 CONCLUSIONS

The five copepod species, which I investigated, are characterised by different life strategies. According to their biochemical and physiological characteristics, however, they separate into only two groups. One group comprises species with high digestive potentials, high digestive adaptability, high metabolic requirements and low energy reserves in terms of lipids. The other group includes species with low digestive potentials, low digestive adaptability, low metabolic requirements and high lipid reserves. It seems likely that the digestive potential and the specific metabolic activity in copepods decline with increasing lipid content. However, in this study only copepod species with pronounced differences in lipid accumulation and size were compared, and therefore future studies should include a wide range of copepod species in order to complete our understanding of physiological adaptive processes in copepods.

The copepods from the North Sea, *Temora longicornis*, *Acartia clausi* and *Centropages typicus*, are exposed to continuous but high variable food availability and composition, but they are not experiencing periods of pronounced food scarcity like in polar regions. These species are able to react fast to nutritional changes on a physiological level optimising food utilization. This is achieved, at least in *T. longicornis*, by expressing diet-specific enzymes. The copepods from the lower St. Lawrence estuary, *Calanus finmarchicus* and *Metridia longa*, are characterised by a low physiological adaptive potential. Both species inhabit environments characterised by seasonal food availability. It can be suggested that these copepods do not respond to changing nutritional conditions within days but rather respond to distinct and long-lasting changes in food supply, i.e. the phytoplankton development in spring after the long winter period characterized by the lack of food items in the water column.

3.2 USE OF FATTY ACID TROPHIC BIOMARKERS

Fatty acids were used extensively during the last decades for the interpretation of consumer-prey relationships in the marine environment (e.g. Lee et al. 1971, Sargent and Whittle 1981, Graeve et al. 1994, Dalsgaard et al. 2003 and references therein). Several studies identified taxon-specific fatty acids which cannot be synthesised *de novo* by its consumers and are therefore suitable as trophic biomarkers (e.g. Ackman et al. 1968, Sargent et al. 1987). However, when using fatty acids as trophic biomarkers, some limitations of this approach must be considered:

(I) Only dietary fatty acids, which are accumulated in neutral lipids of the consumers, are suitable as trophic biomarkers. As components of the cell membranes, polar lipids have primarily structural functions and are characterised by very conservative fatty acid patterns (Sargent et al. 1987). Thus, in organisms with low lipid contents, like *Temora longicornis*, fatty
acids originating from copepods’ polar lipids may mask dietary fatty acids in neutral lipids, when total lipid extract is considered.

(II) Fatty acids are specific only for larger taxa (i.e. diatoms, dinoflagellates, *Calanus* spp.), and usually cannot provide trophic information as precisely as to species-level.

(III) The analysis of fatty acids in the tissue of a consumer does not allow quantitative dietary estimates. In a given food organism, lipid content, and correspondingly the amount of a specific fatty acid biomarker, may vary with the organism’s age, growth phase or nutrient availability (Ackman et al. 1964, Kattner et al. 1983, Morris et al. 1983, Parrish 1987, Støttrup and Jensen 1990, Jónasdóttir 1994, Klein Breteler et al. 2005, publication III). The amount of a trophic biomarker accumulated in the animal thus does not necessarily relate directly to the amount of ingested food organisms.

In spite of these drawbacks, there are clear advantages of fatty acids as a tool for studying trophic relationships, since this approach provides information where other analytical methods fail. Analysis of the consumer’s gut content, for example, allows only to determine recently ingested organisms (snap-shot). Fatty acids, in contrast, integrate the trophic history of a consumer over several weeks, as demonstrated experimentally for *Calanus* spp. fed with different algae (Graeve et al. 1994). Moreover, by means of gut content analysis soft-bodied organisms maybe underestimated due to a faster digestion, whereas hard-bodied dietary components such as diatom shells and exoskeletons may be retained for a longer time in the stomach, resulting in a bias towards the latter group of food items (e.g. Budge et al. 2002). To avoid this problem, feeding experiments with zooplankton and natural seston have been conducted (e.g. reviewed in Kleppel 1993). These experiments are time-consuming, should be performed immediately after capture and do also provide information on short-term feeding only. Analysis of seston composition and phytoplankton abundance provides information on potential diets of copepods. However, copepods feed selectively (e.g. Mullin 1963, O’Connors et al. 1980, Breton et al. 1999, Koski et al. 2005). Their grazing pressure may even limit the development of phytoplankton blooms (e.g. Dam and Peterson 1993, Carlotti and Radach 1996), and hence, specific algae may not be abundant in seston samples, because copepods grazed strongly on these cells, masking their importance as diet. Here, adequate fatty acid biomarkers, accumulated in the consumers’ lipids, will provide information on this feeding event.
3.2.1 TIME-DEPENDENT ACCUMULATION OF DIETARY FATTY ACIDS

Until now, information is scarce concerning the time needed for the accumulation of specific dietary fatty acids in calanoid copepods from temperate regions like *Temora longicornis* and *Centropages typicus* (publications II and III, Brüll 2007). It is known for calanoid copepods and euphausiids from polar regions that dietary fatty acids, which are used as biomarkers, provide trophic information over several weeks (e.g. Graeve et al. 1994, Stübing et al. 2003). Graeve (1993) performed numerous feeding experiments with e.g. *Calanus hyperboreus* from the Arctic. By feeding copepods with algae characterised by different fatty acid patterns, he traced the accumulation of these fatty acids in copepods’ total lipids and wax esters over several weeks and found changes in fatty acid and fatty alcohol patterns after 12 to 35 days. Fraser et al. (1989b) showed for mixed zooplankton, comprising nauplii and early copepodids of *T. longicornis*, *Pseudocalanus elongatus*, *Oithona* spp., *Acartia clausi* and *Microstella norvegica* that fatty acid patterns may change within four days. However, to my knowledge, only one study examined diet-induced changes in a single calanoid copepod species from temperate regions, *A. tonsa* (Veloza et al. 2006). The authors found that dietary fatty acids accumulated in the lipids within five days. Our experimental studies on *T. longicornis* and *C. typicus* showed that dietary fatty acids were accumulated even faster, within only three days (Fig. 10, publications II and III, Brüll 2007). Due to the magnitude of change in fatty acid patterns, however, it is likely that accumulation takes place within even shorter periods, e.g. after one day. In contrast to *Calanus* species, the fatty acid compositions in the species from the North Sea thus provides information on recent feeding events.

In summary, it can be suggested that the time lag after which dietary fatty acids are significantly incorporated in the copepods’ lipids are likely dependent on the metabolic requirements and the biomass turnover rate of a copepod.

3.2.2 FACTORS INFLUENCING THE ACCUMULATION OF DIETARY FATTY ACIDS

The feeding experiments conducted by myself and by Brüll (2007) revealed that feeding of *T. longicornis* and *C. typicus* on the same diet, such as *Rhodomonas baltica*, *Thalassiosira weissflogii* or *Oxyrrhis marina*, does not necessarily result in identical accumulation processes. In both copepod species, feeding on the cryptophycean *R. baltica* caused an increase of the fatty acids 18:2(n-6), 18:3(n-3) and 18:4(n-3). When feeding on the diatom *T. weissflogii*, both species, *T. longicornis* and *C. typicus*, accumulated 18:1(n-7) and 16:2(n-4), but differed in the accumulation of 16:1(n-7) and 18:4(n-4). When feeding on the heterotrophic dinoflagellate *Oxyrrhis marina*, the amount of 22:6(n-3) increased strongly (Fig. 10). Thus, accumulation of specific dietary fatty acids depends on several factors:
(i) The fatty acid patterns of the diets can differ intraspecifically, as e.g. the lipid content in diatoms can increase with development of the culture, and PUFAs reach highest values during the late stationary phase (Fernández-Reiriz et al. 1989). During the feeding experiments conducted with *T. longicornis* (publication III), the biochemical characteristics of the *T. weissflogii* culture changed. Within one month, the lipid content of the *T. weissflogii* culture had increased by a factor of five. In addition, the contents of the PUFAs 16:2(n-4), 16:3(n-4) and 18:4(n-4), as well as of the MUFA 16:1(n-7) increased within one month. Also the *O. marina* culture showed a 3-fold higher lipid content and elevated amount of PUFAs with increasing age.

(ii) Feeding history, lipid content and fatty acid patterns of the copepods in the field influence accumulation processes. *T. longicornis* females fed with *T. weissflogii* even showed a decrease of their 16:1(n-7) and 20:5(n-3) fatty acid concentrations, despite high levels in the diet (Fig. 10, publication III). Copepods in the field were characterised by high portions of these fatty acids. Thus, the amounts of 16:1(n-7) and 20:5(n-3) in the diet were insufficient to cause a further increase of these fatty acids in the copepods. These fatty acids increased in *C. typicus* during the experiment, probably due to comparably low amounts in the copepods from the field (Fig. 10, Brüll 2007). Furthermore, the degree of fatty acid accumulation can differ due to different lipid contents in copepods from the field. *T. longicornis* females, characterised by lower lipid contents in the field, accumulated dietary fatty acids to a much higher extent irrespective of the diet, i.e. *T. weissflogii* and *O. marina* (publication III). Lipid content in these copepods increased by approximately 40%, whereas in individuals with higher initial lipid contents there was no further increase, however, fatty acid patterns changed strongly. *T. longicornis* females seem to use most of the diet directly for reproduction and metabolic processes, and do not extensively accumulate lipids as energy reserves. As the lipid content did not increase beyond 6% DM during the experiments the question arises, whether lipid accumulation reached its physiological limit. This, however, was obviously not the case, since copepods in the field had higher lipid contents (up to 9% DM) and lipid-poor females reached lipid levels of up to 8% DM, when feeding on *O. marina*. Highest lipid accumulation was observed in females with low lipid contents feeding on the culture with lower lipid contents. Therefore, it is suggested that lipid accumulation in copepods not only depends on the biochemical composition of the ingested food type but on the pre-condition of the copepods in terms of their physiological characteristics and needs.

The pronounced diet-induced changes of fatty acid patterns in copepods, independent of increases in lipid contents, underline the excellent suitability of fatty acid trophic biomarkers in copepods like *T. longicornis*, even when investigating recent feeding events.
RESULTS AND SYNOPTIC DISCUSSION

KEY RESULTS AND CONCLUSIONS

- *Temora longicornis* and *Centropages typicus* show distinct diet-induced differences in their fatty acid patterns after only three days of incubation. Such short response times may be explained by the high metabolic requirements and biomass turnover rates of these species.

- The accumulation of dietary fatty acids and diet-induced changes in the copepods’ fatty acid patterns are strongly influenced by various factors: The fatty acid patterns and lipid contents of a diet, e.g. *T. weissflogii* and *O. marina*, can differ intraspecifically, with higher lipid and PUFA levels in older cultures. Feeding history, lipid content and fatty acid patterns of the copepods in the field strongly influence dietary fatty acid accumulation processes. High levels of a specific dietary fatty acid in copepods from the field may mask feeding on a specific diet characterised by this fatty acid in experimental studies. Copepods with high lipid contents in the field do not necessarily accumulate lipids under experimental surplus food conditions even though fatty acid patterns show strong diet-induced changes.

- In conclusion, fatty acid trophic biomarkers are a useful tool to gain information on past feeding events in copepods and they provide information even on recent feeding events in copepods like *Temora longicornis* and *Centropages typicus*; species characterised by low lipid reserves and high biomass turnover rates. These findings will improve the use of this method, when investigating short-term feeding events in these copepods, providing a better understanding of trophic interactions and the resulting changes in the food web.
Fig. 10: Change of fatty acids in % dry mass in *T. longicornis* (I) and *C. typicus* (II) after three days under different nutritional conditions. Females were fed with *R. baltica* (I a, II a), *T. weissflogii* (I b, II b), *O. marina* (I c, II c) or incubated under starving conditions. Fatty acid patterns of diets in % of total fatty acids (% TFA). Resulting increases and decreases of specific fatty acids in % of dry mass (% DM) in copepods compared to females from the field feeding on *R. baltica* (I d, II d), *T. weissflogii* (I e, II e), *O. marina* (I f, II f) or under starving conditions (I g, II g). Diets: n=3, mean values and standard deviation. Copepods: n=3, mean values.
4 PERSPECTIVES

This thesis contributed to our knowledge on physiological adaptations of calanoid copepods in the southern North Sea and in the lower St. Lawrence estuary to changing nutritional conditions in the field. Differences in life strategies were related to different adaptive physiological potentials, resulting in different biochemical characteristics and physiological properties of copepods. Furthermore, experimental studies on North Sea copepods revealed a highly adaptive digestive potential to new nutritional conditions by secreting diet-specific digestive enzymes resulting in pronounced accumulations of dietary fatty acids within few days. From these results, new questions emerged, which provide promising perspectives for future studies which aim at further improving our understanding on physiological processes in copepods. This chapter will shortly summarise some of these new questions.

Adaptive physiological potentials of copepods studied in this thesis resulted in different biochemical and metabolic properties of copepods. Copepods with high potentials to store lipids as energy reserves were characterised by lower adaptive physiological potentials than copepods with low energy reserves. Future studies should continue the investigation of adaptive physiological potentials in other copepod species. Of particular interest are e.g. species with similar size like Temora longicornis, such as Pseudocalanus acuspes, which are characterised by higher potentials to accumulate storage lipids (i.e. wax esters or triacylglycerols) as well as copepods with larger sizes like Calanus finmarchicus but characterised by comparatively low energy reserves. This would clarify whether the specific digestive potential has an effect on the lipid accumulation potential of copepods.

A topic, which could only be investigated briefly in this thesis, is the diet-induced expression of digestive enzymes in copepods. Results showed that T. longicornis secreted specific lipases when incubated with different diets. The next step will be the incubation of different copepod species with a wide range of different diets in order to investigate if a lipase map ("enzymatic fingerprint") can be established which provides information on the ingested diet by copepods. After successful calibration under controlled conditions, this enzymatic fingerprint could be used in the field and may supplement the fatty acid trophic biomarker concept, since digestion of lipids occurs prior to the accumulation of dietary fatty acids.

The efficient digestion of ingested food depends on the consumers’ pool of digestive enzymes. Thus, enzymatic heterogeneity should be a key for the utilisation of a wide food spectrum since every single enzyme has its own characteristics e.g. acting on a specific substrate. Copepods from the North Sea and from the St. Lawrence estuary differed in their responses of digestive activities to changing nutritional conditions. Here, the question arises if the copepods additionally differ in their pool of digestive enzymes. An interesting approach is the
electrophoretic separation of digestive enzymes according to their molecular mass, obtaining qualitative information on the enzymatic pool and, therefore, on the enzyme heterogeneity of different copepod species. This information will provide new insights into the efficient utilisation of different diets and consequently on the digestive adaptive potential of copepods to changing nutritional conditions.
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PHYSIOLOGICAL RESPONSES OF *Temora longicornis* females (CRUSTACEA, COPEPODA) TO CHANGING NUTRITIONAL CONDITIONS IN THE NORTH SEA

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PHYSIOLOGICAL RESPONSES OF *Temora longicornis* FEMALES (COPEPODA, CALANOIDA) TO CHANGING NUTRITIONAL CONDITIONS IN THE NORTH SEA

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Abstract

This study focuses on the physiological response, i.e. digestive, metabolic and reproductive activity, of female *Temora longicornis* to changing feeding conditions during spring in the southern North Sea. From March until May 2005, copepods were sampled off Helgoland once or twice a week and analysed for carbon (C), nitrogen (N), lipid and protein content, fatty acid composition, digestive and metabolic activities as well as egg production rate. Additionally, seston samples were taken as a measure for food quantity and quality and analysed for C, N, chlorophyll \(a\) and lipid content and fatty acid composition. Our results show that females were exposed to changing dietary conditions. During March *T. longicornis* fed omnivorously, the diet consisted of lipid-poor and potentially carbohydrate-rich diatoms, eggs and nauplii. Later in the season (mid-April), with increasing chlorophyll \(a\) concentration, the diet changed towards mainly autotrophic, lipid-rich particles. Related to these changes in diet, the physiology of *T. longicornis* changed. At the beginning of the time series the activities of the digestive enzymes proteinase and amylase were high indicating feeding on protein- and carbohydrate-rich particles. When the diet changed, the digestive activity decreased, particularly amylase activity, suggesting a shift in the enzymatic pattern. However, proteinase activity recovered fast to initial values indicating that protein-rich components were still available during the second period of the time series. At the same time lipid content increased in females indicating accumulation of dietary lipids. The specific metabolic activity correlated with the specific egg production, both showing two peaks during the investigation. This hints to an increase of the metabolic rate, when energy is needed for reproductive processes. Interestingly, highest specific egg production rates were measured under different dietary compositions. These results strongly indicate that *T. longicornis* females react to changing nutritional conditions on
a physiological level optimising the utilization of food. Our results show that *T. longicornis* is well adapted to changing nutritional conditions in the North Sea and that the adaptive potential may be an advantage over species with a less pronounced flexibility in times of climate change.

**Keywords:** *Temora longicornis*, digestive enzymes, metabolism, egg production rates, fatty acids, North Sea

**Introduction**

The North Sea is characterised by high seasonal variability of both abiotic factors, such as light and temperature, and biotic factors such as food availability and quality (e.g. Steele and Henderson 1979, Kiørboe and Nielsen 1994). Copepods, which dominate the zooplankton community for most of the year (Fransz et al. 1991), have to cope with distinct changes in environmental conditions. Recently, Wesche et al. (2006) have shown that *Acartia clausi* overwinters in the Southern North Sea with arrested reproduction, while *Centropages* spp. disappear almost completely and recruit during the following spring, either from resting eggs (*C. hamatus*) or through advection (*C. typicus*). Only *Temora longicornis* continuously reproduces and all developmental stages are present during winter (Wesche et al. 2006). Reproduction of *T. longicornis* peaks, however, usually in April/May, closely linked to the phytoplankton spring bloom (Halsband and Hirche 2001, Arendt et al. 2005, Wesche et al. 2006). Small calanoid copepods such as *T. longicornis* are characterised by low energy reserves and high metabolic requirements (e.g. Mayzaud et al. 1992, Evjemo and Olsen 1997, Helland et al. 2003) and are therefore dependent on a continuous food supply. *T. longicornis* is known as an omnivorous species feeding on phytoplankton, microzooplankton and younger copepodite stages (e.g. Kleppel 1993, Dam and Lopes 2003). Thus, a highly adaptive digestive system is required to efficiently utilize different nutritional sources. However, information is lacking on how this species responds on a physiological level to different types of food.

Many studies focused on the effect of food quality, quantity and diversity on growth and reproduction (e.g. Checkley 1980, Roman 1984, Støttrup and Jensen 1990, Jónasdóttir 1994, Peterson and Kimmerer 1994, Ederington et al. 1995, Kleppel et al. 1998, Klein Breteler et al. 1999, Guisande et al. 2000, Jones et al. 2002, Broglio et al. 2003, Peters et al. 2007). These studies, however, did not address physiological processes during digestion. The influence of internal and external factors on digestive activities has been in the focus of other studies instead. Experiments have shown that activity of digestive enzymes relates to food quantity

Combining detailed knowledge of the dietary composition (input), the digestive responses to the diet and the fuelling of metabolic activity and reproduction (output) in the field, is the next essential step to better understand how diet affects growth and reproduction of copepods in the field. We therefore investigated the nutritional conditions during the spring bloom 2005 in the southern North Sea and the resulting changes in physiological characteristics of Temora longicornis females. To determine the condition of the females we measured prosome length, dry mass, carbon (C), nitrogen (N) and water-soluble protein content, lipid content as well as fatty acid compositions and combined these data with the physiological rates i.e. digestive and metabolic enzyme activities and egg production rates. To characterise the potential diet of T. longicornis we measured chlorophyll a concentration, lipid, C and N content as well as the fatty acid composition in the seston. Despite the fact that T. longicornis feeds on a large range of prey items, this species may feed selectively (Daan et al. 1988, Breton et al. 1999, Cotonnec et al. 2001), and it is thus often difficult to relate physiological rates to ambient potential food items. The present study is part of an integrative research approach studying feeding and growth of dominant zooplankton organisms in the southern North Sea during the spring bloom in 2005. Briefly, Gentsch et al. (submitted) found that the females of T. longicornis have been feeding on a mixed diet, probably with a large contribution of heterotrophic organisms, in March and early April, while females were feeding mainly on autotrophic cells from mid-April through the end of May. This information allows us to elucidate the physiological response of T. longicornis to its varying diet in nature in greater detail than considering solely the biochemical composition of the bulk seston.

Material and Methods

Sampling

Temora longicornis females were sampled once or twice a week from the beginning of March until the end of May 2005 off Helgoland (Helgoland Roads, 54°11’26”N, 07°53’94”E), southern North Sea. A Calcofi net (500 μm mesh size) was towed for 10 min in 10 m depth at a speed of 0.3 m s⁻¹. Immediately after capture, the samples were transferred to buckets containing
surface seawater and transported to the institute. At least 180 healthy looking females were sorted alive under a stereo-microscope. All individuals were briefly rinsed in demineralised water (aqua dem.) and triplicates with ten females each were frozen at –80°C either in pre-weighed Zn cartridges (carbon and nitrogen content), in 1.5 ml Eppendorf reaction tubes (water soluble protein content, enzyme activity) or in pre-cleaned glass vials (dry mass, lipid analysis).

In addition, water samples were taken with a 3 l Niskin bottle in 3 m depth and pre-filtered over 70 μm to remove small zooplankton such as copepod eggs and nauplii. Thereafter, the seawater (100-1000 ml, depending on the amount of particulate matter in the water) was filtered on dried and pre-weighed GF/C filters (0.2 μm mesh size). The filters were stored at –80°C until further analysis of C, N and lipid content and fatty acid composition.

The data for salinity and surface water temperature were provided by Wiltshire et al. (in review) as part of the Helgoland Roads time series data (Franke et al. 2004). Chlorophyll a data were obtained from Gentsch et al. (submitted). The measurements were conducted at the working group of K. Wiltshire (Biologische Anstalt Helgoland, Alfred-Wegener Institute for Polar and Marine Research) according to procedures described by Knefelkamp et al. (2007) and Wiltshire et al. (1998).

**Prosome length**

The prosome length of usually 20 - 50 females from samples preserved in 4% formalin buffered with borax was measured with a stereo microscope (LEICA Mz 125), on March 31 only three females were measured due to extremely low abundance.

**Water-soluble protein content**

Soluble proteins were quantified after Bradford (1976) using a commercial protein assay (BioRad) with bovine serum albumin (BSA, BioRad) as standard. One ml ice-cold demineralised water was added to the deep-frozen samples kept on ice. Homogenisation was carried out in 1.5 ml reaction tubes with a pistil. The homogenates were centrifuged 15 min at 15,000 g (4°C) (Heraeus Instruments, Biofuge fresco). The supernatants were transferred into new reaction tubes and kept on ice until photometrical analysis. Samples and standards (0 to 0.5 mg·ml⁻¹) were measured in triplicates. The optical density of the assay was measured at 595 nm (OD₅₉₅) after 10-15 min of incubation at room temperature (Kontron Instruments, Uvikon Spectrophotometer 941).
Carbon and nitrogen content

The Zn cartridges containing the copepods and the filters with seston were dried for 48 h at 60°C and thereafter weighed on a microbalance (Sartorius, ±2 μg). The filters were then wrapped in Zn foil for further treatment. All samples were analysed in a C:H:N Element Analyzer (EuroVector, Euro EA Element Analyzer).

Dry mass, total lipid content and fatty acid analysis

Dry mass was determined after 24 h of lyophilisation (Leybold-Heraeus, LYOVAC GT2) using a micro-balance (Sartorius, ±2 μg). Lyophilised samples were frozen at –80°C and analysed for total lipid content and fatty acid composition. Prior to the lipid extraction procedure, 1.5 ml (copepods) or 4 ml (filters) of solvent (dichloromethane : methanol 2:1/v:v) were added to the samples, which thereafter were stored at –80°C for 24 h. Then, a defined volume of the internal standard tricosanoic acid (23:0) was added and lipid extraction was performed with minor modifications after Folch et al. (1957) as described in Peters et al. (2006). Sub-samples of total lipids were used for the hydrolysis of lipids in fatty acids and their conversion into methyl ester derivates (FAMEs). This procedure was carried out as described in Kattner and Fricke (1986). Samples were analysed with a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter and 0.25 μm film thickness) operating with a temperature programme. Helium was used as carrier gas.

Enzyme activities

The digestive activity of Temora longicornis females was measured as activity of proteinases (endopeptidases) and amylase. For estimating the metabolic activity two key enzymes were studied, (1) pyruvate kinase, component of the glycolysis, and (2) the citrate synthase, component of the citrate cycle. All enzymatic samples and controls were measured photometrically in triplicates.

Deep-frozen samples were homogenized either in 200 μl ice-cold 0.1 mol l⁻¹ Tris/HCl-buffer (supplemented with 10 mmol l⁻¹ CaCl₂, adjusted to pH 7) (for digestive enzymes) or 50 mmol l⁻¹ Tris/HCl-buffer (adjusted to pH 7) (for metabolic enzymes) with a pistil and shortly centrifuged at 15.000 g (4°C). The supernatants were transferred into new reaction tubes and kept on ice until measurements. Considering the distinct changes in size of the females during our time series, digestive and metabolic activities are expressed as activity per body carbon content.
Digestive enzymes

Total proteinase activity was determined after Saborowski et al. (2004) with minor modifications. The substrate azocasein was used to measure the total proteinase activity. 20 μl of sample (20 μl of buffer for the controls) were incubated in 1.5 ml reaction tubes for 5 min at 30°C. 5 μl azocasein (1% in aqua dem., Fluka 11615) were added and incubated for 1 h at 30°C. Reactions were stopped with 50 μl of 8% TCA (trichloroacetic acid, 8% in aqua dem.) cooled on ice. Immediately after stopping the reaction, the samples were centrifuged at 15.000 g (4°C) for 15 min. Supernatants of samples and controls were then measured photometrically at 366 nm.

Amylase activities were measured with the substrate starch-RBB (Loewe Biochemica GmbH, 4 mg ml⁻¹ solution). Incubation, stopping of reactions and centrifugation were carried out as described for the total proteinase activities, however, with different volumes. Here, 36 μl of sample (36 μl of buffer for the controls, respectively) and 16 μl of starch were used. Reactions were stopped with 16 μl of 1 mol l⁻¹ HCl. Supernatants of both samples and controls were measured photometrically at 600 nm after centrifugation.

Metabolic enzymes

Citrate synthase activity (CS, EC 4.1.3.7) was determined with modifications after Stitt (1984) as described in Saborowski and Buchholz (2002). For the activity measurements, 20 μl DTNB (5,5′-Dithiobis(2-nitrobenzoic acid), 6 mmol l⁻¹ in buffer) (Sigma Aldrich), 20 μl Acetyl-CoA (Acetyl-Coenzyme A trilithium salt, 6 mmol l⁻¹, Roche diagnostics) and 20 μl sample were placed in a cuvette and blended with 520 μl 50 mmol l⁻¹ Tris/HCl-buffer (supplemented with 100 mmol l⁻¹ KCl and 1 mmol l⁻¹ EDTA, adjusted to pH 7.5). After 5 min of incubation at 25°C, 20 μl oxalacetic acid (12 mmol l⁻¹, Sigma Aldrich) were added to start the reaction. The increase in the absorbance was measured continuously for 180 s (25°C) at 405 nm.

Pyruvate kinase activity (PK, EC 2.7.1.40) was determined after Saborowski and Buchholz (2002). For the pyruvate kinase activity measurements, 20 μl NADH (β-NADH reduced disodium salt hydrate, 7 mmol l⁻¹, Sigma Aldrich), 20 μl PEP (Phospho(enol)pyruvic acid (tricyclo-hexylammonium) salt, 16 mmol l⁻¹, Sigma Aldrich), 20 μl LDH (Lactate dehydrogenase, 10 U, Roche Diagnostics) and 20 μl sample were placed in a cuvette and mixed with 500 μl 50 mmol l⁻¹ Tris/HCl-buffer (supplemented with 60 mmol l⁻¹ KCl and 4 mmol l⁻¹ MgSO₄, adjusted to pH 7.5). After 5 min of incubation at 25°C, 20 μl ADP (Adenosine diphosphate, 17 mmol l⁻¹, Roche Diagnostics) were added to start the reaction. The decrease in absorbance was measured continuously for 180 s (25°C) at 340 nm.
**Egg production experiments**

For determination of egg production rates, 24 - 36 females were transferred individually into cell wells (volume 6.3 ml) filled with pre-screened seawater. The females were then incubated for 24 h at ambient temperatures. To reduce the effect of cannibalism, every 8 h the eggs were counted and removed. The specific egg production rate was calculated as egg carbon content per body carbon content of females in %. As we did not measure the carbon content of *T. longicornis* eggs we used the value of 0.04 μg calculated by Huntley and Lopez (1992).

**Statistics**

An arc sine square root transformation was performed for statistical operations that require normal distribution. Data sets were tested for normal distribution using the Shapiro-Wilks test and for variance homogeneity with the Levene’s test. A Kruskal-Wallis ANOVA was used for not normally distributed data sets. In case of normally distributed data showing variance homogeneity an ANOVA was performed followed by the Tukey HSD post-hoc test. The data were analysed with the programme GraphPad Prism version 3.00 (GraphPad Software, San Diego, California).

**Results**

**Hydrographic conditions**

Salinity changed considerably from March until May 2005 (Fig. 1a). From March 8 to March 22, salinity varied between 31.5 and 32.5, increased thereafter and ranged between 33.0 and 33.7 until the beginning of May. Between May 10 and 17, salinity had decreased again strongly to 30.4 – 31.0 and increased again at the end of our time series (32.4). Water temperature increased continuously from approximately 3.5°C at the beginning of March to 10.2°C at the end of May.

**Composition of seston**

The carbon and nitrogen content of the seston <70 μm varied considerably during our time series, as did the C:N ratio (Fig. 1c and d). In March the C:N ratio ranged from 10.4 to 13.0. At this time, the C content was high with a maximum of 561.1 μg l⁻¹, the N content ranged from 24.9 to 43.7 μg l⁻¹. In April and May, the C:N ratio was <10 except for April 15 (11.7) and May 4 (11.3). The C content varied between 171.1 (April 5) and 401.1 μg l⁻¹ (May 19), the N content ranged from 17.8 (April 5) to 60.7 μg l⁻¹ (April 26).

Chlorophyll *a* concentrations in the <70 μm size fraction of the seston ranged between 0.81 μg l⁻¹ (March 22) and 1.29 μg l⁻¹ (April 12) at the beginning of the time series (Fig. 1a). From the
end of April until the end of May, the chlorophyll a content was >2.10 μg l⁻¹, except for May 4 (1.29 μg l⁻¹) and May 24 (0.76 μg l⁻¹). It reached the maximum of 2.75 μg l⁻¹ on May 10.

The lipid content of the seston increased during our time series (Fig. 1b). The lowest content (6.6 μg l⁻¹) was measured on March 15; the maximum (36.0 μg l⁻¹) was reached on May 17, followed by a decrease to 22.0 μg l⁻¹ on May 24.

Not only the amount of lipids in the seston but also the fatty acid (FA) composition changed over the investigation period (Table 1, Fig. 3a - d). Specifically, the amounts of the monounsaturated FA (MUFA) 16:1(n-7) and the polyunsaturated FA (PUFA) 18:4(n-3), 20:5(n-3) and 22:6(n-3) increased over time. All four fatty acids showed highest concentrations on May 17. The concentration of 16:1(n-7) varied between 0.57 (March 15) and 5.22 μg l⁻¹, 18:4(n-3) ranged between 0.04 (March 22) and 2.00 μg l⁻¹, and 20:5(n-3) between 0.10 (March 15) and 1.40 μg l⁻¹. The FA 22:6(n-3) was not detectable during the first month of our investigation and reached its maximum amount with 1.15 μg l⁻¹ on May 17. At the end of May the amounts of these fatty acids decreased within one week by 40 – 50% (16:1(n-7) and 20:5(n-3)) and 80% (18:4(n-3) and 22:6(n-3)).

In contrast to these unsaturated FA the percentage of the saturated FA 18:0 in % of total FA decreased over time, especially during April and May (Fig. 1b). From March 15 until April 15 the percentage ranged between 18.8 and 13.9% FA and decreased strongly thereafter to 7.2% FA on April 26. Between May 4 and May 24, portions ranged between 11.6 and 4.9% FA.

**Physiological condition of the females**

The physiological condition of female *Temora longicornis* changed considerably over time as indicated by all parameters measured.

The female prosome length ranged between 915 and 966 μm from the beginning of the time series until the beginning of April (Fig. 2a). Thereafter, prosome length increased and was >990 μm until April 26 with the maximum of 1128 μm on April 21. In between May 4 and 24 prosome length decreased again and was <910 μm.

The female dry mass was comparably low in March and on 7 April (25.6 – 38.6 μg female⁻¹, Fig. 2d). Correspondingly, the C content was low (8.2 – 14.2 μg C female⁻¹) as was the N content (2.3 – 3.9 μg female⁻¹). From 15 April through 24 May, the females were considerably larger (Fig. 2b); the largest females in terms of body mass were found at 21 April (dry mass 50.4 μg, C content 21.6 μg, N content 5.7 μg female⁻¹). The C:N ratio increased from 3.5 at the beginning of March to 4.4 at the end of May (Fig. 2c).
The water-soluble protein content covered a wide range from 5.6 to 19.0 μg female⁻¹ and, as expected, was significantly related to the N content of the females (p = 0.001, r² = 0.768). Proteins comprised mostly between 25 and 40% of the female dry mass (% DM) (Fig. 2e). Lower percentages were found only at the beginning of our investigation during March with a minimum level on 15 March (16.8% DM), and on 7 April and 12 May (22.5% DM and 21.3% DM, respectively). The maximum of 40.2% DM was measured on 26 April.

Lipids contributed a minor fraction to dry mass. In early March, the lipid content was approximately 2% DM (Fig. 2f). It increased slowly over the course of several weeks to about 4% DM. In between April 21 and 26, however, the lipid content doubled from 4.1 to 8.1% DM and remained higher at 7.5 to 9.0% DM in May.

As in the seston, the fatty acid composition of *T. longicornis* females changed remarkably during our investigation (Table 2), particularly the relative portions of 16:1(n-7), 18:4(n-3), 20:5(n-3) and 22:6(n-3) (Fig. 3a-d). The amount of the monounsaturated fatty acid (MUFA) 16:1(n-7) was very low at our first sampling day with 0.02% DM and ranged between 0.10 and 0.15% DM until April 21. Until mid-May, the percentage of this fatty acid increased continuously to a maximum of 1.30% DM (Fig. 3a). The percentages of the PUFA 18:4(n-3) were also low at the beginning of our time series with 0.02% DM and then increased continuously until April 21 (0.24% DM) (Fig. 3b). In females sampled on April 26, the portion had more than doubled (0.51% DM) and remained relatively constant until the end of May ranging between 0.42 and 0.54% DM.

The PUFAS 20:5(n-3) and 22:6(n-3) also increased in terms of percentage of dry mass (Fig. 3c and d). The portion of the FA 20:5(n-3) increased slowly during March and the first three weeks in April. From April 21 to 26, its percentage almost doubled within a few days and remained relatively high thereafter (1.81 to 2.60% DM). The portion of 22:6(n-3) increased from the beginning of March (0.63%) through May 24 (1.42% DM), however, with high variability.

The proteinase and amylase activities per individual changed remarkably during our investigation period (Fig. 4). From the beginning of the time series until April 5 the proteinase activity ranged between 15.1 and 22.5 dE₃₆₆min⁻¹·10⁻³ ind⁻¹ and decreased thereafter strongly to 10.0 dE₃₆₆min⁻¹·10⁻³ ind⁻¹ on April 15. From April 21 until May 24 proteinase activity was higher again and ranged between 14.4. and 23.4 dE₃₆₆min⁻¹·10⁻³ ind⁻¹. In contrast, amylase activity was highest during March (>17.4 dE₆₀₀min⁻¹·10⁻³ ind⁻¹). On April 5 activity dropped and reached lowest values on April 7 with 6.1 dE₆₀₀min⁻¹·10⁻³ ind⁻¹. Thereafter, amylase activity
increased continuously until May 12 (16.1 $dE_{600} \text{min}^{-1} \cdot 10^{-3} \text{ ind}^{-1}$) and ranged thereafter between 14.3 and 15.9 $dE_{600} \text{min}^{-1} \cdot 10^{-3} \text{ ind}^{-1}$.

In general, the specific activity of the metabolic enzymes ranged most of the time between 4.9±0.2 and 6.8±0.2 (citrate synthase) and 3.8±0.3 and 5.4±0.3 (pyruvate kinase) units $mg_{C}^{-1}$ (units per mg body carbon), respectively. From March 30 to 31, however, the specific citrate synthase activity increased by approximately 35% reaching 8.0±0.2 units $mg_{C}^{-1}$ and increased further to 9.6±0.4 units $mg_{C}^{-1}$ until April 5. At the same time, from March 30 to 31, the pyruvate kinase activity increased by even 70%, and reached 6.9±0.4 units $mg_{C}^{-1}$. On April 7, the activities of both enzymes had decreased again by approximately 30% (citrate synthase) and 20% (pyruvate kinase). On April 26, the specific activities increased sharply again by 48% (citrate synthase) and 41% (pyruvate kinase), respectively.

The egg production rate (EPR) of *T. longicornis* females was low in mid-March with 10.4 egg ind$^{-1}$ d$^{-1}$ and increased continuously to the maximum of 50.1 egg ind$^{-1}$ d$^{-1}$ on April 26 (Fig. 5b). Then, EPR decreased to 25.8 egg ind$^{-1}$ d$^{-1}$ on May 4 and ranged thereafter between 39.9 and 26.6 egg ind$^{-1}$ d$^{-1}$ until the end of May.

In general, the specific EPR corresponded to the individual EPR (Fig. 5 b). Maximum specific production, however, was reached with 13.2% already at April 5 when relatively small females (compare Fig. 2a) produced more than 20 eggs d$^{-1}$.

**Discussion**

*Temora longicornis* is an omnivorous copepod feeding on phytoplankton, microzooplankton, copepod eggs and nauplii (e.g. Klein Breteler 1980, O’Connors et al. 1980, Kleppel 1993, Klein Breteler et al. 2004).

Seston composition is often used as an indicator for feeding conditions (e.g. Mauchline 1998 and references therein). Therefore, we have included biochemical analyses of the particulate matter <70 μm. They revealed that its composition in spring 2005 had changed from primarily detrital, lipid-poor to primarily autotrophic, lipid-rich particles. Throughout March and early April, the detritus content of the seston was apparently high as indicated by high carbon:chlorophyll a ratios and relatively high percentages of the FA 18:0. Levels of saturated FAs are usually elevated in detritus, since they are more stable to biological and chemical remineralisation processes than unsaturated FAs (see e.g. Kattner et al. 1983). At the same time, the amount of both total lipid contents and typical phytoplankton FAs such as 16:1(n-7) and 18:4(n-3) was low, although at least diatoms were abundant at that time (Gentsch et al. submitted). This was likely due to the fact that phytoplankton species often do not synthesize
lipids in large amounts, when nutrients are unlimited (Shifrin and Chisholm 1981, Kattner et al. 1983). In addition, as Gentsch et al. (submitted) describe, the Secchi depth was low, which points to high amounts of re-suspended particulate matter in the water column. During the second half of the time series, from mid-April until the end of May, the chlorophyll a concentration increased, while both, C:N ratio and the percentage of the FA 18:0 decreased, indicating that phytoplankton eventually replaced detritus as a major component of the seston. Moreover, the lipid content in the seston increased strongly, mainly due to increasing amounts of the specific diatom marker FAs 16:1(n-7) and 20:5(n-3) (eicosapentaenoic acid, EPA) and to a lesser extent of the specific dinoflagellate and cryptophyte marker FAs 18:4(n-3) and 22:6(n-3) (docosahexaenoic acid, DHA) (e.g. Sargent et al. 1987, Nichols et al. 1993, Dunstan et al. 1994, Graeve et al. 1994, Dalsgaard et al. in references therein). This increase in lipid contents and phytoplankton specific FA probably was the result of an increase of both total phytoplankton cell number and lipid content of the cells due to nutrient limitation (Kattner et al. 1983).

As *Temora longicornis* often selects for specific dietary components (e.g. Daan et al. 1988, Breton et al. 1999, Cotonnec et al. 2001), it is difficult to compare quantity and quality of the particles in the water column with the condition of females in the field. Our study thus benefits from the parallel study on feeding of *T. longicornis* females by Gentsch et al. (submitted). In March and early April, $\delta^{15}$N values were high (approx. 15‰) and female guts contained often nauplii and diatoms, indicating feeding on a mixed diet, probably with a large contribution of heterotrophic organisms. At this time, females were comparably small (see Fig. 2a, this study) and it is likely that they had developed during winter (Halsband-Lenk et al. 2004, Wesche et al. 2006). From April 7 to 21 female size increased indicating that the replacement of winter females by the first spring generation (Halsband-Lenk et al. 2004). Coincidently, the $\delta^{15}$N decreased to <9‰, which points to feeding mainly on autotrophic cells during ontogenetic development (Gentsch et al. submitted, this study). At the end of April, decreasing female size indicated the appearance of a second spring generation (Halsband-Lenk et al. 2004). The $\delta^{15}$N value of the females, however, did not change, and, thus, the trophic levels of the first and the second spring generation were similar (Gentsch et al. submitted).

Distinct changes in salinity at Helgoland Roads in spring 2005 clearly point to advection processes, however, they were not related to any of the parameters we studied in *T. longicornis*. Advection processes therefore cannot explain the observed morphological and physiological changes in *T. longicornis* over time, and we suggest that past and ambient feeding conditions explain the development of digestive enzyme activity, lipid content and fatty acid composition as observed in spring 2005.
Koski and Klein Breteler (2003) suggest that copepod survival is related to the quality and digestibility of the food source. Therefore, the prerequisite for the successful utilization of different food items is the ability of the digestive system to react quickly to changing nutritional conditions. Digestive enzymes are substrate-specific (e.g. Pandian 1975) and need only 24 to 48 h to acclimate to changing feeding conditions (e.g. Mayzaud and Poulet 1978, Roche-Mayzaud et al. 1991). Variations in the enzyme activity may thus mirror a response to specific substrates (Mayzaud and Conover 1975). It changes, for examples, with copepod species and development stage due to food selection (Hirche 1981), with daytime due to feeding periodicity (Båmstedt 1988), with trophic state due to specific metabolic requirements (Mayzaud 1986, Roche-Mayzaud et al. 1991) and, as suggested by Hassett and Landry (1983), with life cycle strategies. Given that many factors influence the response of digestive enzymes, it is not surprising that in some field and experimental studies on copepods, enzyme activity was positively related to food availability (e.g. Mayzaud and Conover 1975, Mayzaud and Poulet 1978, Cox 1981, Cox and Willasson 1981, Hirche 1981), whereas it was not in others (e.g. Hassett and Landry 1983, 1988, Harris et al. 1986). We focussed on *T. longicornis* females and our samples were always taken between 7 and 9 am, which excludes species, stage and daytime specific responses for the explanation of enzyme activity patterns, and we suggest that changes in proteinase and amylase activity were mainly caused by dietary conditions.

Amylase activity is usually a measure for the potential for starch digestion (e.g. Hasler 1935, Harris et al.1986, Roche-Mayzaud et al. 1991). Amylase in copepods is, however, apparently not restricted to the hydrolysis of starch but can also act on polysaccharides, since Hirche (1981) observed that its activity in *Calanus* species was closely related to the carbohydrate concentration in the seston. This may explain why amylase activity can be linked to phytoplankton concentration (e.g. Mayzaud and Poulet 1978, Hirche 1981), although starch is scarce in autotrophic cells (e.g. Gaudy and Boucher 1983). In our study, amylase activities were highest in March and early April, indicating that then *T. longicornis* females were feeding on a carbohydrate-rich diet. At this time chlorophyll *a* concentration was low. However, diatoms, which can consist of up to 70% of polysaccharides (Haug and Myklestad 1976), were abundant in the phytoplankton community and both, gut content analyses and grazing experiments revealed that *T. longicornis* females were feeding on this algal group (Gentsch et al. submitted). The lack of a relation between amylase activity and chlorophyll *a* concentration is in agreement with Mayzaud (1986), who suggests that special attention should be given to the biochemical composition of the diet - in our case possibly reflected in the taxonomic composition of the phytoplankton - in order to understand the response of digestive enzymes. The total proteolytic potential in *T. longicornis* was estimated via the enzyme activity of the main protein-digesting group in crustaceans, the proteinases or endopeptidases (Gibson and Barker 1979, Garcia-Carreño 1992). Similar to the amylase, also the proteinase activity was high in March and early April, pointing to a protein-rich diet. Diatoms may not only contain
polysaccharides but also proteins in high amounts of up to 50% of organic dry matter (Haug and Myklestad 1976), and besides algae, copepod eggs and nauplii likely provided a protein source for *T. longicornis* at that time (Gentsch et al. submitted). As seen by Daan et al. (1988) under laboratory conditions *T. longicornis* females feed on nauplii stages NI to NV if alternative algal food is low.

In the beginning of April, the amylase and proteinase activity per individual decreased sharply. At this time, the abundance of diatoms had decreased from >15,000 to less than 7,000 cells l⁻¹ (Gentsch et al. submitted), which probably resulted in lower amounts of polysaccharides and proteins and thus in enzyme activities. In part, however, the decrease may also have been due to moulting. Gentsch et al. (submitted) observed a strong increase of the copepodite stage V (CV) of *T. longicornis* as well as of CI-CIV copepodids on April 5. Two days later, the digestive enzyme activities in females had dropped. In decapods, digestive activity changes during moulting cycle, with either highest (e.g. Fernández et al. 1997, Fernández Gimenez et al. 2001) or lowest post-moult activities (Muhlia-Almazán and García-Carreño 2002). Assuming that digestive activity in *T. longicornis* decreases during moulting processes, the decrease in activity may indicate that on April 7 recently moulted females were sampled. However, according to female size and dry mass (this study) and to stable isotope signatures (Gentsch et al. submitted), the new generation developed gradually. Thus, if the digestive enzyme activity was reduced solely due to moulting, the decrease should have been gradually, too. It is therefore more likely that feeding conditions were the decisive factor.

With increasing chlorophyll *a* concentration, the amylase activity per individual increased gradually indicating that polysaccharides again became increasingly available with time. The proteinase activity per individual returned much faster to the previous level. This might be due to the fact that protein availability did not change as distinctly as polysaccharide availability, since heterotrophic prey items, rich in proteins, were constantly available. At least, egg production rates of *Temora longicornis* were still high (this study) and copepod nauplii were frequently found in the water column (Gentsch et al. submitted).

Between April 15 and 26, the lipid content of the seston <70 μm and coincidently the female lipid content had increased. This indicates that then the female lipid metabolism became of importance. Unfortunately, we were not able to measure lipase activities due to limited numbers of females for the analyses but, as only lipases can catalyse the hydrolization of lipids, their activity must have increased considerably. Females strongly accumulated lipids during April and May, apparently by feeding on lipid-rich diatoms and dinoflagellates as indicated by an increase of the specific diatom and dinoflagellate marker FAs 16:1(n-7), 20:5(n-3), 18:4(n-3) and 22:6(n-3) in the female lipids. Coincidently, the δ¹⁵N values of the females decreased, which also points to feeding on autotrophic cells (Gentsch et al. submitted).
submitted). These FAs, which cannot be synthesised de novo by the copepods or only in small amounts (e.g. Fraser et al. 1989, Klein Breteler et al. 2004), are primarily incorporated into neutral (storage) lipids like triacylglycerols and wax esters and comprised only 1% of total FAs in females, which developed during winter. The PUFAs EPA and DHA are, besides being biomarkers for diatoms and dinoflagellates, components of polar lipids. In March and early April, these PUFAs contributed more than 31% of total FAs of the copepod lipid content. At this time, the total lipid content was low in the females (2% DM) and in the seston, both FAs contributed only small fractions or were undetectable, which suggests that EPA and DHA are membrane components in *T. longicornis*. The distinct increase of EPA and DHA in % of dry mass during the second half of the time series, when chlorophyll a concentration increased, indicates that the FAs were then incorporated into neutral lipids. Other studies have also shown that copepods accumulate (n-3) PUFAs in neutral lipids during phytoplankton blooms (e.g. Lee 1975, Falk-Petersen et al. 1987, Fraser et al. 1989), and Kattner et al. (1981) suggest that *T. longicornis* incorporated triacylglycerols, which are typical of a degrading phytoplankton bloom in May.

Females of the second spring generation appearing in May reached higher dry masses at similar prosome lengths than females, which developed during winter. Thus, their water content, which can range between 67 and 92% in copepods, was lower than in winter females, due to accumulation of organic material, e.g. lipids (Bämstedt 1986).

Macromolecules, i.e. proteins, lipids and carbohydrates, provided through digestion fuel not only the accumulation of internal storages but also, of course, basic metabolism, growth and reproduction. Therefore, we documented the overall metabolic performance by determining specific activities of metabolic key enzymes, i.e. pyruvate kinase and citrate synthase (e.g. Vetter 1995, Salomon et al. 2000, Saborowski and Buchholz 2002) and egg production rates. Specific metabolic activities and egg production rates were significantly correlated and both peaked twice, once in March, when heterotrophic organisms were a major food source and once in April, when females were mainly feeding on diatoms and dinoflagellates. Apparently, food of different quality can be transformed efficiently into egg material, and reproduction is not solely dependent on blooms of autotrophic cells. The flexibility may explain why *T. longicornis* can reproduce continuously during winter even though phytoplankton concentration is low (Hickel et al. 1993).

Some fatty acids such as the PUFA 20:5(n-3) and 22:6(n-3) are discussed as essential fatty acids during reproduction, embryogenesis, early naupliar development and growth (e.g. Fraser et al. 1989, Støttrup and Jensen 1990, Jónasdóttir 1994, Laabir et al. 2001). In our study the amount of EPA and DHA, either in the seston samples or in *T. longicornis* females, had no significant effect on the specific egg production rate. However, this may be due to the fact that eggs of *T. longicornis* do not strongly rely on lipids as energy source (Peters et al. 2007).
despite a higher energy content of ca. 39 kJ g\(^{-1}\) for lipids compared to 17 to 18 kJ g\(^{-1}\) calculated for proteins and carbohydrates (see review by Lee et al. 2006). Moreover, *T. longicornis* embryos develop fast and already the nauplius stage II (NII) feeds (Klein Breteler et al. 1990), eggs do not depend on large amounts of lipids as energy source. Peters et al. (2007) suggest that nitrogen-containing compounds may have a higher potential in limiting egg production rates of *T. longicornis* in the North Sea than essential fatty acids. Also, Koski et al. (2006) showed in experiments that the egg production of this species correlated significantly with the particulate organic nitrogen content in the diet, whereas it was independent of EPA and DHA content. The N content of the seston in our time series, however, did not have a significant effect on the specific egg production rate of *T. longicornis*, since highest specific egg production rates were achieved in times of lowest N content. This may indicate that the nitrogen content in the diet was not a limiting factor for specific egg production rates throughout our time series. However, since we only measured the nitrogen content in the <70 μm fraction of the seston it is possible that females obtained nitrogen by feeding on nauplii, eggs and ciliates at least during the first half of the time series.

In conclusion, *T. longicornis* was able to cover its energy demands for growth, energy storage and reproduction by effectively digesting and assimilating different types of food. While it is certainly true that this species feeds selectively (e.g. Tackx et al. 1989), and may thus respond to changing nutritional conditions by its feeding behaviour, our results show that *T. longicornis* is well adapted also on a physiological level. This adaptation may be an advantage over species with a less pronounced flexibility in times of climate change.

Acknowledgements

We especially thank K. H. Wiltshire for providing salinity data and surface water temperatures. We are grateful to C. von Waldthausen for help in the laboratory and for measuring C and N contents. We would like to thank all colleagues at the Biologische Anstalt Helgoland and the Marine Zoology, University Bremen, for their support and laboratory space during experimental and laboratory procedures, and the crew of the R/V “Aade” for careful sampling. This study was funded by the “Struktur- und Investitionsfond des Präsidenten der Helmholtz-Gemeinschaft (President’s Initiative and Networking Fund)”, Germany, VH-NG-058.
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Hasler AD (1935) The physiology of digestion in plankton crustacea II. Further studies on the digestive enzymes of (A) *Daphnia* and *Polyphemus*; (B) *Diaptomus* and *Calanus*. Biol Bull 72: 290-298


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copepodite stage V and VI males and females of Calanus finmarchicus (Gunnerus). Sarsia 67: 63-68


Table 1: Seston: Main fatty acids (>1% of total fatty acids) of the ≤70 μm fraction of water samples (mean ± standard deviations) from 3 m depth (n = 3). tr: traces; –: not detected

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Table 2: *T. longicornis*: Main fatty acids (>1% of total fatty acids) (*n* = 3, mean ± standard deviations) and sum of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). tr: traces; -: not detected

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<td>1.7 ±1.9</td>
<td>tr</td>
<td>tr</td>
<td>1.1 ±0.2</td>
<td>1.8 ±0.2</td>
<td>1.6 ±0.1</td>
<td>1.4 ±0.2</td>
<td>1.7 ±0.1</td>
<td>2.0 ±0.1</td>
<td>2.6 ±0.1</td>
<td>3.1 ±0.1</td>
<td>3.9 ±0.2</td>
<td>3.8 ±0.5</td>
<td>3.5 ±0.2</td>
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<tr>
<td>18:3(n-3)</td>
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<td>tr</td>
<td>tr</td>
<td>1.2 ±0.1</td>
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<td>1.2 ±0.1</td>
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<td>tr</td>
<td>1.2 ±0.4</td>
<td>1.0 ±0.1</td>
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<tr>
<td>18:4(n-3)</td>
<td>1.1 ±0.1</td>
<td>tr</td>
<td>1.9 ±0.1</td>
<td>3.1 ±0.2</td>
<td>4.9 ±0.1</td>
<td>5.0 ±0.4</td>
<td>5.3 ±0.1</td>
<td>5.8 ±0.7</td>
<td>6.8 ±0.4</td>
<td>6.3 ±0.2</td>
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<td>6.0 ±0.7</td>
<td>6.1 ±0.1</td>
<td>5.6 ±0.4</td>
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<tr>
<td>20:1(n-9)</td>
<td>tr</td>
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<td>tr</td>
<td>tr</td>
<td>1.0 ±0.1</td>
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<td>tr</td>
<td>tr</td>
<td>tr</td>
<td>1.1 ±0.1</td>
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<tr>
<td>20:3(n-3)</td>
<td>1.4 ±1.5</td>
<td>tr</td>
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<td>20:4(n-3)</td>
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<td>tr</td>
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<td>1.1 ±0.1</td>
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<tr>
<td>20:5(n-3)</td>
<td>31.5 ±1.6</td>
<td>28.4 ±2.6</td>
<td>30.1 ±2.7</td>
<td>30.8 ±0.7</td>
<td>30.9 ±1.0</td>
<td>35.3 ±0.2</td>
<td>33.6 ±0.6</td>
<td>36.6 ±0.5</td>
<td>31.6 ±3.1</td>
<td>30.1 ±0.8</td>
<td>31.7 ±0.2</td>
<td>26.0 ±1.6</td>
<td>26.4 ±1.4</td>
<td>25.0 ±1.2</td>
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<tr>
<td>22:1(n-9)</td>
<td>1.1 ±0.2</td>
<td>1.3 ±0.1</td>
<td>1.0 ±0.1</td>
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<tr>
<td>22:5(n-3)</td>
<td>1.0 ±0.1</td>
<td>1.2 ±0.2</td>
<td>1.0 ±0.1</td>
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<td>tr</td>
<td>1.3 ±0.1</td>
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<tr>
<td>22:6(n-3)</td>
<td>32.9 ±1.5</td>
<td>30.0 ±1.8</td>
<td>24.2 ±1.3</td>
<td>25.7 ±1.4</td>
<td>23.3 ±1.3</td>
<td>20.2 ±0.6</td>
<td>19.4 ±1.3</td>
<td>18.5 ±0.5</td>
<td>13.8 ±1.9</td>
<td>13.6 ±1.1</td>
<td>12.9 ±0.2</td>
<td>11.5 ±0.3</td>
<td>14.6 ±1.3</td>
<td>17.2 ±0.8</td>
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<tr>
<td>24:1(n-13)</td>
<td>2.6 ±0.3</td>
<td>2.0 ±0.1</td>
<td>1.7 ±0.1</td>
<td>1.6 ±0.1</td>
<td>1.3 ±0.1</td>
<td>1.2 ±0.1</td>
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</table>

SFA: 17.6 ±2.2 | 21.9 ±1.4 | 22.7 ±2.4 | 22.7 ±0.7 | 21.2 ±0.3 | 22.3 ±0.5 | 24.0 ±1.3 | 21.4 ±1.4 | 27.3 ±3.1 | 28.5 ±0.5 | 22.6 ±0.5 | 25.5 ±3.4 | 23.4 ±0.3 | 23.3 ±1.0

MUFA: 9.8 ±0.8 | 11.1 ±1.3 | 13.6 ±1.5 | 11.3 ±0.4 | 12.4 ±0.4 | 10.6 ±0.1 | 10.8 ±0.1 | 10.5 ±0.1 | 12.3 ±0.7 | 14.0 ±0.7 | 16.1 ±0.1 | 20.4 ±0.8 | 20.0 ±2.1 | 18.5 ±0.3

PUFA: 70.4 ±2.9 | 64.0 ±3.8 | 61.9 ±4.0 | 65.2 ±1.3 | 66.4 ±0.5 | 67.1 ±0.6 | 65.3 ±1.2 | 68.1 ±1.3 | 59.6 ±4.5 | 55.9 ±1.2 | 60.6 ±0.5 | 52.4 ±3.9 | 56.0 ±1.4 | 56.3 ±1.9
Figure captions

Fig. 1: Seston: salinity and chlorophyll a content (μg l⁻¹) (a), lipid content (μg l⁻¹) and fatty acid 18:0 (% of total fatty acids) (b), carbon and nitrogen content (μg l⁻¹) (c) and C:N ratio (d) (n = 3, means ± standard deviation, except salinity).

Fig. 2: *T. longicornis* females: prosome length (a), carbon and nitrogen content (b), C:N ratio (c), dry mass (d), water-soluble protein content (e) and lipid content (f) (n = 3, means ± standard deviation, except for prosome length n = 3 – 160 (for details see materials and methods), means ± standard deviation).

Fig. 3: Fatty acids 16:1(n-7) (a), 18:4(n-3) (b), 20:5(n-3) (c) and 22:6(n-3) (d) of *T. longicornis* females (% DM) and seston samples (μg l⁻¹) (n = 3, means ± standard deviation).

Fig. 4: *T. longicornis* females: proteinase and amylase activity per individual (n = 3, means ± standard deviation).

Fig. 5: *T. longicornis* females: Specific citrate synthase and pyruvate kinase activity (a) (n = 3, means ± standard deviation) and the specific egg production rate (EPR) (b) (n = 24 – 36, means).
Fig. 1

(a) Salinity and chlorophyll-a (chl-a) content

(b) Lipid content and 18:0 fatty acid content

(c) C content and N content

(d) C:N ratio
Fig. 3

(a)  16:1(n-7) % DM (females)
16:1(n-7) μg l⁻¹ (seston)

(b)  20:5(n-3) % DM
20:5(n-3) μg l⁻¹

(c)  18:4(n-3) % DM
18:4(n-3) μg l⁻¹

(d)  22:6(n-3) % DM
22:6(n-3) μg l⁻¹
Fig. 4
Fig. 5

(a) Specific enzyme activity (units mgC^{-1})
- Citrate synthase
- Pyruvate kinase

(b) Egg production rate (eggs ind^{-1} d^{-1})
- EPR
- Specific EPR

March April May
22 30 31 5 7 15 21 26 4 10 12 17 19 24

Egg production rate (eggs ind^{-1} d^{-1})
Specific enzyme activity (units mgC^{-1})
SHORT TERM VARIATION OF NUTRITIVE AND METABOLIC PARAMETERS IN *TEMORA LONGICORNIS* FEMALES (CRUSTACEA, COPEPODA) AS A RESPONSE TO DIET SHIFT AND STARVATION

Kreibich T, Saborowski R, Hagen W, Niehoff B

submitted to Helgoland Marine Research
SHORT TERM VARIATION OF NUTRITIVE AND METABOLIC PARAMETERS IN *Temora longicornis* FEMALES (CRUSTACEA, COPEPODA) AS A RESPONSE TO DIET SHIFT AND STARVATION

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Abstract

Changes in fatty acid patterns, digestive and metabolic enzyme activities and egg production rates were studied in the small calanoid copepod *Temora longicornis*. Female copepods were collected in May 2005 off Helgoland (North Sea). In the laboratory one group of copepods was fed with the cryptophycean *Rhodomonas baltica* for a period of three days. Another group of copepods was maintained without food. According to the fatty acid patterns, animals from the field were feeding on a more detrital, heterotrophic and to a minor extent diatom-based diet. Under laboratory conditions, females rapidly accumulated fatty acids such as 18:4(n-3), 18:3(n-3) and 18:2(n-6) which are specific for *R. baltica*. Diatom-specific fatty acids like 16:1(n-7) were strongly reduced. In fed animals the activities of digestive and metabolic enzymes remained constant, and egg production rates (EPR) were highest on day two. Starving animals, in contrast, showed significantly reduced faecal pellet production and egg production rates. Proteolytic enzyme activity decreased rapidly within 24 h and remained at a low level until the end of the experiment. Citrate synthase decreased continuously as well. *T. longicornis* rapidly reacts to changes and food depletion. It has limited energy stores and, thus, strongly depends on continuous food supply.

Keywords *Temora longicornis*, digestive enzymes, metabolism, fatty acids, starvation
Introduction

Copepods are abundant and widespread in the ocean plankton where they constitute a major component of the pelagic food web. As adaptation to different environmental conditions and seasonal cycles they developed a variety of life history traits as well as unique physiological properties. These comprise diapausing, resting eggs, high reproduction rates, vertical migration and efficient energy utilization (e.g. Kiørboe et al. 1985, Dahms 1995, Mauchline 1998, Castellani and Lucas 2003). The utilization of food and the deposition of energy reserves is the fundamental process for survival and reproduction. Feeding and digestion, however, depend on various factors including hydrography, food density, the fitness of the feeder, and its ability to cope with changing trophic conditions (e.g. Mayzaud 1986, Roche-Mayzaud et al. 1991, Kiørboe and Nielsen 1994). Organisms, which are capable of accumulating energy reserves, e.g. lipids, are less vulnerable to fluctuations in food availability. They can survive extended periods with little food or even without food while mobilizing their lipid stores (e.g. Lee 1975, Lee and Barnes 1975, Sargent and Henderson 1986). On the contrary, organisms, which are not able to accumulate energy reserves extremely depend on continuous food supply.

The small calanoid copepod *Temora longicornis* is one of the most abundant species in the North Sea and other regions of the Northeast Atlantic and, thus, it is of outstanding ecological importance (e.g. Fransz et al. 1991, Mauchline 1998). It is known as a species with high metabolic rates but low energy reserves (e.g. Mayzaud et al. 1992, Evjemo and Olsen 1997, Helland et al. 2003). Since the plankton composition may change rapidly in the North Sea (e.g. Kiørboe and Nielsen 1994), *T. longicornis* strongly depend on the ability to react rapidly to altering trophic conditions. The digestive system is immediately affected by fluctuations of food supply. Potential effects of the dietary composition on the expression of digestive enzymes were discussed in earlier studies but different and partly contradicting conclusions were drawn. Some authors suggested that digestive activity increases with increasing food supply, whereas others showed that the digestive activity decreases in copepods under surplus of food (e.g. Mayzaud and Conover 1975, Hirche 1981, Hassett and Landry 1983, Harris et al. 1986, Roche-Mayzaud et al. 1991). As a consequence of food supply the copepods may change their metabolic performance in terms of oxygen consumption or metabolic enzyme activities. Moreover, the reproductive success can change significantly. E. g. egg production rates respond within one day to dietary changes (e.g. Kiørboe et al. 1985, Jónasdóttir and Kiørboe 1996, Hirche et al. 1997).

This study aimed at complementing knowledge on adaptive responses of *T. longicornis* to changing nutritional conditions on different physiological levels. Therefore, we carried out short term feeding and starving experiments. One group of female *T. longicornis* was incubated over three days with the cryptophycean *Rhodomonas baltica*, a high quality food for copepods (e.g.
Klein Breteler 1980, Jónasdóttir 1994, Jónasdóttir and Kiørboe 1996, Koski et al. 1998, Tang et al. 2001, Klein Breteler et al. 2004). Another group of females remained unfed for three days. The parameters we selected covered different physiological levels. From daily samples we analysed the changes of the activities of digestive and metabolic enzymes and egg production rates, whereas changes in fatty acid patterns were investigated at the end of the experiments after three days of incubation.

Material and Methods

Origin of samples

_Temora longicornis_ specimens were sampled in spring (April and May 2005) off Helgoland (54°11’N, 07°54’E) with a CalCoFi plankton net (500 μm mesh size). The net was towed for 10 min at 10 m depth and at a speed of 0.3 m s⁻¹. Immediately after capture the plankton samples were transferred to the laboratories of the Marine Station. For each experiment, a feeding experiment in April and a starving experiment in May, about 450 healthy looking females and 50 males were sorted alive under a stereo-microscope. Due to low numbers of females in the samples we were unable to start both experiments at the same time. Nine groups of ten females each were shock frozen at –80°C after shortly being rinsed in demineralised water. These samples were used for biochemical analysis (dry mass, lipid content, fatty acid composition, metabolic and digestive enzyme activities) representing the _in situ_ condition of the females. Parallel to the zooplankton sampling in April, water samples were taken with a 10 l Niskin bottle in 3 m depth in order to characterise the potential diet of copepods in the field. Water samples were filtered over a 70 μm sieve. Thereafter, triplicates of 500 ml were gently filtered over dried GF/C filters (0.2 μm). The filters were stored at –80°C until analysis of the fatty acid composition.

Algae culturing

_Rhodomonas baltica_ (Cryptophyceae) was grown in f/2 medium (Guillard 1975) over several weeks under continuous illumination and aeration. Each day the bottles with the cultures were gently shaken to maintain cells in suspension. Cell concentrations were measured with a cell counter and analyser system (CASY® Model TTC, Schärfe System GmbH). At the beginning of the feeding experiment a defined volume of the cultures were filtered on dried GF/C filters and stored at –80°C for subsequent fatty acid analysis.

Experiments

Both feeding and starving experiments were carried out with healthy looking females and males. For each experiment, about 150 females and 15 males were placed in each of three
2.5 l beakers containing approximately 2 l of algal suspension or filtered sea-water (0.2 μm). Males were required for the continuous fertilization of females. Copepods were incubated over three days. The cell density of *Rhodomonas baltica* was 20,000 cells ml⁻¹. The beakers were kept in the dark at approximately 5°C throughout the experiment. The temperature was the same as the ambient water temperature. On day two approximately half of the incubation water was exchanged by new medium.

Every day approximately 90 females in total were taken from the beakers. For the measurements of egg and faecal pellet production, 24 females were individually incubated over 24 h in cell wells (6.3 ml volume), filled with filtered sea-water (0.2 μm), at ambient temperature in a constant temperature room. Every 8 h eggs and faecal pellets were counted and removed from cell wells in order to avoid cannibalism on eggs and feeding on pellets. The other females were frozen in groups of ten at –80°C for biochemical analysis of digestive and metabolic enzymes. On day three additionally three groups of ten females were taken from the beakers and were frozen for the analysis of fatty acids.

**Enzyme analysis**

The digestive potential of *T. longicornis* females was represented by total proteolytic activity. As representatives of metabolic activity, citrate synthase (CS; EC 4.1.3.7) the key enzyme of the triacetic acid cycle was selected. All enzymatic assays were carried out in triplicates.

Total proteinase: deep-frozen samples (10 individuals) were homogenized in 200 μl of ice-cold buffer (0.1 mol l⁻¹ Tris/HCl pH 7, supplemented with 10 mmol l⁻¹ CaCl₂). Proteinase activity was determined with modifications after Saborowski et al. (2004). The copepods were thoroughly squeezed with a micropestle and the extract was centrifuged at 15,000 g for 10 min (4°C). Thereafter, the supernatants were transferred into new microtubes and were kept on ice. The total proteinase activity was measured with Azocasein as substrate. Twenty μl of sample (20 μl of buffer for the control assays) were incubated in microtubes for 5 min at 30°C. Then 5 μl of azocasein solution (Fluka 11615, 1% in aqua dem.) were added and the tubes were incubated for 1 h at 30°C. The reactions were stopped by the addition of 50 μl of 8% trichloroacetic acid (TCA, 8% in aqua dem.) and cooling on ice. The microtubes were centrifuged at 15,000 g (4°C) for 15 min. The optical density of the supernatants was measured at 366 nm.

Citrate synthase: deep-frozen samples were homogenized as described above except that Tris/HCl (50 mmol l⁻¹, pH 7) was used as extraction buffer. CS activity was determined with modifications after Stitt (1984) as described in Saborowski and Buchholz (2002). Twenty μl DTNB (5,5'-Dithiobis(2-nitrobenzoic acid), 6 mmol l⁻¹ in buffer) (Sigma D8130), 20 μl Acetyl-CoA (Acetyl-Coenzyme A tri-lithium salt, 6 mmol l⁻¹) (Roche diagnostics, 10101893001) and
20 μl sample were placed in a cuvette and mixed with 520 μl of 50 mmol l⁻¹ Tris/HCl-buffer, pH 7.5 (supplemented with 100 mmol l⁻¹ KCl and 1 mmol l⁻¹ EDTA). After 5 min of incubation at 30 °C, 20 μl of oxalacetic acid (12 mmol l⁻¹) (Sigma O 4126) were added to start the reaction. The increase of absorbance was measured continuously for 180 s at 405 nm and at 30 °C.

Dry mass, lipid content and fatty acid analysis

The copepods were lyophilised for 24 h (Leybold-Heraeus, LYOVAC GT2). The dry mass was measured with a micro-balance (Sartorius, ±2 μg). The lyophilised samples were then stored at −80 °C.

Prior to the lipid extraction procedure, organic solvent (dichloromethane:methanol, 2:1/v:v) was added to the copepods (1.5 ml) and to the filter samples (4 ml) and the samples were left for 24 h at −80 °C. Thereafter, a defined amount of the internal standard tricosanoic acid (23:0) was added to the samples for the determination of total lipid content. Lipid extraction was performed with minor modifications after Folch et al. (1957) as described by Peters et al. (2006). Sub samples of the total lipid extracts were used for fatty acids analysis after Kattner and Fricke (1986). The fatty acids were first transformed into methyl ester derivates (FAMEs). Then they were separated with a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter and 0.25 μm film thickness) operating with a temperature program. Helium was used as carrier gas. Chromatograms were processed and analysed with the software KromaSystem 2000 version 1.83 (Bio-Tek Kontron Instruments). Peaks were identified by means of reference standards and by comparison of relative retention times.

Statistics

An arc sine square root transformation was performed for statistical operations, which require normal distribution of data sets. The Kolmogorov-Smirnov test (with Lilliefors’ correction) was used to test data for normality. Variance in homogeneity was tested with the Levene’s test. Significant differences between groups were tested either with a Student’s t-test or a one-way ANOVA followed by the Holm-Sidak post-hoc test. The data were analysed with the programme SigmaStat version 3.5 (Systat Software, Inc.).

Results

Mortality, dry mass and lipid content of copepods

The mortality was less than 10% during the entire duration of the experiments. Dry masses (DM) and lipid contents of in situ females were not significantly different from females fed with *R. baltica* or starved females (t-tests, p>0.05) due to high variance. Dry masses were
approximately 27 μg ind⁻¹ (feeding experiment) and 40 μg ind⁻¹ (starving experiment). Lipid content in the feeding experiment amounted to 3.4±0.7% DM in in situ females and 2.8±0.3% DM in females fed with *R. baltica*. Lipid contents decreased from 6.6±0.7% DM in the animals from the field to 4.7±1.4% DM after three days of starvation (Table 1).

**Faecal pellet production and egg production rate**

Females, which were incubated immediately after capture produced 23.3 faecal pellets female⁻¹ d⁻¹ (Fig. 1). After one day of feeding the faecal pellet production rate (FPR) remained at the same level but increased at day two (60.6 FP female⁻¹ d⁻¹). At day three, it dropped to 29.2 FP female⁻¹ d⁻¹. FPR in starved females decreased strongly from 10.3 faecal pellets female⁻¹ d⁻¹ to 2.2 faecal pellets female⁻¹ d⁻¹ on day one and stayed low throughout the experiment not exceeding 4.2 faecal pellets female⁻¹ d⁻¹ (Fig. 1).

In fed females, egg production rate (EPR) increased within two days from 17.7 eggs female⁻¹ d⁻¹ to 41.6 eggs female⁻¹ d⁻¹ (Fig. 2). On day three, only 25.6 eggs female⁻¹ d⁻¹ were produced. The EPR in starved females decreased throughout the experiment from initially 24.6 eggs female⁻¹ d⁻¹ to 11.1 eggs female⁻¹ d⁻¹ on day three (Fig. 2).

**Proteinase activity of copepods**

Proteinase activity was 2.0±0.2·10⁻² dE₃₆₆ min⁻¹ ind⁻¹ in animals from the field. In fed animals, the activity was slightly reduced after one day to 1.4±0.2·10⁻² dE₃₆₆ min⁻¹ ind⁻¹ (Fig. 3) but increased constantly in the following days to 2.3±0.3·10⁻² dE₃₆₆ min⁻¹ ind⁻¹. Significant differences appeared between day one and day three (one way ANOVA p<0.05, Holm-Sidak post hoc test). In starved animals, proteinase activity decreased constantly from 2.1±0.1·10⁻² dE₃₆₆ min⁻¹ ind⁻¹ (in situ) to 0.3±0.2·10⁻² dE₃₆₆ min⁻¹ ind⁻¹ on day three. The reduction was already significant after one day under starving conditions (one way ANOVA p<0.001, Holm-Sidak post hoc test).

**Citrate synthase**

The citrate synthase activity of fed females did not change significantly over time (one way ANOVA p>0.05), and ranged between 9.3·10⁻² (in situ) and 8.2·10⁻² U ind⁻¹ (day three) (Fig. 4).

In starved females CS activity decreased constantly during the experiment from 8.5·10⁻² to 6.1·10⁻² units ind⁻¹. The differences between the activities of in situ females and starving females were statistically significant on day three (one way ANOVA p<0.01, Holm-Sidak post hoc test).
Fatty acid compositions of diets and copepods

The seston and the *Rhodomonas baltica* culture showed distinct differences in their fatty acid composition. Composition of the seston was characterised by high portions of the saturated fatty acids (SFA) 16:0 (34.0±0.5% of total fatty acids (FA)) and 18:0 (15.3±0.5% FA) (Fig. 5a). The monounsaturated fatty acid (MUFA) 18:1(n-9) reached 11.4±0.3% FA, followed by 16:1(n-7) (7.8±0.8% FA), the polyunsaturated fatty acid (PUFA) 20:4(n-3) (7.6±0.4% FA) and the MUFA 18:1(n-7) (5.8±1.2% FA). In *Rhodomonas baltica* (Fig. 5a), major fatty acids were the PUFAs 18:4(n-3) with 28.0±1.8% FA, followed by 18:3(n-3) (13.3±1.0%), 18:2(n-6) (11.5±0.8%), 22:6(n-3) (9.2±1.4%) and the MUFA 18:1(n-7) with 8.3±0.6% FA. The SFAs 16:0 (7.0±2.6% FA) and 18:0 (1.7±1.9% FA) and the MUFA 18:1(n-7) (4.5±0.4% FA) showed relatively low amounts.

The fatty acid pattern of *T. longicornis* changed considerably after three days of feeding on *R. baltica* (Fig. 5b). The females from the field showed elevated amounts of the PUFAs 20:5(n-3) with 29.9±0.8% and 22:6(n-3) with 23.5±2.0% FA. The SFA 16:0 reached 20.1±0.9%. After feeding on *R. baltica*, the amount of the FA 20:5(n-3) had decreased significantly to 21.9±1.1% (t-test, p<0.01), whereas FA 22:6(n-3) increased to 28.2±1.2% FA (t-test, p<0.05). The FA 16:0 decreased significantly to 13.5±0.2% (t-test, p<0.001). Most distinct changes were observed in the amounts of 18C fatty acids (t-tests, p<0.01). The amount of 18:4(n-3) increased from 3.4±0.2% to 7.8±0.5%, 18:3(n-3) from 0.7±0.1% to 4.3±0.2% and 18:2(n-6) from 1.2±0.4% to 3.9±0.6% FA. The MUFA 16:1(n-7), which reached 3.5±0.3% in females from the field, was reduced in fed females to 0.7±0.1% FA.

Specific fatty acid accumulation and reduction was calculated as the difference in % of the amount *in situ* in % DM and the amount in % DM after three days of incubation. After feeding on *R. baltica* the strongest relative accumulation was observed in 18:3(n-3) with approximately 430%, followed by 18:2(n-6) (153%) and 18:4(n-3) (87%). The strongest decrease was observed in 16:1(n-7) and 16:3(n-4), which were both reduced by approximately 80% (Fig. 6). The amounts of all fatty acids in starved females were reduced after three days of incubation, reduction of single fatty acids ranged between approximately 10 and 54% of the *in situ* amount. Strongest reduction were measured for 14:0 (54%), 18:4(n-3) (49%), 16:2(n-4) (46%), 16:1(n-7) (44%) and 18:3(n-3) (42%) (Fig. 6).

Discussion

*Temora longicornis* showed rapid and distinct physiological responses to changes of diet and to starvation. The dietary shift was reflected by a drastic change in fatty acid patterns while starvation caused a significant decrease of digestive proteinase activity, CS activity, and egg production rates. These results excellently complement our view about the trophic interactions and the ecological function of *T. longicornis* in the southern North Sea.
The duration of the experiments (three days) was chosen to exert a significant stress on the animals but to avoid excessive mortality. Indeed, in each of the experiments the mortality of copepods was less than 10%. Many copepod species show a close correlation between food concentration on one side and ingestion rates and faecal pellet production on the other site (Mauchline 1998 and references therein). The same was true in our experiments for *T. longicornis*. Feeding on *R. baltica* increased faecal pellet production above *in situ* rates while starvation drastically reduced faecal pellet production. This is a good confirmation that *T. longicornis* accepted *R. baltica* as food. Moreover, *T. longicornis* continuously increased egg production rates, which, in turn, show that food quality was sufficient to maintain physiological integrity and reproductive processes at a high level. In contrast, starving copepods immediately stopped faecal pellet production and continuously reduced the production of eggs.

The shift to *Rhodomonas* diet did not cause adverse physiological reactions in *T. longicornis*. The average dry mass (DM) as well as the total lipid contents remained at the same level as in the animals from the field. In starving animals, however, the lipid content decreases significantly. *T. longicornis* does not accumulate high amounts of lipids. In our samples the lipid values were less than 5% DM. In contrast, copepod species from high latitudes may accumulate lipids up to 75% of their dry mass (Lee et al. 2006). Accordingly, *T. longicornis* depends on the continuous supply of food and suffers in periods of starvation. A clear indication for starvation is the rapid and almost entire depletion of storage lipids. Merely polar lipids remained in the samples after three days. These, however, significantly contribute to structural cell components and, thus, cannot be metabolised without risking cellular integrity (e.g. Sargent et al. 1987).

The effects of the dietary composition or starvation on the activities of digestive enzymes of copepods were discussed controversially (e.g. Mayzaud and Conover 1975, Hirche 1981, Hassett and Landry 1983). Some authors reported that digestive enzyme activities rise when food concentrations increased while others showed the opposite. In our study proteinase activities in *T. longicornis* females remained at the same level between females from the field and those fed with *R. baltica*. In contrast, starving animals lost almost their entire proteolytic activity within two days. The reduction of the digestive potential may be seen as an immediate mechanism to save metabolic energy. Upon sufficient food supply the copepods synthesize and release digestive enzymes to efficiently utilize the ingested food. Rapid ingestion and, thus, faster gut passage causes a significant loss of enzymes via the release of faecal pellets (e.g. Nott et al. 1985). In order to maintain a high digestive potential the copepods have to keep enzyme synthesis rates high as well. As soon as food supply ceases, high enzyme synthesis rates are physiologically adverse: their synthesis is metabolically costly and high activities of particularly proteolytic enzymes may cause lesions in the animals. Assuming that *T. longicornis* is capable of increasing digestive enzyme synthesis rates after short periods of
hunger as rapid as reducing it, then this reaction appears to be a primarily and efficient mechanism for saving metabolic energy without a lasting loss of physiological performance.

Citrate synthase is an important metabolic key enzyme of the tricarboxylic acid cycle. In several studies alterations in enzyme activity was shown to be related to the nutritional state of the animals (e.g. Clarke and Walsh 1993, Meyer et al. 2002). The *T. longicornis* females in our experiments, which were fed with *Rhodomonas*, maintained CS activities at an almost constant level. Accordingly, it can be suggested that these females maintained metabolic capacities similar to those from animals in the field. In contrast, a strong reduction of particularly CS activity was evident when the animals starved. These results are in accordance with previous work by Clarke and Walsh (1993) who showed that citrate synthase activity decreased in starving *T. longicornis* already after 24 hours. Apparently, starving *T. longicornis* reduce their capacity for aerobic energy generation due to both the lack dietary substrates and the lack of extensive storage products. The rapid reduction of metabolic rates enables the animals to extend their time of survival and reproduction.

The trophic biomarker concept is based on the observation that specific dietary fatty acids are incorporated largely unmodified into the lipid pool of the consumer (e.g. Sargent and Whittle 1981, Sargent et al. 1987, Graeve et al. 1994, Dalsgaard et al. 2003). In calanoid copepods from Polar regions they provide trophic information over a time scale of several weeks (e.g. Graeve et al. 1994). However, for small calanoid copepods like *Temora longicornis* information about time scales for the incorporation of dietary fatty acids is scarce. We found that the fatty acid composition changed rapidly in *T. longicornis*. The seston in the field contained high amounts of saturated fatty acids (16:0 and 18:0), which are characteristic for detritus (e.g. Kattner et al. 1983). Moreover, 18:1(n-9), which is a major fatty acid in most marine animals (e.g. Sargent and Falk-Petersen 1981, Falk-Petersen et al. 1990) accounted for about 11% of total fatty acids in the seston. This indicates that the seston predominantly contained detritus of poor nutritive quality and heterotrophic organisms. On the other hand, *Rhodomonas* spp. are characterised by the presence of polyunsaturated fatty acids with 18 C atoms, like 18:4(n-3), 18:3(n-3) and 18:2(n-6), and essential fatty acids such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (e.g. Jónasdóttir 1994) and sterols (Klein Breteler et al. 2004). Veloza et al. (2006) showed in feeding experiments with *Acartia tonsa* that dietary fatty acids are accumulated within five days. We observed an accumulation of specific *R. baltica* fatty acids 18:1(n-7), 18:2(n-6), 18:3(n-3) (linolenic acid), 18:3(n-6), 18:4(n-3) and 22:6(n-3) (DHA) already within three days. It can be suggested that the dynamics of trophic marker accumulation in *T. longicornis* may be even more rapid. As an exception the amount of the fatty acid 20:5(n-3) (EPA) decreased in the copepods although it showed higher values in *R. baltica* than the seston. The seston was pre-filtered over a 70 μm sieve. Therefore, larger organisms, which might contain elevated amounts of 20:5(n-3) and served as food for *T. longicornis* may have been excluded from the sample.
Several authors discussed that long chain (n-3)PUFAs are important for growth and development in marine calanoid copepods (e.g. Jónasdóttir 1994; Jónasdóttir and Kiorboe 1996). Therefore, the decrease of EPA and, to a lower extent, DHA may indicate that these fatty acids were used for reproductive processes, since egg production rate increased in females fed with R. baltica. In feeding experiments with A. tonsa Veloza et al. (2006) suggest that low amounts of EPA in A. tonsa may indicate that EPA is catabolised by the copepod. Jónasdóttir (1994) suggested that Acartia spp. females utilize energy from lipids to fuel biosynthesis of egg-yolk, and that specific dietary fatty acids, probably 20:5(n-3) and 22:6(n-3), are straight directed to vitellogenesis. Both fatty acids EPA and DHA are essential to copepods, since metazoans are not able at all or only to a low degree to synthesise these fatty acids de novo by e.g. elongation of linolenic acid (e.g. Brett and Müller-Navarra 1997). In our study the portion of EPA decreased in % FA as well as in % DM in copepods despite the fact that potential precursors such as 18:3(n-3) and 18:4(n-3) reached high amounts in R. baltica and were accumulated in T. longicornis. Therefore, we suggest that in T. longicornis EPA is the main fatty acid, which is metabolised during periods of elevated egg production rates.

In conclusion our results show that T. longicornis rapidly reacts to changes and food depletion and, thus, strongly depends on continuous food supply. It has limited energy stores and can survive only short periods of starvation. Upon starvation a sequence of successive physiological reactions appeared to save metabolic energy. These are a strong decrease in digestive proteases activity, followed by a decrease of metabolic enzyme activity and reduced egg production rates. A nutritional shift entailed a rapid change in the fatty acid composition, which again reflects the limited compensatory ability of T. longicornis. Due to these physiological preconditions it appears consistent that the distributional range of T. longicornis comprises predominantly the coastal areas and shallow waters (Krause et al. 1995 and references therein), which are rich in nutrients and, thus, provide continuously sufficient food.

Acknowledgements

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Figure legends

Fig. 1: Faecal pellet production rate (FPR, faecal pellets female\(^{-1}\) d\(^{-1}\)) of T. longicornis females under in situ conditions (day 0) and during the experiments incubated with Rhodomonas baltica or under starving conditions (n=24, mean ± standard deviation)

Fig. 2: Egg production rate (EPR, eggs female\(^{-1}\) d\(^{-1}\)) of fed (R. baltica) and starved T. longicornis females (n=24, mean ± standard deviation)

Fig. 3: Proteinase activity (dE\(_{366}\) min\(^{-1}\)·10\(^{-2}\) ind\(^{-1}\)) of fed (R. baltica) and starved T. longicornis females (n=3, mean ± standard deviation)

Fig. 4: Citrate synthase activity (units·10\(^{-2}\) ind\(^{-1}\)) of fed (R. baltica) and starved T. longicornis females (n=3, mean ± standard deviation)

Fig. 5: Fatty acid composition (% of total fatty acids) of seston and R. baltica (a), and of in situ T. longicornis and fed with R. baltica over three days (b) (n=3, mean ± standard deviation)

Fig. 6: Change in amounts of fatty acids of T. longicornis females after three days of incubation with R. baltica and under starving conditions in % of in situ amounts (amounts calculated in % dry mass)
Table 1: Dry mass (μg ind⁻¹) and lipid content in % dry mass (DM) of *in situ* females, females fed with *R. baltica* and starved females on day three (n = 3; mean ± standard deviation).

<table>
<thead>
<tr>
<th></th>
<th>dry mass (μg ind⁻¹)</th>
<th>lipid (% DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. baltica</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in situ</td>
<td>27.6 ± 6.8</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>d3</td>
<td>26.4 ± 9.6</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>starved</td>
<td></td>
<td></td>
</tr>
<tr>
<td>in situ</td>
<td>40.5 ± 2.9</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>d3</td>
<td>39.0 ± 3.3</td>
<td>4.7 ± 1.4</td>
</tr>
</tbody>
</table>
**Figure 1**

![Graph showing FPR (faecal pellets female$^{-1}$ d$^{-1}$) for R. baltica and starved conditions.](image)

**Figure 2**

![Graph showing EPR (eggs female$^{-1}$ d$^{-1}$) for R. baltica and starved conditions.](image)
Figure 3

Proteinase activity (D₆₅₀ min⁻¹·10⁻² ind⁻¹) over time for R. baltica and starved conditions.

Figure 4

Citrate synthase activity (units·10⁻² ind⁻¹) over time for R. baltica and starved conditions.
Figure 5

(a) % of total fatty acids in seston and R. baltica females.

(b) % of total fatty acids in situ females and R. baltica females.

Fatty acids:
- 14:0
- 16:0
- 16:1(n-9)
- 16:1(n-7)
- 16:2(n-4)
- 16:3(n-4)
- 18:0
- 18:1(n-9)
- 18:1(n-7)
- 18:2(n-6)
- 18:3(n-3)
- 18:3(n-6)
- 18:4(n-3)
- 20:4(n-3)
- 20:5(n-3)
- 22:6(n-3)

Legend:
- Seston
- R. baltica
- R. baltica females

in situ females
Figure 6

The figure shows the change in various fatty acids (% of total fatty acids) in the species *R. baltica* under starved conditions compared to normal conditions. The fatty acids listed include:

- 14:0
- 16:0
- 16:1(n-9)
- 16:1(n-7)
- 16:2(n-4)
- 16:3(n-4)
- 18:0
- 18:1(n-9)
- 18:1(n-7)
- 18:2(n-6)
- 18:3(n-3)
- 18:3(n-6)
- 18:4(n-3)
- 20:4(n-3)
- 20:5(n-3)
- 22:6(n-3)

The bars indicate the percentage change for each fatty acid under starved conditions, with the grey bars representing *R. baltica* and the striped bars representing the starved condition.
Influence of physiological conditions and feeding history on digestion, metabolism and fatty acid composition of Temora longicornis females (Crustacea, Copepoda) under different nutritional conditions

Kreibich T, Saborowski R, Hagen W, Niehoff B

planned for submission to Journal of Experimental and Marine Ecology
INFLUENCE OF PHYSIOLOGICAL CONDITIONS AND FEEDING HISTORY ON DIGESTION, METABOLISM AND FATTY ACID COMPOSITION OF *Temora longicornis* FEMALES (CRUSTACEA, COPEPODA) UNDER DIFFERENT NUTRITIONAL CONDITIONS

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Abstract

This experimental study focused on the physiological adaptation of *Temora longicornis* females to new dietary conditions by investigating changes in fatty acid (FA) patterns, digestive and metabolic activities as well as egg and faecal pellet production rates. In April and May 2005, during the spring phytoplankton bloom in the southern North Sea, we conducted two experiments over 3 days in each incubating *T. longicornis* females with either the diatom *Thalassiosira weissflogii*, the heterotrophic dinoflagellate *Oxyrrhis marina* or under starving conditions. Our results show that *T. longicornis* females react rapidly and very variably to changing nutritional conditions in order to cover their energetic demands. Feeding history and physiological conditions of females prior to the experimental period strongly influenced the response of the digestive activities, lipid accumulation processes, as well as catabolism of different energetic compounds during starvation. However, independent of physiological conditions, dietary FAs were rapidly incorporated into copepods’ lipids resulting in fast changes of FA patterns. Hence, the digestive system must be characterised by a strong adaptive potential and high plasticity. Fed females in April increased their proteinase activity, whereas females in May reduced their digestive activity. These different reactions were due to different initial activities in the field, showing that females were able to adjust digestion specifically to changing conditions. For the first time lipase/esterase patterns were analysed in copepods qualitatively by SDS-PAGE showing a diet-induced expression of specific lipases/esterases in copepods. This strong adaptive potential led to fast changes in functional responses of copepods, i.e. egg production rate (EPR). EPR increased under surplus food conditions which points to a rapid digestion of dietary components and an efficient transformation into egg material and/or use to fuel metabolic processes during both experiments.
Key words: Temora longicornis, physiological adaptation, feeding, digestion, metabolism, fatty acids, lipase

Introduction

The North Sea is characterised by high seasonal variability in abiotic and biotic factors (e.g. Gieskes and Kraay 1984, Kiørboe and Nielsen 1994, Krause et al. 1995), generally showing two phytoplankton blooms, one in spring and another in autumn (e.g. Fransz et al. 1991, Skogen and Moll 2000). Calanoid copepods constitute the most important portion of the zooplankton in the North Sea in terms of both biomass and abundance (Hickel 1975, Fransz et al. 1991). The small calanoid copepod Temora longicornis, a temperate and neritic species, is one of the most abundant calanoid copepod species in the North Sea (e.g. Krause et al. 1995, Halsband-Lenk et al. 2002). It feeds on phytoplankton, microzooplankton and younger copepod stages (e.g. Kleppel 1993, Dam and Lopes 2003).

Due to their physiological properties, i.e. rapid turnover of biomass and simultaneously low capacity to build up energy stores of lipids (e.g. Båmstedt 1986, Mayzaud et al. 1992, Evjemo and Olsen 1997, Helland et al. 2003), T. longicornis relies on continuous food supply and cannot starve over prolonged time. Thus, a high adaptive digestive potential is crucial to digest a wide spectrum of dietary compounds in order to provide the organism with energetic components. During the last decades several studies focused on the adaptation of digestive activities of copepods to changing nutritional conditions in the field as well as in the laboratory. Different reactions of the digestive system of copepods were observed. In some studies an increase of digestive activity due to increasing food supply was shown (Mayzaud and Conover 1975, Mayzaud and Poulet 1978, Cox 1981), whereas other authors observed a decrease of the activity (Hassett and Landry 1983, Harris et al. 1986), or even no changes appeared despite increasing food concentration (e.g. Head et al. 1984, Kreibich et al. submitted). To explain these contradictions, some authors proposed that metabolic requirements, life strategies and feeding history have a major influence on the adaptive digestive potential (Hassett and Landry 1983, Roche-Mayzaud et al. 1991, Mayzaud et al. 1992). Therefore, one aim of the present study was to investigate the response of the digestive proteinase and lipase/esterase to changing nutritional conditions in T. longicornis females with different feeding histories.

Proteinases are the major group of protein-digesting enzymes in crustaceans (Gibson and Barker 1979). They catalyse the hydrolysis of peptide bonds within proteins (e.g. García-Carreño 1992). However, information on the total proteolytic potential of copepods is scarce (Mayzaud and Conover 1975). Most studies focussed on a single serine proteinase, i.e.
trypsin (e.g. Hirche 1981, Harris et al. 1986, Båmstedt 1988). Dietary lipids are digested by lipases and esterases (e.g. Mayzaud 1986, Díaz et al. 1999). However, limited information is available concerning lipase/esterase activities in copepods (Knotz et al. 2006). This is surprising, since lipids and fatty acids (FAs) in copepods received great attention during the last decades due to their function e.g. during reproduction, ontogeny or buoyancy, or as nutritional biomarkers (e.g. Dalsgaard et al. 2003 and references therein, Lee et al. 2006 and references therein). Therefore, for the first time, a method is presented characterising lipases/esterases qualitatively in copepods in order to gain information on possible diet-induced changes in lipase/esterase patterns which may indicate whether copepods are capable of adapting to nutritional changes on a qualitative enzymatic level.

We performed two experiments over three days by feeding *T. longicornis* females either with the diatom *Thalassiosira weissflogii*, the heterotrophic dinoflagellate *Oxyrrhis marina*, which was fed with *Rhodomonas baltica*, or incubated females under starving conditions. The assimilation of ingested food was traced via FA trophic biomarkers (e.g. Lee et al. 1971, Sargent and Whittle 1981, Sargent et al. 1987, Pond et al. 2002). In a recent study by Kreibich et al. (submitted) it was shown that *Temora longicornis* females react fast to changing nutritional conditions by largely accumulating dietary fatty acids (FAs) already within three days. However, since algal cultures, even of the same species, can differ in their FA composition and their lipid content (e.g. Ackman et al. 1964, Kattner et al. 1983 Jónasdóttir 1994), the question arises whether these fluctuations influence accumulation processes in copepods. In order to answer this question copepods were fed with algal cultures of different ages.

In addition to the measurements of digestive activities and FA compositions of copepods and diets, egg and faecal pellet production rates as well as metabolic activities were measured in order to document the efficiency of the transformation of ingested food into egg material.

**Materials and Methods**

**Sampling**

*Temora longicornis* females were sampled in April (Experiment 1) and May 2005 (Experiment 2) at Helgoland Roads (54°11'N, 07°54'E), southern North Sea. A CalCoFi net (500 μm mesh size) was towed for 10 min in 10 m depth at a speed of 0.3 m s⁻¹. Immediately after capture, specimens were transferred to the institute. Approximately 1500 healthy looking females were sorted out alive under a stereo-microscope. Females were shortly rinsed in demineralised water and shock-frozen at -80°C in groups of ten for subsequent analyses of their physiological condition in the field. Additionally, water samples were taken in 3 m depth at the
sampling station with a 10 l Niskin bottle and filtered over a 70 μm sieve. Thereafter, volumes of 500 ml were filtered on precombusted GF/C filters (0.2 μm mesh size). The filters were stored at −80°C for fatty acid (FA) analysis and carbon and nitrogen measurements.

**Experimental work**

**Algae cultures**

The diatom *Thalassiosira weissflogii* and the heterotrophic dinoflagellate *Oxyrrhis marina* were grown in 1 to 10 l bottles with f/2 medium (Guillard 1975) over several weeks under constant light. The heterotrophic dinoflagellate *O. marina* was fed with *Rhodomonas baltica*. Every day, bottles were gently shaken two to three times in order to keep the cells in suspension. All bottles were constantly supplied with air. Cell concentrations of the diets were measured using a cell counter (CASY® Model TTC, Schärfe System GmbH). At the beginning of each experiment, a defined volume of the cultures was filtered on precombusted GF/C filters in triplicates and stored at -80°C for FA, carbon and nitrogen analysis.

**Experiments**

*T. longicornis* females were fed with *Thalassiosira weissflogii* and *Oxyrrhis marina* for three days. Additionally, females were kept starving in 0.2 μm filtered seawater. Two days before the feeding of *T. longicornis* started, feeding of *O. marina* was stopped to reduce the density of *R. baltica* cells. In every treatment about 150 healthy looking females were incubated in each of three 2.5 l beakers. Algal densities were 8,000 and 2,000 cells ml⁻¹ (*T. weissflogii* and *O. marina*, respectively) to ensure surplus food. Beakers were kept in the dark and at constant temperature of 6 and 9°C, respectively, according to the outdoor water temperature. Every day groups of 10 females were sorted and stored at –80°C for later analysis. On the second day, approximately half of the incubation water was replaced by new filtered seawater and algae.

**Analytical work**

**Carbon and nitrogen content of seston and cultures**

For the determination of carbon and nitrogen content filters with seston and cultures were dried for 48 h at 60°C and weighed on a microbalance (Sartorius, ±2 μg), wrapped in Zn-foil and analysed in a C:H:N Element Analyzer (EuroVector, Euro EA Element Analyzer).

**Dry mass, protein, total lipid content and fatty acid analysis**

Dry mass of copepod samples was measured after 24 h of lyophilisation (Leybold-Heraeus, LYOVAC GT2) using a micro-balance (Sartorius, ± 2 μg). Lyophilised samples were stored at -80°C for analysis of the FA composition.
Water-soluble proteins were quantified after Bradford (1976) using a commercial protein assay (BioRad, 500-0006), as described by Kreibich et al. (in prep.). Bovine serum albumin (BSA) was used as standard. Samples were homogenised in demineralised water, centrifuged and supernatants were measured for protein content.

Lipid extraction and transesterification of seston, algal culture and copepod samples were carried out as described in Kreibich et al. (in prep.). Briefly, lipids were extracted in a defined volume of dichloromethane:methanol (2:1/v:v) at -80°C for 24h. Thereafter a defined volume of the internal standard tricosanoic acid (23:0) was added to the samples which were then sonicated and further treated essentially after Folch et al. (1957) as described in Peters et al. (2006). Sub-samples of total lipids were used for the hydrolysis of lipids and their conversion into FA methyl esters (FAMEs, Kattner and Fricke 1986).

**Measurement of enzyme activities**

The digestive potential of *Temora longicornis* females was represented by the activities of proteinases (endopeptidases), amylase and lipase/esterase. The metabolic activity was estimated by means of the metabolic key enzymes, pyruvate kinase (PK) and citrate synthase (CS). All samples and controls were assayed photometrically or fluorometrically in triplicates.

Total proteinase activity was measured as described by Kreibich et al. (submitted). Briefly, deep-frozen samples were homogenised in buffer, centrifuged and supernatants were incubated with azocasein as substrate. After incubation of samples and controls, the absorbance of the supernatants was measured at 366 nm.

The lipolytic activity (carboxylic ester hydrolases (E.C. 3.1.1)) of *Temora longicornis* females was determined fluorometrically in single individuals with 4-Methylumbelliferyl butyrate (Fluka BioChemika, 19362) as substrate (Knotz et al. 2006). Standard curves were prepared with 4-Methylumbelliferone (Sigma, M1381). Deep-frozen individuals were homogenised in 200 μl ice-cold 0.1 mol l⁻¹ Tris/HCl-buffer (pH 7.0) with a micropestle and centrifuged for 10 min at 15,000 g (4°C). Thereafter, the supernatants were transferred into new microtubes and kept on ice. Assays were run at 25°C with 10 μl of sample. Fluorescence was measured at 360 nm (ex) and 450 nm (em) after 30 min of incubation with a Kontron SFM 25 device. The rate of autolysis was determined and subtracted from the results. Samples and blanks were run in triplicates. Enzyme activities were expressed in nmol h⁻¹ ind⁻¹.

The metabolic enzyme activity was measured photometrically. Citrate synthase activity (CS; EC 4.1.3.7) was determined after Stitt (1984) with modifications as described by Saborowski and Buchholz (2002).
Electrophoretic analysis

Proteins were separated by discontinuous SDS-PAGE (Laemmli 1970, Schägger and von Jagow 1987) on mini-gels (80 x 100 x 0.75 mm, Hoefer, SE 250). The acrylamide concentration of the stacking gel was T=4%, C=2.6% and that of the separation gel was T=12.3%, C=2.6%. The electrode buffer was a 0.025 mol l⁻¹ Tris/HCl buffer with 0.192 mol l⁻¹ glycine and 0.1% SDS at pH 8.3. Individual copepods from Experiment 2 were homogenised in 15 μl a. dem. and 15 μl sample buffer (25% stacking gel buffer (0.5 mol l⁻¹ Tris/HCl, pH 6.8), 20% bromphenol blue, 30% glycerine, 4% SDS, 21% a. dem.) with a micropestle. The tubes were centrifuged for 10 min (15000 g, 4°C). Ten μl of the supernatants and a molecular weight marker (Sigma, M4038) were applied onto the gels. Two gels were loaded with the same samples and were run at the same time at 150 V, 30 mA, and 2°C. After the run lipolytic bands were made visible on the gels as described by Díaz et al. (1999). Gels were gently washed with a. dem. and incubated in 2.5% Triton X 100® for 30 min at room temperature. Afterwards the gels were washed briefly in 50 mmol l⁻¹ phosphate buffer (pH 7.0) and subsequently incubated for 5-10 min in a 100 μmol l⁻¹ MUF-butyrate solution (diluted in 50 mmol l⁻¹ phosphate buffer, pH 7.0). Images were taken with a ChemiDoc XRS System (Bio-Rad) at UV illumination and analysed with the Quantity One Software (Bio-Rad). The gels were washed briefly with a. dem., subsequently stained with the PlusOne Silver Staining Kit (GE Healthcare, 17-1150-01) and photographed.

Statistics

The Kolmogorov-Smirnov test (with Lilliefors' correction) was used to test for normal distribution of data sets. If statistical tests required normal distribution then the data sets were arc sine square root transformed. Homology of variances was tested with the Levene's test. Differences between groups were tested either with a Student’s t-test or one-way ANOVA followed by the Holm-Sidak post-hoc test. All analyses were carried out with the programme SigmaStat version 3.5 (Systat Software, Inc.).

Results

Experiment 1 (April 15-18)

Dry mass, protein and lipid content of copepods

Dry mass and protein content of females from the field and females used in the experiment were not significantly different. They ranged between 30.5 and 42.3 μg ind⁻¹, and between 11.3 and 14.9 μg ind⁻¹, respectively (Table 1) (one-way ANOVA, p>0.05). Lipid contents amounted to 1.8 μg ind⁻¹ in females from the field and increased to 2.3 and 2.8 μg ind⁻¹ in
females fed with *T. weissflogii* and *O. marina*, respectively. In starving females lipid content decreased to 1.0 μg ind⁻¹. The increase in females fed with *O. marina* was statistically significant compared to females from the field (*t*-test, *p*<0.01). The number of replicates of females fed with *T. longicornis* and starving females were insufficient in order to perform statistical tests (one and two samples, respectively).

**Fatty acid compositions of diets and copepods**

Seston lipids on April 15 were dominated by the saturated fatty acids (SFAs) 16:0 with 34.2% of total fatty acids (TFA) and 18:0 with 13.9% TFA, followed by the monounsaturated fatty acids (MUFTs) 18:1(n-9), 16:1(n-7) and 18:1(n-7), which ranged between 11.1 and 7.1% TFA (Fig. 1a). The diatom *T. weissflogii* was characterised by high levels (>15% TFA) of 16:0, 20:5(n-3) and 16:3(n-4); 16:1(n-7) reached 9.3% TFA. The polyunsaturated fatty acid (PUFA) 18:4(n-4), not detectable in seston and *O. marina*, represented 5.9% TFA. The FA pattern of the heterotrophic dinoflagellate *O. marina* was dominated by 22:6(n-3), 16:0 and 16:1(n-7) (>16% TFA), 20:5(n-3) amounted to 10.4% TFA. The total lipid content of *T. weissflogii* was 23.1 μg mgC⁻¹, and of *O. marina* 52.9 μg mgC⁻¹ (Table 2).

The females from the field were characterised by high portions of 20:5(n-3) (33.4% TFA), followed by 22:6(n-3) and 16:0 (19.3 and 18.2% TFA, respectively). Fed and starving females showed clear differences in FA patterns (Fig. 1b). In females fed with *T. weissflogii* the PUFA 22:6(n-3) was reduced to 15.0% TFA and 20:5(n-3) to 24.0% TFA. 16:3(n-4) increased strongly from 1.0 to 8.3% TFA and the MUFA 16:1(n-7) from 3.6 to 6.7% TFA. 18:4(n-4) was present with 4.0% TFA. The amount of the PUFA 22:6(n-3) increased in females fed with *O. marina* to 33.0% TFA, 20:5(n-3) decreased to 15.8% TFA. The MUFTs 16:1(n-7) and 18:1(n-7) reached 10.7% TFA and 4.8% TFA, respectively. Compared to females from the field starving females showed a similar FA pattern, except for 22:6(n-3), which had a relatively higher amount of 28.0% TFA, and the FAs 16:1(n-7) and 18:4(n-3), which were reduced to 1.8 and 2.9% TFA, respectively.

Mass-specific FA accumulation and reduction was calculated as the difference in % of the amount in females from the field in % DM and the amount in % DM after three days of incubation (Fig. 2). Females fed with *T. weissflogii* showed highest accumulation of 16:3(n-4), 16:0, 16:1(n-7) and 18:4(n-4), ranging between 0.47 and 0.24% DM, and reduced the amount of 18:4(n-3) (-0.14% DM). In contrast, in females fed with *O. marina* there was a strong increase of the FAs 22:6(n-3), 16:0 and 16:1(n-7), which ranged between 1.73 and 0.68% DM, and a decrease of 20:5(n-3) (-0.22% DM). Starving females reduced the amount of 20:5(n-3), 16:0, 18:4(n-3) and 16:1(n-7) (-0.17 to -0.08% DM), but had a higher level of 22:6(n-3) as compared to the females from the field.
Digestive enzyme activity of copepods

Proteinase activity differed significantly between the groups during the experiment (Fig. 3a). Females from the field showed proteinase activities of $10.0 \cdot 10^{-3} \text{ (dE}_{366} \text{ min}^{-1} \text{ ind}^{-1})$. In fed females, activity increased significantly already within the first day (one-way ANOVA, $p<0.001$). The activity in starving females decreased from day two on. Highest values were measured on day two for females fed with $O. marina$ with $22.1 \cdot 10^{-3} \text{ (dE}_{366} \text{ min}^{-1} \text{ ind}^{-1})$, and lowest values for starving females on day three ($6.6 \cdot 10^{-3} \text{ dE}_{366} \text{ min}^{-1} \text{ ind}^{-1}$).

Citrate synthase activity of copepods

Citrate synthase (CS) activities reached $11.4 \mu \text{U ind}^{-1}$ in females from the field (Fig. 4a) and did not change significantly under laboratory conditions, ranging then between 8.1 and $13.7 \pm 1.1 \mu \text{U ind}^{-1}$. CS activity was slightly elevated in fed females compared to starving females during the experiment (one-way ANOVA, $p<0.05$).

Egg production rate and faecal pellet production

Egg production rate (EPR) in females from the field was $38.4 \text{ eggs ind}^{-1} \text{ d}^{-1}$ and increased in fed females, ranging between 41.0 and 82.1 eggs ind$^{-1} \text{ d}^{-1}$ (Fig. 5a). Highest EPR was measured in females fed with $O. marina$ on day one. EPR in starving females decreased during the experiment with a minimum of $9.5 \text{ eggs ind}^{-1} \text{ d}^{-1}$ on day three. Faecal pellet production (FPR) was $4.7 \text{ pellets ind}^{-1} \text{ d}^{-1}$ in females from the field and increased in females fed with $O. marina$ and $T. weissflogii$ to 55.3 and 40.5 pellets ind$^{-1} \text{ d}^{-1}$ on day one, respectively (Fig. 5b). Thereafter, FPR decreased slightly in both groups to approx. 36 pellets ind$^{-1} \text{ d}^{-1}$ on day three, each. FPR in starving females decreased during the experiment and reached lowest values on day two (1.9 pellets ind$^{-1} \text{ d}^{-1}$).

Experiment 2 (May 12-15)

Dry mass, lipid and protein content of copepods

The dry mass of the females from the field, fed and starving females did not differ significantly (one-way ANOVA, $p>0.05$) and ranged between 45.3 and 38.2 $\mu \text{g ind}^{-1}$ (Table 1). Lipid contents ranged between 2.72 and 1.83 $\mu \text{g ind}^{-1}$ and were significantly higher in females from the field and those fed with $T. weissflogii$ compared to females fed with $O. marina$ and starving ones (one-way ANOVA, $p<0.05$). The water-soluble protein content was significantly higher in females fed with $T. weissflogii$ ($20.1 \mu \text{g ind}^{-1}$) than in the other groups, where it ranged between 10.7 and 15.2 $\mu \text{g ind}^{-1}$ (one way ANOVA, $p<0.001$) (Table 1).
**Fatty acid compositions of diets and copepods**

The seston on May 12 was characterised by a heterogeneous FA composition (Fig. 6a). Only two FAs were present in elevated amounts, the SFA 16:0 (28.4% TFA) and the MUFA 16:1(n-7) (15.6% TFA). Others did not exceed 7.0% TFA. *T. weissflogii* was characterised by a high portion of 16:3(n-4) with 20.5% TFA, followed by 16:0, 16:1(n-7), 20:5(n-3) and 18:4(n-4), which ranged between 14.7 and 11.4 % TFA. The FA pattern of *O. marina* was characterised by mainly two FAs, the PUFA 22:6(n-3) with 41.2% TFA and the SFA 16:0 with 23.6% TFA. The FA content of *T. weissflogii* during this experiment was 118.2±12.3 μg mg⁻¹ C, and of *O. marina* 152.5±7.6 μg mg⁻¹ C (Table 2).

*T. longicornis* females showed remarkable differences in their FA pattern between females from the field and after three days of incubation (Fig. 6b). The MUFA 16:1(n-7) was highest in females from the field (10.6% TFA) and was reduced in all three treatments, however, significant differences were detected only between females fed with *O. marina* (3.4% TFA) and the other three groups (one-way ANOVA, p<0.01, Holm-Sidak post hoc test). The PUFAs 16:2(n-4) and 16:3(n-4) reached significantly higher amounts in females fed with *T. weissflogii* with 2.3% TFA and 4.2% TFA, respectively (one way ANOVA, p<0.01). The PUFA 18:4(n-3) was reduced significantly in all three treatments (one way ANOVA, p<0.01) and ranged between 2.3 and 4.3% TFA compared to the females from the field with 6.2% TFA. In females fed with *T. weissflogii* the FA 18:4(n-4) reached 5.6% FA, and was not detectable in the other groups. The PUFA 22:6(n-3) increased strongly in females fed with *O. marina* up to 35.9% TFA compared to the other groups (ANOVA, p<0.01). Here, this PUFA ranged between 13.5% and 17.3% TFA.

Females fed with *T. weissflogii* accumulated 18:4(n-4), 16:3(n-4) and 16:2(n-4) in a range between 0.35 and 0.08% DM (Fig. 7). Strongest decrease was observed for 20:5(n-3) (-0.52% DM). Females fed with *O. marina* accumulated exclusively 22:6(n-3) (0.79% DM), whereas 20:5(n-3) and 16:1(n-7) were markedly reduced (-0.98 and -0.53% DM, respectively). The strongest decrease in starving females occurred in 20:5(n-3), 16:1(n-7), 16:0 and 18:4(n-3) ranging between -0.56 and -0.20% DM.

**Quantitative digestive activity of copepods**

The proteinase activity changed significantly during the experiment (Fig. 3c). The highest value was measured in females from the field (21.1·10⁻³ dE₃₆₆ min⁻¹ind⁻¹). The activity decreased significantly in all three treatments already at day one (one way ANOVA, p<0.001). After three days of incubation, the proteinase activity in females fed with *T. weissflogii* and fed with *O. marina* reached 16.5·10⁻³ and 12.0·10⁻³ (dE₃₆₆ min⁻¹ind⁻¹), respectively. In starving females, the proteinase activity was reduced to 3.4·10⁻³ (dE₃₆₆ min⁻¹ind⁻¹).
No significant changes in the lipase activity were detected between females from the field and fed females: activities ranged between 35.7 and 41.4 nmol h\(^{-1}\) ind\(^{-1}\) (Fig. 8). In starving females the lipase activity decreased significantly to 19.9 nmol h\(^{-1}\) ind\(^{-1}\) (one way ANOVA, p<0.01).

**Qualitative lipolytic enzyme activity of copepods**

The SDS-PAGE analyses on single copepods incubated under different nutritional conditions revealed changes in the lipolytic enzyme patterns (butyrate esterase, Fig. 9). In total, nine activity bands were detected with apparent molecular weights ranging from 185 kDA (band 1) to 22 kDA (band 9). Four main activity bands were detected in females from the field and fed females (band 1-3 and 7). In starving females only band 1 was detected in all three individuals. In females fed with *T. weissflogii* a specific band appeared at approx. 85 kDA (band 5). Females fed with *O. marina* were characterised by a strong activity band at approx. 100 kDA (band 4). Starving females showed a high individual variability in bands and their intensity.

**Citrate synthase activity of copepods**

The citrate synthase (CS) activity in fed females did not change significantly compared to values in females from the field and ranged between 7.8 and 10.8 μU ind\(^{-1}\) (Fig. 4b). In contrast, CS activity in starving females decreased continuously during the experiment and reached 6.1 μU ind\(^{-1}\) on day three (one way ANOVA, p<0.01).

**Egg production rate and faecal pellet production**

Females from the field produced 24.6 eggs ind\(^{-1}\)d\(^{-1}\). EPR increased when females were fed with *O. marina* and *T. weissflogii* and reached the maximum on day one with 52.9 and 62.8 eggs ind\(^{-1}\)d\(^{-1}\), respectively (Fig. 5c). Thereafter, EPRs decreased and reached 21 eggs ind\(^{-1}\)d\(^{-1}\) on day three. EPR of starving females decreased continuously during the experiment to the minimum of 11.1 eggs ind\(^{-1}\)d\(^{-1}\) on day three.

Females from the field produced 10.3 faecal pellets ind\(^{-1}\)d\(^{-1}\). When fed with *O. marina* and *T. weissflogii* FPR increased strongly and reached 39.0 and 36.2 faecal pellets ind\(^{-1}\)d\(^{-1}\) already on day one (Fig. 5d). Thereafter, FPR decreased strongly in females fed with *O. marina* and reached lowest values on day two with 10.7 faecal pellets ind\(^{-1}\)d\(^{-1}\). In contrast, FPR in females fed with *T. weissflogii* was high throughout the entire experiment with maximum values on day two (41.6 faecal pellets ind\(^{-1}\)d\(^{-1}\)). FPR in starving females decreased already on day one, FPR ranged between 2.2 and 4.2 faecal pellets ind\(^{-1}\)d\(^{-1}\) during the experiment.
Discussion

Changes in fatty acid patterns

Our study showed that *Temora longicornis* females were able to adapt very fast to changing nutritional conditions including the whole range of physiological parameters tested. They showed rapid change of fatty acid (FA) composition as well. These results confirm other studies on diet-dependent FA accumulation in small copepods (Veloza et al. 2006, Kreibich et al. submitted). In addition, the present results indicate that the degree of FA accumulation depends on several factors other than the FA composition of the diet. These include the initial lipid content as well as the FA pattern and feeding history of the consumer.

Females feeding on the diatom *Thalassiosira weissflogii* showed an increase in the polyunsaturated FAs (PUFAs) 16:2(n-4), 16:3(n-4) and 18:4(n-4) which are typical for the diatom culture used in these experiments. Simultaneously, they showed a reduction of e.g. 18:4(n-3) which are specific for dinoflagellates (Sargent et al. 1987, Graeve et al. 1994). The unusual occurrence of 18:4(n-4) in the cultures (confirmed by GC-MS, Graeve unpubl. data) has, to our knowledge, not yet been reported in marine algae. The origin of this FA is unknown and further investigations are needed to clarify this appearance.

Although copepod females were feeding on the same algae cultures in both experiments, differences were detected in the degree of FA accumulation. In Experiment 1, FAs were accumulated to a much higher extent than in Experiment 2 (see Figs. 2 and 4), probably due to lower initial lipid contents in females from the field (Table 1). On April 15 (Experiment 1), the seston was characterised by lower lipid contents and higher portions of saturated fatty acids (SFAs) than on May 12 (Experiment 2). Higher contents of SFAs indicate the dominance of detritus in seston samples (Kattner et al. 1983). In May, in contrast, lipid-rich diatoms and dinoflagellates dominated the seston community, indicated by high levels of diatom- and dinoflagellate-specific FAs such as the monounsaturated fatty acid (MUFA) 16:1(n-7) and the PUFAs 20:5(n-3) (eicosapentaenoic acid, EPA), 18:4(n-3) and 22:6(n-3) (docosahexaenoic acid, DHA) (e.g. Dalsgaard et al. 2003 and references therein). FA composition of copepods indicates that females caught in May were feeding on diatoms and dinoflagellates, since copepods are not able to synthesise these long chain PUFAs *de novo*, or only to a small extent, but directly incorporate them from the diet (Sargent and Whittle 1981, Sargent et al. 1987, Dalsgaard et al. 2003 and references therein). It is likely that feeding on these algal species resulted in an increase of total lipid contents in copepods from April until May. Presumably due to higher lipid content, these females did not accumulate lipids at surplus food but showed a shift in their FA pattern, whereas females caught during April increased their lipid content by approx. 45 to 80% when feeding on *T. weissflogii* and *O. marina*, respectively. As the lipid content did not increase beyond 6% DM during the second
experiment the question arises if lipid accumulation reached its physiological limit. This, however, was obviously not the case, since copepods in the field were characterised by higher lipid contents (up to 9% DM) and females with low initial lipid contents reached lipid levels of up to 8% DM when feeding on O. marina.

Surprisingly, the content of 16:1(n-7) in % DM as well as in % TFA in females feeding on T. weissflogii in Experiment 2 decreased although this FA reached approximately 13% of TFA in the culture. This maybe due to high portions of this FA in both copepods and seston in the field in May than in our culture (Fig. 3). We suggest that high contents of a specific dietary FA in copepods from the field may mask feeding on a specific diet characterised by this FA in experimental studies. These results indicate that feeding history of copepods should be considered when interpreting FA accumulation processes in copepods during recent feeding events, e.g. during experiments.

Besides feeding history and copepod’s lipid content influencing the degree of FA accumulation in copepods, the lipid content and FA composition of a culture obviously has an effect on accumulation processes. As documented by e.g. Fernández-Reiriz et al. (1989) lipid content in diatoms can increase with the development of the culture and PUFAs reach highest values during the late stationary phase. This is in accordance with our results showing that T. weissflogii and O. marina cultures reached approx. 3- to 5-fold higher lipid levels and higher PUFA contents in May than in April. The Oxyrrhis marina cultures differed in addition in their 16:1(n-7) content. This FA, which actually is a specific diatom FA, reached high portions in the culture used in Experiment 1. In previous studies, 16:1(n-7) was detected in only low amounts (≤1% TFA) or was even absent in O. marina fed with Rhodomonas spp. (Klein Breteler et al. 1999, Veloza et al. 2006). This could indicate that diatoms contaminated the cultures. However, cultures were frequently checked under a microscope at least every week and always prior to an experiment but no diatom cells were visually detected. Thus, we cannot rule out that O. marina might have the potential to synthesize this FA de novo, to test this hypothesis, however, was beyond the scope of the present paper. Due to changes in the FA composition of the cultures, a direct comparison of the dietary effects of O. marina on T. longicornis is limited. Nevertheless, both O. marina cultures induced strong changes in the fatty acid patterns of the copepods, mainly resulting in increasing contents of 22:6(n-3) in copepods’ lipids.

Despite of higher lipid contents of both T. weissflogii and O. marina cultures fed during Experiment 2, females accumulated larger lipid amounts during Experiment 1. We therefore suggest that lipid accumulation not only depends on the biochemical composition of the food type ingested but on the pre-condition of the copepods in terms of their physiological characteristics and needs.
The lipid content in starving females in both experiments decreased strongly. Mainly diatom- and dinoflagellate-specific FAs such as 16:1(n-7), 18:4(n-3), 20:5(n-3) and 18:2(n-6) were reduced indicating that these were rapidly metabolised. With decreasing biomass by -28%, the relative content of the essential membrane compound DHA increased in the copepods’ lipids during Experiment 1. Its total amount remained almost constant since it has not been metabolised for energy production as most of the other FAs. Upon starvation A. clausi as well as T. longicornis catabolise endogenous proteins (Mayzaud 1976, Helland et al. 2003). However, in Experiment 1 of the present study protein and lipid content decreased only slightly which indicates that females catabolised both proteins and lipids in order to provide enough energy for metabolic processes. Interestingly, females in Experiment 2 showed a decrease in their lipid content by almost 28%, whereas protein content and dry mass did not change significantly. However, it should be considered that higher standard deviations may mask differences in dry mass of copepods after starvation. The decrease in lipid content is in accordance with a previous study on T. longicornis (Evjemo 2001 cited in Helland et al. 2003). The author showed that starvation for 64 h can result even in a loss of approx. 50% of total lipids in T. longicornis. We assume therefore that starving T. longicornis females are able to catabolise different types of macromolecules in order to provide energy for metabolic processes. It seems likely that primarily lipids are used to fuel basal physiological processes during starvation when copepods accumulated lipid reserves. After the depletion of the lipid reserves starving copepods may progressively catabolise endogenous proteins.

Response in digestive activity

In earlier studies, responses in digestive enzyme activities of copepods to different feeding conditions led to controversial conclusions. Some authors observed an increase of activities in copepods or bulk extracts of total zooplankton communities to increasing concentrations of substrates in the diet, e.g. proteins and carbohydrates (i.e. Mayzaud and Conover 1975, Mayzaud and Poulet 1978, Cox 1981, Cox and Willasson 1981, Hirche 1981). Other studies showed negative relationships between concentration of potential food and digestive enzyme activities (e.g. Hassett and Landry 1983, Harris et al. 1986, Hassett and Landry 1988). Our results on Temora longicornis show that both reactions under surplus food conditions can occur, even when the copepods were feeding on the same diet. In Experiment 1, proteolytic activity of fed females increased, whereas activities decreased in Experiment 2. At first sight, this seems contradictory. However, later studies in the 1990s discussed factors others than food concentration, which may influence digestive enzyme activities under changing nutritional conditions. Roche-Mayzaud et al. (1991) suggested for Acartia clausi that feeding is likely controlled by feedback mechanisms which operate to meet the metabolic requirements of individuals. In our study, proteinase activity was significantly lower in females caught in April
than in May, indicating different feeding conditions.Apart from this difference, females reached similar proteolytic activities in both experiments when feeding on *T. weissflogii*. This may indicate that the digestive system adapted similarly to this monoculture. Feeding on a monoculture may result in a small spectrum of enzymes needed for the digestion of this specific diet, which may lead to similar proteinase activities. However, whether or not this proteolytic activity is actually diet-induced needs further confirmation.

It is suggested that digestive enzymes of copepods need at least 24 h to acclimate to different conditions (e.g. Mayzaud and Poulet 1978, Roche-Mayzaud et al. 1991, Mayzaud et al. 1992, Mayzaud et al. 1998). Our data, however, have shown pronounced changes in activities after one day, and this suggests that the digestive system is able to adapt faster than previously thought. But the study of Smith and Hall (1980) on *Temora longicornis* indicates that copepods seem to be able to adapt within few hours to changing nutritional conditions. By applying radioisotope tracers, the authors showed that already after 4 h radioactive grains were deposited to some extent in the cells of the gut-wall of the copepods. We assume that copepods were able to adjust their digestive system within few hours to new feeding conditions. Therefore, we propose to test the adaptive digestive potential in copepods in short time scaled experiments.

Even under extreme conditions, i.e. starvation, the response of the digestive enzyme system may vary. Starving females varied in their response of the digestive activity to new conditions. Some studies showed that the course of digestive activities in starving animals is characterised by an initial period of stability or small increase followed by a decrease (Boucher and Samain 1974), whereas others observed that the activity decreases in absence of food without an initial period of stability (Cox and Willason 1981). Mayzaud and Mayzaud (1985) showed for *A. clausi* that the response of the digestive activity under starving conditions depends on the investigated enzyme. In our study, both scenarios were observed and therefore we cannot support the observations made by Mayzaud and Mayzaud (1985) but suggest for *T. longicornis* that the reaction depends on the initial enzyme activity copepods exhibited in the field. A 24 h period of stability in proteinase activity was observed in copepods, which had lower activities in the field, whereas copepods with higher initial activities reduced their activity already within the first 24 h.

The prerequisite for the observed accumulation of dietary FAs is the retrieval of lipid hydrolysing enzymes. Our investigation shows that, despite of similar quantitative lipase/esterase activities in fed and in females from the field, feeding on different diets results in different qualitative lipase/esterase patterns. Lipases specifically hydrolyse ester bonds in lipids (Christie 1973). E.g. phospholipase A acts on phosphatidylcholine with the release of unsaturated FAs (Hanahan et al. 1960). Since phospholipids in dinoflagellates are
predominately composed of the FAs 22:6(n-3) and 16:0 (Leblond and Chapman 2000), we assume that the specific lipase/esterase band in *T. longicornis* females fed with *O. marina* constitutes a phospholipase. This lipase may hydrolyse the ester bonds in phospholipids releasing the PUFA 22:6(n-3), which reached high portions in *O. marina* and was strongly accumulated in females’ lipids. When feeding on *T. weissflogii*, females showed another lipase band which seems to be diet-induced as well, responsible for the hydrolisation of lipids containing *T. weissflogii*-specific FAs like 16:2(n-4), 16:3(n-4) and 18:4(n-4). Polar lipids are the most abundant lipid class in microalgae such as diatoms, which indicates the presence of another specific phospholipase hydrolysing phospholipids of *T. weissflogii*.

Theoretically, these diet-specific lipases in *T. longicornis* can be obtained via the ingested diets as well. Pohnert (2002) showed that Phospholipase A2 in *Thalassiosira rotula* triggers the wound-activated chemical defence in diatoms. Thus, it is possible that the new lipase/esterase bands in fed females are obtained through the diet. To test the origin of these lipases, additional gels were run with homogenates of *O. marina* and *T. weissflogii* with different concentrations. The resulting zymograms revealed that lipase bands in both cultures had different molecular weights than the bands in fed *T. longicornis*. Additionally, bands in the diets were only detectable when cultures were highly concentrated (>200,000 cells ml⁻¹). In order to exclude that individual variability of copepods was responsible for the different patterns additional gels with homogenates of ten females for each treatment were run. Results showed that diet-specific patterns appeared in both feeding groups, confirming that these bands were indeed diet-induced.

**Egg production rate and metabolic activity**

The different diets in this study did not have a measurable effect on citrate synthase (CS) activities in females, indicating that females maintained metabolic capacities similar to those from females from the field, as seen in a previous study (Kreibich et al. submitted). In contrast, CS activity was strongly reduced under starving conditions, which is in accordance with other studies (Clarke and Walsh 1993, Kreibich et al. submitted). After 24 h feeding on both diets, egg production rates of females increased. This shows that dietary components were rapidly digested and efficiently transformed into egg material and used to fuel metabolic processes. As summarised in Tester and Turner (1990) copepods convert ingested food to eggs in a lag
period of 6 to 24h. In Experiment 1, the *O. marina* culture induced highest EPR in *T. longicornis*. Klein Breteler et al. (1999) showed that *O. marina* enhance copepod growth by “trophic upgrading”, i.e. by ameliorating the food quality. They provide essential compounds such as highly unsaturated FAs and sterols to the copepods. Our results indicate that *O. marina* enhances egg production as well. However, in Experiment 2, females feeding on *O. marina* showed a lower EPR than females feeding on *T. weissflogii* and a sharp decrease of the faecal pellet production on day 2. This indicates that the ingestion of *O. marina* cells were reduced, possibly due to a methodological problem.

Earlier studies attempted to better describe food quality by focusing on different biochemical compounds, such as essential fatty acids (EFAs) like 20:5(n-3) (EPA) and 22:6(n-3) (DHA), and their influence on EPR and hatching success (e.g. Støttrup and Jensen 1990, Jónasdóttir 1994, Klein Breteler et al. 1999, Hazzard and Kleppel 2003, Thor et al. 2007). Some authors suggest that EPA and DHA are rapidly catabolised by small calanoid copepods and directed straight into vitellogenesis (Jónasdóttir 1994, Veloza et al. 2006, Kreibich et al. submitted). This was also observed for EPA in the present study. In fed females EPA was not accumulated and was even reduced despite of similar or higher portions in the diets than in seston samples. At the same time, EPR of copepods increased. In addition, as our results indicate, EPA seems to play a major role also with regard to lipid utilisation to sustain vital metabolic processes since *T. longicornis* showed a decrease of EPA under starvation.

**Summary**

Our results showed that *T. longicornis* females react rapidly to changing nutritional conditions in order to cover their energetic demand. Feeding history, physiological and biochemical conditions of females influence strongly lipid accumulation processes, as well as catabolism of different energetic compounds during starvation. However, independent of physiological conditions, dietary FAs are rapidly incorporated into copepods’ lipids resulting in fast changes in FA patterns. This is ensured by the strong adaptive potential and high plasticity of the digestive system. The inducibility of diet-dependent changes in the lipase/esterase patterns indicates that enzyme expression and activities are tightly regulated with changing nutritional conditions in copepods. This strong adaptive potential leads to fast changes in functional responses of copepods to food availability, i.e. egg production rate.

**Acknowledgements**

We would like to thank the crew of the R/V Aade for taking copepod and seston samples, U. Tillmann for kindly providing the algae cultures and the staff at the Biologische Anstalt Helgoland for logistic support. We thank E. Gentsch, B. Hansen and C. von Waldthausen for
their support during experiments and laboratory work. This study was funded by the “Struktur- und Investitionsfond des Präsidenten der Helmholtz-Gemeinschaft (President’s Initiative and Networking Fund)”, Germany, VH-NG-058.

References


Evjemo JO, Olsen Y (1997) Lipid and fatty acid content in cultivated live feed organisms compared to marine copepods. Hydrobiologia 358: 159-162


Tables

Table 1: Dry mass, lipid and protein content of *in situ* *Temora longicornis* females and after three days of incubation (d3) under different nutritional conditions (*Thalassiosira weissflogii*, *Oxyrrhis marina* and starvation). Protein content was additionally measured on days one and two (d1, d2). Mean ± standard deviation, n=3, except lipid contents of females fed with *T. weissflogii* (n=1) and starved females in Experiment 1 (n=2).

<table>
<thead>
<tr>
<th>day</th>
<th>food type</th>
<th>dry mass (μg ind⁻¹)</th>
<th>lipid (μg ind⁻¹)</th>
<th>lipid (% DM)</th>
<th>protein (μg ind⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>in situ</td>
<td>42.3 ± 4.5</td>
<td>1.8 ± &lt;0.1</td>
<td>4.3 ± 0.5</td>
<td>12.7 ± 3.1</td>
</tr>
<tr>
<td></td>
<td><em>T. weissflogii</em></td>
<td>34.4 ± 3.5</td>
<td>2.3</td>
<td>6.2</td>
<td>14.8 ± 1.3</td>
</tr>
<tr>
<td>d3</td>
<td><em>O. marina</em></td>
<td>36.0 ± 5.5</td>
<td>2.8 ± 0.6</td>
<td>7.8 ± 1.2</td>
<td>13.7 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>starved</td>
<td>30.5 ± 9.2</td>
<td>1.0</td>
<td>3.8</td>
<td>11.3 ± 2.3</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>in situ</td>
<td>40.5 ± 2.9</td>
<td>2.6 ± 0.2</td>
<td>6.6 ± 0.7</td>
<td>10.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td><em>T. weissflogii</em></td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>17.0 ± 1.7</td>
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<tr>
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<td><em>O. marina</em></td>
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<td>n.d.</td>
<td>n.d.</td>
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</tr>
<tr>
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<td>starved</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>13.5 ± 1.3</td>
</tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>in situ</td>
<td>45.3 ± 6.2</td>
<td>2.7 ± 0.3</td>
<td>6.0 ± 0.3</td>
<td>20.1 ± 0.9</td>
</tr>
<tr>
<td></td>
<td><em>T. weissflogii</em></td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>19.4 ± 0.8</td>
</tr>
<tr>
<td>d2</td>
<td><em>O. marina</em></td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
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</tr>
<tr>
<td></td>
<td>starved</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>12.5 ± 2.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>in situ</td>
<td>38.2 ± 3.1</td>
<td>1.9 ± 0.3</td>
<td>4.9 ± 1.0</td>
<td>13.4 ± 0.9</td>
</tr>
<tr>
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<td><em>O. marina</em></td>
<td>39.0 ± 3.3</td>
<td>1.8 ± 0.4</td>
<td>4.7 ± 1.4</td>
<td>12.1 ± 0.7</td>
</tr>
</tbody>
</table>

n.d.=not determined
Table 2: Carbon (C), nitrogen (N) content, C:N ratio and lipid content of seston samples and of *Thalassiosira weissflogii* and *Oxyrrhis marina* cultures in Experiment 1 and 2. Mean ± standard deviation (n=3), except for C and N content and C:N ratio April 15, May 12 of *O. marina* in Experiment 2 (n=2).

<table>
<thead>
<tr>
<th></th>
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<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April 15</td>
<td>May 12</td>
<td>T. weissflogii</td>
</tr>
<tr>
<td>C content (μg l⁻¹)</td>
<td>207.7 ± 12.2</td>
<td>340.0 ± 12.2</td>
<td>1680.2 ± 30.0</td>
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<tr>
<td>N content (μg l⁻¹)</td>
<td>17.8 ± 1.8</td>
<td>44.4 ± 1.8</td>
<td>191.4 ± 17.1</td>
</tr>
<tr>
<td>C:N ratio</td>
<td>11.7 ± 0.4</td>
<td>7.7 ± 0.4</td>
<td>8.8 ± 0.9</td>
</tr>
<tr>
<td>lipid content (μg l⁻¹)</td>
<td>10.5 ± 2.2</td>
<td>25.2 ± 0.6</td>
<td>38.9 ± 9.7</td>
</tr>
<tr>
<td>lipid content (μg mgC⁻¹)</td>
<td>50.5 ± 10.6</td>
<td>74.2 ± 1.6</td>
<td>23.1 ± 5.8</td>
</tr>
</tbody>
</table>
Figure captions

Fig. 1: Experiment 1: Fatty acid composition (% of total FAs) of (a) seston (field), *T. weissflogii* (*T.w.*) and *O. marina* (*O.m.*), and (b) of *T. longicornis* from the field (field), fed with *T. weissflogii* and *O. marina* or starved over three days (n=3, mean ± standard deviation, females fed with *T. weissflogii* n=1, starved females n=2).

Fig. 2: Change of main fatty acids in % dry mass in *T. longicornis* after three days feeding on *T. weissflogii* and *O. marina*, and 3 days under starving conditions in Experiment 1 as compared to values from the field (n=3, mean values).

Fig. 3: Proteinase activity of *T. longicornis* in Experiment 1 (a) and 2 (b). Activities in females from the field and activities after one, two and three days of incubation with *T. weissflogii* (*T.w.*), *O. marina* (*O.m.*) and under starving conditions (n=3, mean ± standard deviation).

Fig. 4: Citrate synthase activity of *T. longicornis* females in Experiment 1 (a) and 2 (b). Activities in females from the field and activities after one, two and three days of incubation with *T. weissflogii* (*T.w.*), *O. marina* (*O.m.*) and under starving conditions (n=3, mean ± standard deviation).

Fig. 5: Egg production rates and faecal pellet production of *T. longicornis* in Experiment 1 (a and b) and 2 (c and d). Activities in females from the field and activities after one, two and three days of incubation with *T. weissflogii* (*T.w.*), *O. marina* (*O.m.*) and under starving conditions (n=36).

Fig. 6: Experiment 2: Fatty acid composition (% of total FAs) of (a) seston (field), *T. weissflogii* and *O. marina*, and (b) of *T. longicornis* from the field (field), fed with *T. weissflogii* (*T.w.*) and *O. marina* (*O.m.*) or under starving conditions over three days (n=3, mean ± standard deviation, females fed with *T. weissflogii* n=1, starved females n=2).

Fig. 7: Change of main fatty acids in % dry mass in *T. longicornis* after three days feeding on *T. weissflogii* or *O. marina*, or three days of starvation in Experiment 2 as compared to values from the field (n=3, mean values).

Fig. 8: Lipase activity (nmol h⁻¹ ind⁻¹) of *T. longicornis* females from the field, fed with *T. weissflogii*, *O. marina* or starving females (n=3, mean ± standard deviation).

Fig. 9: Experiment 2: SDS-PAGE with lipase active bands of individual *T. longicornis* females. Lane 1: molecular marker (range 205-6.5 kDA), lanes 2-4: females from the field, lanes 5-7: females fed with *T. weissflogii*, lanes 9-11: females fed with *O. marina*, lanes 12-14: starving females.
Fig. 1

(a) and (b) show the percentage of total fatty acids in different samples. The x-axis represents the percentage of total fatty acids, and the y-axis lists various fatty acids, including 14:0, 16:0, 16:1(n-7), 16:2(n-4), 16:3(n-4), 18:0, 18:1(n-7), 18:1(n-9), 18:2(n-6), 18:3(n-3), 18:4(n-4), 18:4(n-3), 20:5(n-3), and 22:6(n-3).

Legend:
- field
- T.w.
- O.m.
- starved
Fig. 2

Change fatty acid (% dry mass)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>T. weissflogii</th>
<th>O. marina</th>
<th>starved</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td></td>
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<tr>
<td>16:1 (n-7)</td>
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<tr>
<td>16:2 (n-4)</td>
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<tr>
<td>16:3 (n-4)</td>
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<td></td>
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<tr>
<td>18:1 (n-7)</td>
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<tr>
<td>18:1 (n-9)</td>
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<td>18:2 (n-6)</td>
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<tr>
<td>18:3 (n-3)</td>
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<td>18:4 (n-3)</td>
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<td>18:4 (n-4)</td>
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<tr>
<td>20:5 (n-3)</td>
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<td></td>
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<tr>
<td>22:6 (n-3)</td>
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</tbody>
</table>

Change fatty acid (% dry mass)
Fig. 3

![Graph a](image1.png)  ![Graph b](image2.png)

Fig. 4

![Graph a](image3.png)  ![Graph b](image4.png)
Fig. 5

(a) Egg production rate (eggs ind\(^{-1}\) d\(^{-1}\))

(b) Faecal pellet production (ind\(^{-1}\) d\(^{-1}\))

(c) Egg production rate (eggs ind\(^{-1}\) d\(^{-1}\))

(d) Faecal pellet production (ind\(^{-1}\) d\(^{-1}\))
<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Field</th>
<th>T.w.</th>
<th>O.m.</th>
<th>Starved</th>
</tr>
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<tbody>
<tr>
<td>14:0</td>
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<tr>
<td>16:0</td>
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<tr>
<td>16:1(n-7)</td>
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<td>16:2(n-4)</td>
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<td>16:3(n-4)</td>
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<td>22:6(n-3)</td>
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</tbody>
</table>

**Fig. 6**

The bar chart shows the percentage of total fatty acids for different fatty acids in various conditions. The x-axis represents the percentage of total fatty acids, ranging from 0 to 40, while the y-axis lists the fatty acid names. The bars are color-coded to indicate different conditions: field, T.w., O.m., and starved.
Fig. 7

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>T. weissflogii</th>
<th>O. marina</th>
<th>starved</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>16:0</td>
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<tr>
<td>22:6(n-3)</td>
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Change fatty acid (% dry mass)
Fig. 8

Lipase activity (nmol h\(^{-1}\) ind\(^{-1}\))

<table>
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<tr>
<th></th>
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<th>O.m.</th>
<th>starving</th>
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<tr>
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<td>205.0</td>
<td>97.0</td>
<td>14.2</td>
<td>116.0</td>
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</table>

Fig. 9

kDA marker field T.w. O.m. starving

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<th>3</th>
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<td>97.0</td>
<td>84.0</td>
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<td>55.0</td>
<td>45.0</td>
<td>36.0</td>
<td>29.0</td>
<td>24.0</td>
<td>20.0</td>
<td>14.2</td>
<td>6.5</td>
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PUBLICATION IV

FEEDING STRATEGIES, DIGESTIVE ACTIVITIES AND REPRODUCTION OF

CALANUS FINMARCHICUS AND METRIDA LONGA

IN THE LOWER ST. LAWRENCE ESTUARY, QUÉBEC, CANADA

Kreibich T, Plourde S, Joly P, Starr M, Auel H, Niehoff B

planned for submission to Polar Biology
FEEDING STRATEGIES, DIGESTIVE ACTIVITIES AND REPRODUCTION OF
CALANUS FINMARCHICUS AND METRIDIA LONGA IN THE LOWER ST.
LAWRENCE ESTUARY, QUÉBEC, CANADA

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Abstract

This study compares feeding behaviour and digestive, metabolic and reproductive activity of Calanus finmarchicus and Metridia longa females in relation to feeding conditions from May until August 2006 in the lower St. Lawrence estuary. The copepods were exposed to highly variable nutritional conditions throughout the time series, as indicated by distinct changes in carbon, nitrogen, chl a and lipid content, fatty acid composition and stable isotope signatures of the seston and in phytoplankton and protist abundances. During the time series, phytoplankton concentration peaked twice, mainly due to increasing diatom abundance, once in July and once in August. C. finmarchicus and M. longa adapted differently to these conditions. Spawning of C. finmarchicus females started one week after the first peak had developed whereas the reproductive period of M. longa started two weeks later. In both species, the increasing egg production was accompanied by increasing metabolic activities, probably in order to cover the energy demands for gonad maturation and spawning. C. finmarchicus fed mainly on diatoms and dinoflagellates as indicated by increasing contents of the specific fatty acids 16:1(n-7) and 18:4(n-3). Besides feeding also on diatoms and dinoflagellates, M. longa apparently preyed upon Calanus spp. eggs as indicated by the accumulation of Calanus-specific fatty acids and alcohols (20:1(n-9), 22:1(n-11)) in the female. Moreover, M. longa females were more enriched by 1.5‰ of the stable isotope ¹⁵N than C. finmarchicus females, indicating that M. longa fed on a higher trophic level than C. finmarchicus. The interspecific differences and the temporal changes in food availability and composition, however, did not relate to digestive enzyme activities. This suggests that digestive enzyme activities in these two species do not change with food availability and quality but seem to be adapted to longer seasonal time scales.

Key words: Calanus finmarchicus, Metridia longa, physiology, fatty acid composition, digestion, metabolism, reproduction
Introduction

*Calanus finmarchicus* and *Metridia longa* are abundant in artic zooplankton communities, and both species are well adapted to the environmental conditions prevailing in higher latitudes, characterized by food scarcity during winter and the development of a phytoplankton bloom during spring (e.g. Siegel et al. 2002). Due to the accumulation of depot lipids, i.e. wax esters, *C. finmarchicus* and *M. longa* can starve over prolonged periods (e.g. Lee 1975, Falk-Petersen et al. 1987, Miller et al. 1991, Kattner and Hagen 1995, Albers et al. 1996). Their over-wintering strategies, however, differ. Usually the copepodite stage V (CV) of *C. finmarchicus* migrates to deep-water layers and spends the winter in a diapause (Hirche 1996). Prior to the spring phytoplankton bloom in spring, the CV return to the surface and moult to adults (e.g. Miller et al. 1991, Diel and Tande 1992). *M. longa*, in contrast, overwinters as physiologically active adult (Tande and Grønvik 1983, Båmstedt and Ervik 1984, Båmstedt and Tande 1988). *C. finmarchicus* is considered to be rather herbivorous and accordingly reproduction is usually closely coupled to the phytoplankton bloom (e.g. Marshall and Orr 1955, Diel and Tande 1992, Plourde and Runge 1993). However, also feeding on microzooplankton may fuel gonad maturation and spawning (Ohman and Runge 1994). Low reproductive rates have been found prior to the bloom (e.g. Niehoff et al. 1999), and it has been shown that *C. finmarchicus* may use internal body reserves for reproduction when food is scarce (Irigoien et al. 1996, Niehoff 1998). *M. longa* feeds on eggs and nauplii of other copepod species besides autotrophic organisms and is less dependent on the phytoplankton bloom (e.g. Falk-Petersen et al. 1987, Norrbin et al. 1990, Albers et al. 1996). Reproduction in *M. longa*, thus, may commence well after the onset of the spring phytoplankton bloom (Tande 1982, Tande and Grønvik 1983). Some studies, however, have shown that reproduction can start with the beginning of the phytoplankton bloom as well (Båmstedt and Tande 1988).

Although numerous studies concentrated on life-cycle strategies in both species, physiological adaptive processes were less studied, particularly in *Metridia longa*. Digestion and its ability to respond to changing nutritional conditions is a key physiological process influencing the success of a species. Digestive enzymes hydrolyse dietary constituents and, thus, provide the organism with energy as well as with essential macromolecules, which are used for anabolic processes of lipids and proteins. In order to digest the diet efficiently, specific enzymes need to be provided. The digestive activity in copepods is influenced by both, internal and external factors. Studies identified inter-, intraspecific and ontogenetic differences as well as seasonal and diurnal variations in copepods’ digestive activities (e.g. Hirche 1979, Hallberg and Hirche 1980, Hirche 1981, Mayzaud et al. 1984, Båmstedt 1988, Båmstedt and Tande 1988, Hirche 1989). However, most studies, investigating digestive enzyme activities in copepods, disregarded that metabolic requirements of copepods and life strategies may influence

This study therefore aimed at investigating interspecific physiological adaptive processes and functional responses of *Calanus finmarchicus* and *Metridia longa* females to changing nutritional conditions in order to obtain detailed information on processes driving population dynamics. To study physiological adaptive processes in *C. finmarchicus* and *M. longa*, we performed a field campaign during the spring phytoplankton bloom 2006 in the lower St. Lawrence estuary (LSLE), Canada. The LSLE is a very dynamic marine environment characterised by a two-layer circulation pattern with a surface freshwater runoff from the St. Lawrence River, compensated by a slower advection of deep water (e.g. Zakardjian et al. 1999, Zakardjian et al. 2000). Tidal mixing transports periodically nutrient-rich deep cold water to the surface. This upwelling process supports the spring phytoplankton bloom (Therriault et al. 1990 cited after Plourde et al. 2001, Levasseur et al. 1984, Plourde and Runge 1993). Thus, the zooplankton community, with *C. finmarchicus* and *M. longa* as typical members (Plourde and Runge 1993, Plourde et al. 2001), is exposed to high variable nutritional conditions and need to adapt to these changes to ensure the survival of the species.

To determine the female condition we measured dry mass, carbon, nitrogen, lipid and water-soluble protein content. The trophic level of both species was determined by measuring stable isotope signatures, and the diet they were feeding on was traced via fatty acid trophic biomarkers. We combined these data with physiological rates of the copepods, i.e. digestive and metabolic enzyme activities and egg production rates. To define the seston composition we measured stable isotope signatures, chlorophyll *a* concentration, lipid, carbon and nitrogen content as well as the fatty acid composition and we analysed phytoplankton and protist abundances.

**Materials and Methods**

**Field work**

Temperature (30 m, 100 m, 200 m and 300 m depth) and salinity data (30 m depth) were measured with an Applied Microsystems CTD-12 at the monitoring Station off Rimouski in the St. Lawrence estuary, Québec, Canada (48°40’ N, 68°35’ W, approximately 330 m depth).

Zooplankton and seston were sampled once a week from May 24 until August 9 2006. Water samples were collected with Niskin bottles (4 l and 10 l) at a depth of 10 m and filled into bottles, which were kept in the dark until filtration of samples. The two copepod species, *Calanus finmarchicus* and *Metridia longa*, were sampled with plankton nets (202 and 333 μm
mesh size) vertically towed from 320 to 0 m at a speed of <0.5 m s⁻¹. Immediately after capture, zooplankton samples were transferred from the restricted-flow cod-end into 4 l jars filled with filtered seawater. Bottles and jars were kept ice-cooled during transport to the laboratory at the Maurice-Lamontagne Institute, Mont-Joli.

At least 250 healthy looking females of each species were sorted alive under a stereo-microscope. All individuals were briefly rinsed in demineralised water and triplicates of batches of ten females each were frozen immediately in liquid nitrogen either in 1.5 ml reaction tubes for analyses of water soluble protein content and enzyme activity or in pre-cleaned glass vials for determination of dry mass and lipid analysis. For the analysis of stable isotopes, triplicates with batches of five to seven copepods were frozen immediately in liquid nitrogen in 1.5 ml reaction tubes. Twenty-four females were individually transferred in pre-weighed Zn-cartridges for the analysis of carbon (C) and nitrogen (N) contents.

The water samples (10 l Niskin bottles) for the analyses of the stable isotopes δ¹⁵N and δ¹³C (SI), C, N and lipid content and fatty acid composition were pre-filtered over a 125 μm mesh-sized sieve to remove small zooplankton organisms. Sub-samples (750-1000 ml, depending on the amounts of particulate matter) were filtered on dried and pre-weighted GF/C filters (0.2 μm mesh size). Triplicate samples for each parameter were frozen in liquid nitrogen and stored at -80° C before being transported to the laboratories in Germany on dry ice or in liquid nitrogen. For the analysis of chlorophyll a concentration, two sub-samples of each water sample (4 l Niskin bottles) were filtered on Whatman GF/F glass-fiber filters (<0.7 μm mesh size) for subsequent measurements at the Maurice Lamontagne Institute.

To obtain phytoplankton and protist species composition and number, sub-samples were taken from the water samples and preserved with an acid Lugol's solution (Parsons et al. 1984). These samples were concentrated by settling and analysed under a phase-contrast inverted microscope (Lund et al. 1958).

For determination of in situ egg production rates, 40 females were transferred individually into petri dishes filled with pre-screened seawater. The females were then incubated for 24 h at ambient temperatures in the dark. After incubation females were removed and eggs were counted.

**Analytical work**

For the determination of carbon and nitrogen content Zn-cartridges and filters were dried for 48 h at 60 °C and weighed with a microbalance (Sartorius M2P, ±1 μg). After weighing, the
filters were wrapped in Zn foil for further treatment. The samples were analysed in a C:H:N
Element Analyzer (EuroVector, Euro EA Element Analyzer).

Prior to the measurement of the stable isotopes, copepods were transferred from reaction
tubes into pre-weighed Zn-cartridges. Then, copepod samples and seston filter were dried and
weighed. Filters were wrapped in Zn foil. The stable isotope ratios $^{15}\text{N}/^{14}\text{N}$ ($\delta^{15}\text{N}$) and $^{13}\text{C}/^{12}\text{C}$
($\delta^{13}\text{C}$) were analysed by AGROISOLAB Isotopenlabor (Jülich, Germany).

Measurements of chlorophyll $a$ concentrations were made via the fluorometric method for
acetone-extracted samples as described by Parsons et al. (1984). The filters were extracted in
90% acetone in the dark at 4°C for 24 h. The extinction of the sample extracts was measured
with a Turner Designs fluorometer calibrated against pure chlorophyll $a$ extract (Sigma
Chemicals).

For the determination of dry mass, copepod samples were lyophilised for 24h using a
LYOVAC GT2 (Leybold-Heraeus) and then weighed with a micro-balance (Sartorius, ±2 μg).
Lyophilised samples were frozen again at -80°C and used for analyses of total lipid content
and fatty acid composition. Lipid analyses were also conducted with seston samples. Twenty-
four hours prior to the extraction procedure, 1.5 ml (copepods) or 4 ml (seston samples) of
solvent (dichloromethane:methanol 2:1/v:v) were added to the samples. Thereafter samples
were stored again at -80°C. Lipid extraction was performed with minor modifications after
Folch et al. (1957). A defined volume of the internal standard tricosanoic acid (23:0) was
added to the samples after Peters et al. (2006). Seston samples were extracted as described
in Kreibich et al. (submitted). Copepod samples were homogenised as described by Hagen
(2000), however, without using butylhydroxytoluene as antioxidant. Sub-samples of total lipids
were used for the hydrolysis of lipids in fatty acids and their conversion into methyl ester
derivates (FAMEs). This procedure was carried out as described in Kattner and Fricke (1986).
Samples were analysed with a gas chromatograph (HP 6890A) equipped with a DB-FFAP
column (30 m length, 0.25 mm inner diameter and 0.25 μm film thickness) operating with a
temperature programme. Helium was used as carrier gas.

In this study we focussed on fatty acids as trophic biomarkers in order to trace feeding of
copepods on different components of the seston. The monounsaturated fatty acid (MUFA)
16:1(n-7) and the polyunsaturated fatty acid (PUFA) 20:5(n-3) are known as biomarkers for
diatoms, and 18:4(n-3) and 22:6(n-3) are typical for dinoflagellates (e.g. Sargent et al. 1987,
Nichols et al. 1993, Dunstan et al. 1994, Graeve et al. 1994). The MUFAs 20:1(n-9) and
22:1(n-11) as well as the fatty alcohols 20:1(n-9) and 22:1(n-11) are specific for Calanus spp.
(e.g. Sargent and Henderson 1986, Kattner and Hagen 1995).
Soluble proteins were quantified after Bradford (1976) using a commercial protein assay (BioRad), as described in Kreibich et al. (submitted). Bovine serum albumin (BSA, BioRad) was used as standard.

The digestive activity of *Calanus finmarchicus* and *Metridia longa* females was measured as activity of proteinases (endopeptidases) and amylase. For the measurement of the metabolic activity two key enzymes were measured, (1) pyruvate kinase (PK, EC 2.7.1.40), component of the glycolysis, and (2) the citrate synthase (CS, EC 4.1.3.7), component of the citrate cycle. All enzyme samples and controls were measured photometrically in triplicates. The proteinase and amylase activities were determined as described in Kreibich et al. (submitted). Pyruvate kinase activities were measured as described in Saborowski and Buchholz (2002), and citrate synthase activities were determined after Stitt (1984) with minor modifications after Saborowski and Buchholz (2002). All protocols for the determination of the enzyme activities measured in this study are listed in Kreibich et al. (submitted).

**Statistics**

An arc sine square root transformation was performed for statistical operations that require normal distribution. Data sets were tested for normal distribution using the Kolmogorov-Smirnov test and for variance homogeneity with the Levene’s test. For normally distributed data showing variance homogeneity an ANOVA was performed followed by the Holm-Sidak post-hoc test. A Kruskal-Wallis ANOVA was used for not normally distributed data sets, followed by a Tuckey post hoc test. In case of unequal treatment group sizes a Dunn’s post hoc test was performed. A Pearson’s correlation for normally distributed data or Spearman’s rank correlation for not normally distributed data were used. The data were analysed with the programme SigmaStat 3.5 (Systat Software, Inc.).

**Results**

*Hydrography*

Sea surface temperature (SST) in the upper 30 m varied over the investigation period from May 24 until August 9 (Fig. 1a). Minimum values of 4.8 to 5.3°C were recorded at the end of May, followed by an increase to 7.0°C on June 12 and again lower temperatures of approximately 5.5°C from end of June until July 11. On July 19, another maximum of 7.1°C occurred, followed by another decrease to 6.0°C at the end of July and in early August, before the absolute highest SST of 7.5°C was measured on August 9, on the last day of the time series. Thus, there was only a slight seasonal increase, but much stronger short-term
variability in SST, possibly related to the lateral advection of surface waters by wind and currents.

In contrast to the SST, the water temperature of sub-surface layers was almost constant throughout the study period without any significant variability (Fig. 1a). In 30 to 100 m depth, water temperature remained at 1 to 2°C throughout the study period with highest values measured in May and early June. Higher temperatures of 2.6 to 3.6°C occurred in 100 to 200 m depth; even higher temperatures of 4.5 to 5.1°C prevailed in 200 to 300 m depth.

The salinity of the upper 30 m layer was very variable at the beginning of the time series with minima of 26.7 and 26.5 psu on May 24 and June 12, respectively and a maximum of 28.4 psu on May 31 (Fig. 1b). From the end of June until August, generally higher salinities of 28.4 to 29.4 prevailed with less variability. In general, surface salinity seemed to be inversely related to SST; salinity maxima on May 31, July 5 and August 1 coincided with minima in SST, while salinity minima on May 24, June 12, July 19 and August 9 occurred together with local maxima in SST.

**Seston composition**

Total abundance of phytoplankton and protist cells was lowest in May with \(<1 \times 10^6 \text{ l}^{-1}\) and remained below \(3 \times 10^6 \text{ l}^{-1}\) until the end of June (Fig. 2). In the first week of July, a pronounced phytoplankton bloom developed with a 5-fold increase in cell numbers to a maximum of \(11 \times 10^6 \text{ l}^{-1}\) on July 5. However, the bloom collapsed within a few days so that phytoplankton abundance was below \(3 \times 10^6 \text{ l}^{-1}\) from July 11 until August 1. On the last day of the time series, another bloom seemed to develop with increased cell numbers of \(6 \times 10^6 \text{ l}^{-1}\).

The trends in phytoplankton abundance and especially the development of blooms were strongly coupled to diatom abundance (Fig. 2). Diatoms were almost absent at the beginning of the study period in May, increased during June to ca. 40% of total phytoplankton abundance and reached a maximum dominance of 90% at the day of the phytoplankton bloom on July 5. After that, relative diatom abundance decreased again to ca. 40% in mid of July and to less than 10% at the end of July and on August 1, in association with the decrease in total cell number. The second maximum in phytoplankton abundance at the end of the study period coincided again with an elevated abundance of diatoms (60% of total phytoplankton). Dinoflagellates were present throughout the study period, but comprised \(<10\%\). Cryptophyceans and prymnesiophyceans generally contributed between 8 and 20% and even less during bloom events. Besides diatoms, unidentified flagellates dominated cell numbers with up to 60% especially in May. They generally comprised 20 to 40% except during the two bloom situations on July 5 and August 9. Heterotrophic cells, such as ciliates and
heterotrophic flagellates, and other low-numbered taxa are summarized under ‘others’ and contributed 2 to 18% of total cell number during the time series.

In general, the biochemical composition of seston mirrored the trends in phytoplankton abundance (Fig. 3). Chl \(a\) concentration started at very low levels of 10 to 25 \(\mu g\) \(l^{-1}\) in May, increased to medium values of 80 to 140 \(\mu g\) \(l^{-1}\) during June and reached a maximum of 230 \(\mu g\) \(l^{-1}\) at the time of the phytoplankton bloom on July 5 (Fig. 3a). In contrast to phytoplankton abundance, which collapsed immediately after the bloom (Fig. 2), chl \(a\) remained at high concentrations of 215 \(\mu g\) \(l^{-1}\) on July 11, before it decreased to less than 100 \(\mu g\) \(l^{-1}\) in late July and to a minimum of 35 \(\mu g\) \(l^{-1}\) on August 1. The second bloom event on August 9 (Fig. 2) was accompanied by a slight increase in chl \(a\) concentration to 75 \(\mu g\) \(l^{-1}\). The seston lipid content reached its maximum of 89 \(\mu g\) \(l^{-1}\) during the first phytoplankton bloom on July 5 (Fig. 3a). Besides another maximum of 75 \(\mu g\) \(l^{-1}\) coinciding with the second phytoplankton bloom, lipid content of seston was below 30 \(\mu g\) \(l^{-1}\) in May and June and below 45 \(\mu g\) \(l^{-1}\) in July and August.

Carbon and nitrogen content of seston were strongly correlated, suggesting that the protein content, which we did not measure, increased as the lipid content did (Fig. 3b, \(r = 0.965\)). Both parameters reached their maxima during the two phytoplankton blooms. The C:N ratio decreased from 9 at the end of May to 5.3 in early August. The stable isotope ratio \(\delta^{13}C\) seemed to follow the trend of carbon content with a maximum of -21.4 during the first phytoplankton bloom (Fig. 4a), whereas \(\delta^{15}N\) showed substantial oscillations between minima of 4.7 and maxima of 7.4 but without any apparent seasonal signal (Fig. 4b).

**Body mass and biochemical composition of C. finmarchicus and M. longa**

In both copepod species, individual dry mass increased over the study period (Fig. 5a). In late May, *C. finmarchicus* started with 265 to 305 \(\mu g\) ind\(^{-1}\). From June 21 onwards, body mass increased and reached a maximum of 455 \(\mu g\) ind\(^{-1}\) on August 1, whereas substantially lower values of 380 \(\mu g\) ind\(^{-1}\) were measured on August 9. Similarly, *M. longa* gained body mass between the minimum of 262 \(\mu g\) ind\(^{-1}\) on June 21 and the maximum of 417 \(\mu g\) ind\(^{-1}\) on August 1. Accordingly, carbon content almost doubled in both species from ca. 125 \(\mu g\) ind\(^{-1}\) in late May (for *C. finmarchicus*) and June 21 (for *M. longa*) to maxima of 245 \(\mu g\) ind\(^{-1}\) in late July and early August (Fig. 5b). A comparison of trends in lipid versus protein content (Fig. 5c and e) demonstrates that most of the increase in dry mass during the time series was related to the accumulation of lipids. In both species, lipid content increased from ca. 25 \(\mu g\) ind\(^{-1}\) in June to 6-times higher levels of ca. 115 \(\mu g\) ind\(^{-1}\) on August 1, whereas protein and nitrogen content (Fig. 5d) were rather stable at 40 – 60 \(\mu g\) ind\(^{-1}\) and 30 – 40 \(\mu g\) ind\(^{-1}\), respectively, in both species from June onwards. Consequently, there was a slight increase in the C:N ratio from June to August (Fig. 5f).
In both copepod species, strongest increase in body dry mass and lipid accumulation coincided with the timing of the first phytoplankton bloom in early July. However, there are species-specific differences concerning the start of the lipid accumulation and gain in body mass. In particular the trends in body dry mass and lipid content indicate a time lack of two to three weeks concerning the begin of lipid accumulation in *M. longa* as compared to *C. finmarchicus*. The minima in body dry mass were recorded on May 31 for *C. finmarchicus* and on June 21 for *M. longa* (Fig. 5a), while the lowest lipid content of *M. longa* occurred on June 29, one to two weeks later than in *C. finmarchicus* (Fig. 5c).

Despite substantial variations in stable isotope signatures of seston throughout the time series, δ¹³C and δ¹⁵N ratios of *C. finmarchicus* and *M. longa* remained rather constant during the study period. Both species showed similar δ¹³C ratios of -21 to -22 (Fig. 4a). In contrast, they deviated with regard to δ¹⁵N values. *M. longa* had consistently higher δ¹⁵N ratios of 9.1 to 9.9 than *C. finmarchicus* with 7.7 to 8.4 (Fig. 4b).

**Fatty acid and alcohol composition of seston and copepods**

The fatty acid composition of seston samples mirrored the seasonal succession of the phytoplankton community. Short-chain saturated FAs 16:0 and 18:0 were moderately abundant throughout the study period, whereas the typical FA biomarkers of diatoms 16:1(n-7) and 20:5(n-3) increased from traces <0.5 μg l⁻¹ in May to maximum values of 15.3 μg l⁻¹ and 20.1 μg l⁻¹, respectively, at the time of the phytoplankton bloom on July 5 emphasising that this bloom was mainly composed of diatoms (Table 1). Both diatom markers remained at relatively high levels throughout July and reached a second maximum during the second phytoplankton bloom on August 9. The typical FAs of flagellates 18:4(n-3) and 22:6(n-3) also started at low levels of 0.2 to 0.3 μg l⁻¹ in May and increased to 6 to 8 μg l⁻¹ during the two phytoplankton blooms on July 5 and August 9 (Table 1).

The fatty acid composition of *Calanus finmarchicus* was dominated by a mixture of the short-chain saturated FAs 14:0 and 16:0, the polyunsaturated FAs 20:5(n-3) and 22:6(n-3) and mono-unsaturated FAs of varying length, i.e. 16:1(n-7), 20:1(n-9) and 22:1(n-11) (Table 2). These seven FAs contributed between 4 and 23% of total FAs each throughout the time series. Among the fatty alcohols 20:1(n-9) and 22:1(n-11) (Fig. 7a, b) were most important throughout the study period, while 16:0 contributed at substantial fraction only from July 11 onwards (Table 2).

Similarly, seven FAs mainly made up the body lipids of *Metridia longa* (Table 3). However, in this case 14:0 was less important, while 18:1(n-9), which is generally considered a marker for carnivorous feeding, occurred in larger amounts in *M. longa* than in *C. finmarchicus*. Thus, FA
composition of *M. longa* was dominated by 16:0, 16:1(n-7), 18:1(n-9), 20:1(n-9), 20:5(n-3), 22:1(n-11) and 22:6(n-3), all contributing between 4 and 20% of total FAs each. In contrast to *C. finmarchicus*, the fatty alcohol composition of *M. longa* was more variable with the five fatty alcohol species 14:0, 16:0, 20:1(n-9), 22:1(n-9) and 22:1(n-11) contributing substantial, but variable amounts (Table 3, Fig. 7a, b).

Figure 6 illustrates the shifts in fatty acid composition of both copepod species during the time series. In order to correct for changes in body mass, data are presented as % of dry mass. Both copepod species accumulated storage lipids derived from dietary input as is evident from the increase in the amounts of the diatom markers 16:1(n-7) and 20:5(n-3) (Fig. 6a, b) as well as of the flagellate marker 18:4(n-3) (Fig. 6c). In contrast, the FA 22:6(n-3), which mainly acts as a structural component of biomembranes, remained rather stable, especially in *C. finmarchicus* (Fig. 6d). The two long-chain monounsaturated FAs 20:1(n-9) and 22:1(n-11), which are generally considered to be synthesised by *Calanus*, showed different and rather complex trends (Fig. 6e, f). In May, *C. finmarchicus* had rather low levels of 22:1(n-11), but accumulated this FA until early August. By contrast, rather high amounts of 20:1(n-9) in May were depleted by mid of June and only slightly increased thereafter. *Metridia longa*, on the other hand, had rather high levels of 22:1(n-11) in June, which fell to intermediate levels later in the season. 20:1(n-9) in *M. longa* started at high levels in June, decreased to a minimum on June 29 and July 5 and increased again during July and early August.

**Enzyme activities in Calanus finmarchicus and Metridia longa**

Absolute digestive enzyme activities per individual were almost constant throughout the study period and measured 46.0 ± 4.5 $dE_{366}$ min$^{-1}$ 10$^{-3}$ ind$^{-1}$ and 23.5 ± 2.3 $dE_{600}$ min$^{-1}$ 10$^{-3}$ ind$^{-1}$ for proteinase and amylase in *C. finmarchicus* (Fig. 8a). In *M. longa* digestive activities were constant throughout the time series, as well, and reached lower values than in *C. finmarchicus* with 25.8 ± 4.1 $dE_{366}$ min$^{-1}$ 10$^{-3}$ ind$^{-1}$ and 13.0 ± 2.0 $dE_{600}$ min$^{-1}$ 10$^{-3}$ ind$^{-1}$ for proteinase and amylase (Fig. 8b).

Absolute activities of pyruvate kinase and citrate synthase increased in *C. finmarchicus* from 19.9 and 25.1 units 10$^{-2}$ ind$^{-1}$ on May 24 to 49.4 and 88.7 units 10$^{-2}$ ind$^{-1}$ on July 26, before they decreased again (Fig. 9a). In *M. longa*, the absolute activity of citrate synthase almost doubled from 60.8 units 10$^{-2}$ ind$^{-1}$ on June 21 to 109.0 units 10$^{-2}$ ind$^{-1}$ on July 26 (Fig. 9b), whereas there was only a very slight increase in the activity of pyruvate kinase during the same period from 36.3 units 10$^{-2}$ ind$^{-1}$ on June 21 to 41.9 units 10$^{-2}$ ind$^{-1}$ on July 26.
Egg production rates of C. finmarchicus and M. longa

In C. finmarchicus, egg production rate (EPR) increased from low values of 8 to 17 eggs ind\(^{-1}\) d\(^{-1}\) in May via intermediate EPRs of 30 to 43 eggs ind\(^{-1}\) d\(^{-1}\) in June and early July to a maximum of 76 eggs ind\(^{-1}\) d\(^{-1}\) on July 11, immediately after the first phytoplankton bloom (Fig. 10). From mid-July until August 1, EPR remained relatively high at 50 to 60 eggs ind\(^{-1}\) d\(^{-1}\), followed by a decrease towards 38 eggs ind\(^{-1}\) d\(^{-1}\) on August 9. Thus, a rather strong coupling between chl \(a\) concentration and EPR of C. finmarchicus was apparent at least until mid of July. Thereafter, egg production of C. finmarchicus remained higher than what could be expected from chl \(a\) concentrations, possibly fuelled by internal energy reserves accumulated during the previous phytoplankton bloom.

In contrast, M. longa had very low EPRs of \(<10\) eggs ind\(^{-1}\) d\(^{-1}\) until mid of July (Fig. 10). A pronounced maximum of 47 eggs ind\(^{-1}\) d\(^{-1}\) occurred on July 26, followed by a steep decline via 21 eggs ind\(^{-1}\) d\(^{-1}\) on August 1 to 4 eggs ind\(^{-1}\) d\(^{-1}\) on August 9. Similarly to the trends in body dry mass and carbon content, there seemed to be a time lack of ca. 15 days between the maxima in EPR of C. finmarchicus and M. longa.

Discussion

The high variability of the hydrographic regime in the lower St. Lawrence estuary (LSLE) exposed Calanus finmarchicus and Metridia longa to a highly variable food supply, as indicated by changing phytoplankton and protist abundances, chl \(a\) concentration and seston composition in terms of carbon, nitrogen and lipid contents, fatty acid composition as well as high variability in stable isotope (SI) signatures of the seston. The inverse relation between sea surface temperature and surface salinity indicates that upwelling processes periodically transported nutrient-rich cold deep water to the surface. These processes support the development of phytoplankton blooms in the lower St. Lawrence estuary (LSLE), which usually persist into September (Levasseur et al. 1984, Therriault and Levasseur 1985).

In the LSLE, the phytoplankton bloom usually does not develop until late spring and until then, food limited conditions prevail (Plourde and Runge 1993, Plourde et al. 2001). Also during our study, egg production rate (EPR) of C. finmarchicus were low in May. The content of fatty alcohols, which are components of wax esters, were relatively high during this month. This lipid class is primarily used for over-wintering and for early gonad maturation (e.g. Lee et al. 1970, Lee and Hirota 1973, Sargent and Henderson 1986, Hagen and Kattner 1998, Lee et al. 2006 and references therein, Niehoff 2007). In addition, wax esters are used for the synthesis of yolk, which is mainly composed of lipovitellin, a lipoprotein. Phosphatidylcholine, the predominant lipovitellin lipid, has to be converted from female’s storage lipids (review in Lee et
al. 2006). This may explain the decreasing fatty alcohol portions in *C. finmarchicus* females at the beginning of June, when egg production increased. In addition, the content of the fatty acid (FA) 16:1(n-7) decreased, which is known to be a component of *C. finmarchicus*’ wax esters (Albers et al. 1996). In May, *Metridia longa* was not abundant and thus measurements of this species were not possible.

On June 12, phytoplankton abundance and chl a concentration had increased, mainly due to diatoms and, to a lesser extent, to dinoflagellates. Simultaneously, EPR of *C. finmarchicus* increased and, thus, was synchronised with the timing of the phytoplankton bloom corroborating that females in addition to internal energy reserves use ambient food for the final maturation of ovaries (e.g. Tande 1982, Diel and Tande 1992). The phytoplankton bloom triggered the initiation of the reproductive period in *C. finmarchicus* in the Gulf of St. Lawrence as shown previously by Plourde and Runge (1993). In other areas, however, where chlorophyll a concentrations prior to the bloom are higher, reproduction starts significantly earlier than the bloom (e.g. Niehoff et al. 1998, Campbell and Head 2001).

During this first reproductive peak of *C. finmarchicus*, lipid content was lower in females compared to earlier sampling dates. Despite similar dry masses, the fatty alcohol content decreased by more than 50%. This hints to mobilisation of wax esters for reproductive processes, corroborated by decreasing C:N ratio. At the same time, total lipid content decreased, indicating that the first phytoplankton peak did not provide enough energy to additionally accumulate neutral lipids, i.e. triacylglycerols and wax esters, and compensate for the loss of wax esters during the final maturation of ovaries. In contrast, EPR in *M. longa* remained low during June. The fatty acid pattern of *M. longa* sampled on June 12 suggests that females were feeding on *Calanus* spp. eggs and nauplii, due to elevated contents of the FAs 22:1(n-11) and 20:1(n-9) (e.g. Sargent and Henderson 1986, Kattner and Hagen 1995). The contents decreased thereafter, indicating that *M. longa* used other components of seston from mid-June onwards.

On July 5 the phytoplankton concentration peaked. From then onwards, both copepod species strongly accumulated typical diatom and dinoflagellate fatty acids throughout the time series. These FAs, mainly 16:1(n-7), 18:4(n-3) and 20:5(n-3) (eicosapentaenoic acid, EPA) are accumulated in the copepods’ neutral lipids. The FA 22:6(n-3) (docosahexaenoic acid, DHA) was additionally accumulated in *M. longa*, however, to a lesser extent. DHA is mainly known as a component of polar lipids, which have a membrane function in cells (see Dalsgaard et al. 2003 and references therein), however, the increase of DHA in % dry mass in *M. longa* suggests an accumulation in neutral lipids.
The continuing occurrence of the FAs 22:1(n-11) and 20:1(n-9) in *M. longa* lipids indicate that this species continuously fed on *Calanus* eggs and nauplii, however to a lesser extent than during mid-June. From the beginning of July until the beginning of August the FA 18:1(n-9) increased strongly in *M. longa*, which may indicate that females fed on other metazoans during this time, in addition to *Calanus* eggs and nauplii. This FA is used as indicator for carnivory, however, it can be synthesized *de novo* by all metazoans including the copepods (e.g. Falk-Petersen et al. 1990), and is, thus, not unambiguous as indicator of carnivorous feeding (Dalsgaard et al. 2003). Our results should therefore be interpreted carefully. However, higher enrichment of the heavier $^{15}$N isotope in *M. longa* by approximately 1.5‰ compared to *C. finmarchicus* underlines stronger omnivorous feeding behaviour by *M. longa* throughout the time series. The striking differences between the highly variable $\delta^{15}$N signature of seston and the constant signatures in both copepod species suggests that stable isotope analysis does not provide information on possible changes of the trophic level of these species on time scales of days or weeks. This is corroborated by the study of Tamelander et al. (2006), who fed *C. glacialis* with algae cultures characterised by different $\delta^{15}$N values. Their results showed that the $\delta^{15}$N signal in copepods did not change significantly over a period of 33 days, independent of the diet. It is, however, also possible that the constant $\delta^{15}$N values reflect that the trophic position of both species just did not change significantly over the course of the time series, especially considering that metabolic activity was high in both species indicated by high egg production rates and metabolic enzyme activities.

The increase in dry mass of both species during the second period of the time series may be explained by increased food availability and lipid accumulation during and after the main phytoplankton bloom on July 5. However, we cannot exclude that advection of females of other populations with different life histories may have contributed to the pronounced increase in dry mass of *C. finmarchicus* between June 21 and July 5 and of *M. longa* between July 5 and 11. In the adjacent Gulf of St. Lawrence (GSL), *C. finmarchicus* starts to reproduce earlier in the year (late April – early May) than in the LSLE (late June) (Plourde and Runge 1993, McLaren et al. 2001). As proposed by Plourde et al. (2001), *C. finmarchicus* copepodites CV, which over-winter in the GSL, can be advected into the LSLE with deep water. In spring of the following year, these copepodites would arise from diapause and moult to adults constituting the bulk of females in late spring and early summer. In contrast, copepodites CV originating from the local summer production in the LSLE enter diapause in later summer and emerge from diapause in the following year two months later than copepods originating from the GSL. Thus, these females would constitute the bulk of *C. finmarchicus* females in late summer during July and August in the LSLE. The new population of *M. longa* would have been advected from arctic regions and passes the GSL where phytoplankton bloom starts earlier in the year than in the LSLE (Zakardjian et al. 2000). This would lead to earlier accumulation of dietary lipids than in the LSLE which may explain the observed differences in lipid contents.
The main phytoplankton bloom on July 5 led to increasing EPRs of *C. finmarchicus*. Highest EPR in *C. finmarchicus* was reached approximately one week after the distinct increase in phytoplankton abundance and chlorophyll *a* content. This one week lag corresponds to the time required for the final maturation of oocytes (Plourde and Runge 1993). Reproduction is an energy demanding process and requires higher metabolic activities in organisms. Accordingly, pyruvate kinase increased by a factor of 2.5 and citrate synthase activity by a factor of 3.5 in times of high EPR. In contrast, the EPR of *M. longa* was not synchronised with phytoplankton biomass, but reached maximum on July 26. The pyruvate kinase activity did not change considerably during the time series, however, citrate synthase activity increased by a factor of 1.8 during the reproductive period. The delayed reproductive onset of *M. longa* females may be associated with their over-wintering strategy. They over-winter as physiologically active adults (Tande and Grønvik 1983, Båmstedt et al. 1985), and may have not sufficient energy reserves left for early reproduction. Thus, they must feed for approximately one month during the spring bloom prior to spawning (Båmstedt and Tande 1988).

Seasonal changes in digestive enzyme activities have been observed in bulk zooplankton extracts (e.g. Mayzaud and Conover 1975, Mayzaud and Conover 1984) and in calanoid copepods, e.g. *Calanus finmarchicus*, *C. helgolandicus*, *M. longa* and *Temora longicornis* (e.g. Hirche 1981, Båmstedt 1988, Kreibich et al. submitted), in relation to the concentration of diatoms and dinoflagellates and of substrates such as proteins or carbohydrates in the diet. In our study, however, digestive enzyme activities in both *C. finmarchicus* and *M. longa* were elevated already prior to the phytoplankton bloom and remained almost constant throughout the time series. This may indicate that digestive enzymes are triggered by internal factors, e.g. the transition from diapause to activity. It is also possible that already low food concentrations are sufficient to trigger the synthesis of digestive enzymes and that higher concentrations do not alter activities. The latter has been shown for *Calanus pacificus* digestive enzyme activities, which, once developed, remained high even when feeding rates were declining (Hassett and Landry 1990). Thus, it is possible that *C. finmarchicus* and *M. longa* developed their digestive potential prior to our time series and activities remained high independent of nutritional conditions. Our data indicate that both species do not respond to dietary changes on time scales of days to one week as previous suggested for several copepod species including *C. finmarchicus* and *M. longa* (Mayzaud and Poulet 1978, Hirche 1981, Båmstedt 1988, Roche-Mayzaud et al. 1991, Kreibich et al. submitted). Changes can then be observed on longer time scales only. Hallberg and Hirche (1980), for example, showed that over-wintering *C. finmarchicus* copepodids are characterised by a reduced gut epithelium and low digestive enzyme activities. During emergence from diapause, CVs re-develop those cells of the gut epithelium, which are responsible for the secretion of digestive enzymes, and the activities of their digestive enzyme increases (Hallberg and Hirche 1980).
SUMMARY

This study showed that *Calanus finmarchicus* and *Metridia longa* in the lower St. Lawrence estuary differed in their feeding behaviour and in the onset of the reproductive period. In *C. finmarchicus*, the development of the phytoplankton bloom apparently triggered the onset of the reproductive period, whereas reproduction of *M. longa* started with a delay of approximately two weeks. *M. longa* seemed to depend on phytoplankton, on *Calanus* spp. – probably eggs and nauplii - as well as on other metazoans to start egg production. In times of higher EPR metabolic enzyme activity increased in both species, probably to cover the energy-demanding reproductive processes. Although both species are exposed to a highly dynamic system with distinct changes in potential food supply, digestive activities did not change considerably during the phytoplankton bloom. We suggest therefore that proteinase and amylase activities in these calanoid copepods from the LSLE, once developed, do not respond to short-term changes of nutritional conditions, maybe due to higher thresholds which was not reached during this time series. The activities seem to be adapted to longer seasonal time scales. Thus, our investigation indicates that adaptive processes of the digestive system do not only depend on food quantity and quality, but that life strategies represent important factors which should be considered when interpreting digestive enzyme activities in copepods under changing environmental conditions.

Acknowledgements

We are very grateful to S. Lessard for analysing the phytoplankton-microzooplankton samples, C. von Waldthausen for her help in the laboratory (measurement of C and N contents, and enzyme activities). This study was funded by the “Struktur- und Investitionsfond des Präsidenten der Helmholtz-Gemeinschaft (President’s Initiative and Networking Fund)”, Germany, VH-NG-058.

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Lee RF (1975) Lipids of Arctic Zooplankton. Comp Biochem Physiol 51B: 263-266


Ohman MD, Runge JA (1994) Sustained fecundity when phytoplankton resources are in short supply: Omnivory by Calanus finmarchicus in the Gulf of St. Lawrence. Limnol Oceanogr 39: 21-36


## Tables

Table 1: Main fatty acids and alcohols of seston samples in μg l⁻¹ (>0.1 μg l⁻¹, mean ± standard deviation, n = 3, except June 21 n = 2 and August 1 n = 1), and saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and total alcohols in % of total lipid content (TL) tr = traces, - = not detected.

<table>
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<tr>
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<th>May</th>
<th>June</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
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<tr>
<td>14:0</td>
<td>0.3 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>15:0</td>
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<td>0.2</td>
<td>tr</td>
</tr>
<tr>
<td>16:0</td>
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<td>4.1 ± 0.2</td>
<td>3.4</td>
<td>2.2 ± 0.2</td>
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<td>1.4 ± 0.1</td>
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<td>0.8 ± 0.1</td>
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<td>0.4</td>
<td>0.2 ± &lt;0.1</td>
</tr>
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<td>0.4 ± &lt;0.1</td>
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<td>2.9</td>
<td>2.7 ± 0.1</td>
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<td>tr</td>
<td>tr</td>
</tr>
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<td>0.2 ± &lt;0.1</td>
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<td>3.2 ± &lt;0.1</td>
<td>1.2</td>
<td>0.9 ± &lt;0.1</td>
</tr>
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</table>

% TL

| SFA        | 33.8 ± 3.7 | 26.4 ± 3.8 | 34.0 | 29.4 ± 1.0 | 20.1 ± 0.2 | 21.3 ± 1.1 | 25.0 ± 0.6 | 17.6 ± 0.4 | 22.1 ± 0.6 |
| MUFA       | 22.7 ± 2.2 | 15.9 ± 1.3 | 16.0 | 16.8 ± 0.2 | 20.8 ± <0.1 | 18.8 ± 0.9 | 24.2 ± 0.3 | 14.9 ± 2.0 | 20.0 ± 0.8 |
| PUFA       | 19.3 ± 2.4 | 44.9 ± 1.3 | 39.6 | 45.4 ± 1.1 | 57.0 ± 0.3 | 53.4 ± 1.1 | 46.5 ± 1.1 | 32.9 ± 0.4 | 45.5 ± 0.2 |

<table>
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<th>21</th>
<th>29</th>
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<td>0.3</td>
<td>0.2 ± &lt;0.1</td>
<td>0.2 ± &lt;0.1</td>
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<td>-</td>
<td>tr</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>20:1 (n-9)</td>
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<td>0.3</td>
<td>0.2 ± &lt;0.1</td>
<td>-</td>
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<td>0.7 ± 0.1</td>
<td>-</td>
<td>3.8 ± 0.3</td>
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</table>

alcohols % TL

| 18.2 ± 1.6 | 7.3 ± 0.5 | 8.9 | 7.7 ± 0.5 | 2.2 ± 0.1 | 5.2 ± 0.4 | 4.3 ± 0.2 | 17.6 ± 1.2 | 12.0 ± 0.1 |
Table 2: Main fatty acids and fatty alcohols of *C. finmarchicus* in % total fatty acids and % total fatty alcohols (>1% of total fatty acids/fatty alcohols, mean ± standard deviation, n = 3).

SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids. TL total lipid content. tr: traces, -: not detected

<table>
<thead>
<tr>
<th>fatty acids</th>
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<th>July</th>
<th>August</th>
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<td>3.7 ± 3.3</td>
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<td>22:1(n-11)</td>
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<td>20:1(n-9)</td>
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<td>57.4 ± 1.0</td>
<td>63.0 ± 9.0</td>
<td>59.7 ± 7.0</td>
</tr>
</tbody>
</table>

| alcohols % TL | 41.2 ± 3.2 | 43.1 ± 0.9 | 20.6 ± 7.1 | 23.4 ± 12.7 | 31.3 ± 1.4 | 31.2 ± 6.6 | 29.5 ± 3.7 | 30.1 ± 4.3 | 36.6 ± 2.2 | 36.6 ± 1.8 |
Table 3: Main fatty acids and fatty alcohols of *M. longa* in % total fatty acids and % total fatty alcohols (>1% of total fatty acids/fatty alcohols, mean ± standard deviation, n = 3, except June 29 n = 2). SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids. TL total lipid content. tr: traces, -: not detected

<table>
<thead>
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<td>1.8 ± 0.2</td>
<td>1.6 ± 0.2</td>
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| SFA         | 10.8 ± 1.5 | 14.4 ± 4.0 | 17.2   | 19.2 ± 2.5 | 13.7 ± 1.1 | 12.3 ± 1.2 | 10.7 ± 1.5 |
| MUFA        | 55.3 ± 3.5 | 45.7 ± 13.0 | 30.5   | 35.7 ± 4.8 | 47.9 ± 4.2 | 47.9 ± 2.3 | 48.3 ± 3.6 |
| PUFA        | 33.4 ± 2.1 | 39.9 ± 9.2 | 52.3   | 45.1 ± 2.3 | 38.5 ± 3.7 | 39.8 ± 1.9 | 41.0 ± 2.8 |

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Figure captions

Fig. 1: Water temperature in the surface layer (0 – 30 m) and in the sub-surface layers (30 – 100 m, 100 – 200 m, 200 – 300 m) (a) and salinity in the surface layer (0 – 30 m) (b) during the time series at Rimouski Station.

Fig. 2: Seston composition (%) (a) and total abundance of phytoplankton and protist cells (b) during the time series. Seston grouped in diatoms, dinoflagellates, cryptophyceans, prymnesiophyceans, undefined autotrophic flagellates, and others (comprising groups with low abundances, such as heterotrophic flagellates, choanoflagellates and ciliates).

Fig. 3: Chlorophyll a (chl a) and lipid content (a) and carbon (C), nitrogen (N) content and C:N ratio (b) in seston samples (mean ± standard deviation, n = 3, except for data points without standard deviation n = 2).

Fig. 4: Stable isotope signatures of seston and copepods (a) δ13C, (b) δ15N during the time series (mean ± standard deviation, n = 3).

Fig. 5: *Calanus finmarchicus* and *Metridia longa*. Dry mass (a), carbon (b), lipid (c) nitrogen (d) and protein content (e) in μg ind⁻¹ and C:N ratio (f) (mean ± standard deviation, n = 3, except for data points without standard deviation n = 2).

Fig. 6: *Calanus finmarchicus* and *Metridia longa*. Fatty acids 16:1(n-7) (a), 20:5(n-3) (b), 18:4(n-3) (c), 22:6(n-3) (d), 22:1(n-11) (e) and 20:1(n-9) (f) in % dry mass during the time series (mean ± standard deviation, n = 3, except for data points without standard deviation n = 2) note different scaling.

Fig. 7: *Calanus finmarchicus* and *Metridia longa*. Fatty alcohols 22:1(n-11) (a) and 20:1(n-9) (b) in % dry mass during the time series (mean ± standard deviation, n = 3, except for data points without standard deviation n = 2) note different scaling.

Fig. 8: Proteinase and amylase activity per individual of *Calanus finmarchicus* (a) and *Metridia longa* (b), (mean ± standard deviation, n = 3, except for data points without standard deviation n = 2).

Fig. 9: Pyruvate kinase (PK) and citrate synthase (CS) activity per individual of *Calanus finmarchicus* (a) and *Metridia longa* (b) (mean ± standard deviation, n = 3, except for data points without standard deviation n = 2).

Fig. 10: Egg production rates (eggs ind⁻¹ d⁻¹) of *Calanus finmarchicus* and *Metridia longa* females (n = 24), and chlorophyll a content (μg l⁻¹) of seston.
Fig. 1

(a) Temperature (°C) at different depths (30 m, 100 m, 200 m, 300 m) over the months of May to August.

(b) Salinity (psu) over the same period.
Fig. 2

![Graph a](image1)

![Graph b](image2)

Graph a: Percentage of total phytoplankton (% of total phytoplankton)

- Autotrophic flagellates
- Prymnesiophyceans
- Cryptophyceans
- Dinoflagellates
- Others
- Diatoms

Graph b: Total cells (10^6 l^-1)

- 24: May
- 31: June
- 12: July
- 21: August
- 29: May
- 5: June
- 11: July
- 19: August
- 28: May
- 1: June
- 9: July

Legend:
- Others
- Autotrophic flagellates
- Prymnesiophyceans
- Cryptophyceans
- Dinoflagellates
- Diatoms

**Note:** The graphs illustrate the monthly distribution of phytoplankton types in terms of percentage and total cell count.
Fig. 3

(a) Lipid content and chl a content over time from May to August.

(b) C content, N content, and C:N ratio over time from May to August.
Fig. 4

(a) δ^13C

(b) δ^15N
Fig. 5

(a) Protein content (μg ind⁻¹)
(b) Lipid content (μg ind⁻¹)
(c) Carbon content (μg ind⁻¹)
(d) Nitrogen content (μg ind⁻¹)
(e) Protein content (μg ind⁻¹)
(f) C:N ratio

Graphs showing the content of proteins, lipids, carbon, nitrogen, protein, and C:N ratio for different months.
Fig. 6

a. C. finmarchicus and M. longa

b. 20:5(n-3)

c. 18:4(n-3)

d. 22:6(n-3)

e. 22:1(n-11)

f. 20:1(n-9)
Fig. 7

![Graph showing fatty alcohol content of C. finmarchicus and M. longa over time.](image)
Fig. 8

**a**

- Pyruvate kinase activity (units $10^{-2}$ ind$^{-1}$)
- Citrate synthase activity (units $10^{-2}$ ind$^{-1}$)

**b**

- Pyruvate kinase activity (units $10^{-2}$ ind$^{-1}$)
- Citrate synthase activity (units $10^{-2}$ ind$^{-1}$)
Fig. 9

(a) Proteinase activity ($dE_{366} \text{min}^{-1} \times 10^3 \text{ ind}^{-1}$) vs. amylase activity ($dE_{600} \text{min}^{-1} \times 10^3 \text{ ind}^{-1}$) over the months of May to August.

(b) Proteinase activity ($dE_{366} \text{min}^{-1} \times 10^3 \text{ ind}^{-1}$) vs. amylase activity ($dE_{600} \text{min}^{-1} \times 10^3 \text{ ind}^{-1}$) over the months of May to August.
Fig. 10

The figure shows a line graph with the following data:

- **X-axis**: Dates from May 24 to August 9.
- **Y-axis**: EPR (eggs ind$^{-1}$ d$^{-1}$) and chlorophyll a (μg l$^{-1}$).
- **Graph Lines**:
  - **Solid black line**: C. finmarchicus.
  - **Dashed black line**: M. longa.
  - **Dotted black line**: Chlorophyll a.

The data points indicate a seasonal trend with peaks and troughs for each category over the specified months.
DIETARY SHIFTS IN THE NORTH SEA COPEPOD Temora longicornis IN SPRING 2005 – EVIDENCE FROM STABLE ISOTOPE SIGNATURES, FATTY ACID BIOMARKERS AND FEEDING EXPERIMENTS

Gentsch E, Kreibich T, Hansen B, Hagen W, Niehoff B

submitted to Marine Ecology Progress Series
DIETARY SHIFTS IN THE NORTH SEA COPEPOD *Temora longicornis* IN SPRING 2005 – EVIDENCE FROM STABLE ISOTOPE SIGNATURES, FATTY ACID BIOMARKERS AND FEEDING EXPERIMENTS

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²Marine Zoology (FB 2), University of Bremen, P.O. Box 330440, D-28334 Bremen, Germany

Abstract

The calanoid copepod *Temora longicornis* occurs abundantly throughout the year in the southern North Sea. Due to its inability to store significant amounts of energy for periods of low food availability, *T. longicornis* relies on a constant supply of food. Phytoplankton standing stock is low in winter and early spring. Therefore, in order to maintain its metabolic processes, *T. longicornis* needs to utilize other food sources as well. We combined weekly incubation experiments from March to May 2005 using natural <70 μm plankton assemblages with gut content analyses, stable isotope signatures (δ¹⁵N and δ¹³C) and fatty acid biomarkers to get a broad view on the feeding preferences of *T. longicornis* females. The results show that *T. longicornis* females are omnivorous, and switch their feeding mode to more herbivorous feeding with increasing phytoplankton concentrations in spring. The δ¹⁵N signatures measured for *T. longicornis* females in early spring are the highest reported for this species so far and agree with the gut content analyses, indicating feeding on nauplii. The incubation experiments revealed selective feeding on dinoflagellates in early spring, but not on the abundant diatoms. The latter were selectively ingested later in spring, when they were less abundant. Also the trophic marker fatty acids 16:1(n-7) for diatoms and 18:4(n-3) for dinoflagellates indicated increased feeding on these algae with the onset of the productive season. *T. longicornis* females ingested cells of three size fractions (<12.5 μm; 12.5–30 μm and >30 μm), with strongest selection for the larger cells. In summary, *Temora longicornis* females adjust their feeding modes to ambient food conditions and utilize a broad range of potential food sources ranging from phytoplankton to zooplankton, such as nauplii.
Introduction

The calanoid copepods *Temora longicornis*, *Acartia clausi*, *Pseudocalanus elongatus*, *Paracalanus parvus* and *Centropages* spp. contribute a major fraction of zooplankton abundance in the North Sea (Krause & Martens 1990, review in Fransz et al. 1991, Greve et al. 2004). In contrast to larger copepods from high latitudes, these species are generally not capable of storing significant amounts of lipids as energy source to sustain longer periods of low food availability (Lee et al. 2006), but are dependent on a continuous food supply. These copepods play a major role in the North Sea food web throughout the year and may have a substantial impact on the phytoplankton standing stock (Maar et al. 2004). *T. longicornis*, as a representative of this group, is reproducing year-round and exhibits highest abundances in the North Sea between fall and spring (reviewed in Fransz et al. 1991). Off Helgoland it is abundant throughout the year (Halsband & Hirche 2001, Greve et al. 2004, Halsband-Lenk et al. 2004).

Laboratory studies with specific diets and field studies with natural particulate matter (PM) have revealed that *T. longicornis* is omnivorous, feeding on phytoplankton as well as on other zooplankton and ciliates (Marshall & Orr 1966, Kleppel 1993, Dam & Lopes 2003).

Feeding experiments and gut content analyses are widely used methods to study feeding in copepods (e.g. Gauld 1951, Marshall & Orr 1966, O’Connors et al. 1980, Baars & Fransz 1984, Kiorboe et al. 1985, Dam & Lopes 2003). However, these methods reveal information on the ingested food items on a short time scale only. Therefore, analyses of carbon and nitrogen stable isotopes as well as fatty acid trophic markers have recently become important tools, since these methods reveal the longer-term feeding history of a species (Minagawa & Wada 1984, Post 2002, reviewed in Dalsgaard et al. 2003, Stübing & Hagen 2003, Lee et al. 2006). The analysis of stable nitrogen ($\delta^{15}$N) and carbon ($\delta^{13}$C) isotopes elucidates the trophic position of an organism within a food web ($\delta^{15}$N) and its basic carbon source ($\delta^{13}$C) (Post 2002, Sato et al. 2002, Gorokhova et al. 2005, Sommer et al. 2005). Various studies in different ecosystems have shown an average increase in $\delta^{15}$N of approximately 3.4 ‰ (Minagawa & Wada 1984, reviewed in Post 2002) and of 0.5 to 1 ‰ for $\delta^{13}$C (Michener & Schell 1994) between trophic levels. Specific fatty acids (FA) can be used as trophic biomarkers of food items, as these FA cannot be synthesized by the animal itself and their unmodified incorporation and accumulation in the lipid stores of the animal indicates the ingestion of specific food items. For examples, the fatty acids 16:1(n-7) and 18:4(n-3) are indicative of diatoms and dinoflagellates, respectively (Kates & Volcani 1966, Harrington et al. 1970). Hence, the food spectrum of an animal may be derived from its fatty acid composition. Even though general knowledge about the feeding behaviour of *T. longicornis* is broad, information about the response of this species to different food regimes in nature, e.g. before, during and after the spring diatom bloom, is still scarce. This, however, is essential to quantify...
the specific role of this species in the pelagic food web and to estimate its influence on the phytoplankton community. The aim of our study was, therefore, to elucidate whether and how *T. longicornis* changes its foraging strategy according to changing food conditions in the North Sea. We studied, on a weekly basis, grazing, gut contents, stable isotope signatures and fatty acid compositions in female *T. longicornis* from March to May 2005 at Helgoland, North Sea. By combining these different methods, we present a comprehensive approach on the foraging behaviour of *T. longicornis* during spring in the North Sea.

**Material and Methods**

The time series was sampled from 8 March to 24 May 2005 on a weekly basis at the Biologische Anstalt off the island of Helgoland (Germany) in the North Sea. This included weekly sampling for the analysis of stable isotope signatures (δ15N and δ13C), carbon (C) and nitrogen (N) content as well as fatty acids compositions of both particulate matter (PM) and *Temora longicornis* females. In addition, water samples were analysed for chlorophyll *a* (chl *a*) content as well as phytoplankton composition and abundance. All samples were taken onboard RV "Aade" at the 20 m depth line (54° 11´26´´N, 7° 53´94´´E), unless weather conditions were inappropriate. In the latter case, samples were taken at the station Kabeltonne, Helgoland Roads (54° 11´18´´N; 7° 54` E).

Water samples were collected with a 3 L NISKIN bottle from 3 m depth. Zooplankton was caught using Nansen- and Calcofi nets with 150 μm and 500 μm mesh size, respectively. Vertical hauls from the bottom to the surface were taken with the Nansen net, while the Calcofi net was towed horizontally for 10 min. All samples were immediately transferred to the laboratory where they were stored in the dark at ambient temperature until further processing.

In the laboratory, water samples were screened over 70 μm mesh to remove zooplankton and copepod eggs. The Nansen net sample was immediately preserved in 4 % borax-buffered formalin for the determination of copepod abundances. Copepods for the time series and the experiments were sorted from the Calcofi net samples in order to get sufficient numbers of fit animals.

Data for salinity, temperature and nutrients were provided by Wiltshire et al. (in review) as part of the Helgoland Reede time series data (Franke et al. 2004). For explanation of changes in the salinity, we used the DRIFT Model programme by Greve (pers. comm.) to elucidate the particle drift and thereby the movement of the water body around Helgoland during the time series. Computation was done with a 14- and 7-day backwards tracking of particles.
point was Helgoland Roads, from where the particle movement history was calculated. This was done for each sampling date during the time series.

Analysis of the particulate matter in the water column

On each sampling date, triplicates of water sub-samples (500 ml each) were filtrated over pre-combusted (500 °C, 24 h) and pre-weighed GF/C filters (Ø 25 mm). Salt was washed off the filters with some drops of distilled water. For the analysis of C and N content, stable isotope signatures and fatty acid compositions (see below), three sub-samples were used for each analysis. To determine pigment concentrations as a proxy for food amount and composition, on each sampling date additional three water sub-samples (500 ml each) were filtrated on Whatman nylon membrane filters (0.2 μm pore size). All filters were immediately frozen at −80 °C.

Carbon and nitrogen content and stable isotope analysis

For analysis, frozen samples were dried at 60 °C for at least 48 h before they were weighed on a Sartorius M2P microbalance (0.001 mg accuracy). After weighing, the filters were wrapped in Zn foil for further treatment. The stable isotope signatures of δ¹⁵N and δ¹³C were analysed at the AGROISOLAB Isotopenlabor in Jülich/Germany. C and N content were measured with an elemental autoanalyser (EURO EA Elemental Analyzer (EuroVector), Hekatech, Germany).

High Pressure Liquid Chromatography pigment analysis

For pigment analysis, the Whatman filters were transferred into Falcon tubes with 2 ml analytical acetone added. Filters were immediately frozen at −80 °C and stored for a minimum of 48 h prior to further analysis. Measurements were conducted at the Biologische Anstalt Helgoland at the working group of K.H. Wiltshire according to procedures described by Knefelkamp et al. (2007) and Wiltshire et al. (1998). For measurements, analytical quartz sand was added to the falcon tubes and the filters were ground to extract the pigments. The tubes were then placed in a sonification bath for 90 min before the supernatant was extracted into analytical vials for measurements using a Waters 2695 Separation Module.

Fatty acid composition

Triplicate sub-samples of PM were analysed for fatty acids (FA) on a gas chromatograph (HP 6890A) equipped with a DB-FFAP column, as described in detail by Peters et al. (2006). In this study, we focussed on FA that are potential trophic biomarkers. These FA are obtained from the diet and incorporated directly and unmodified in the organisms lipids. The mono-unsaturated FA (MUFA) 16:1(n-7) is known as potential marker for diatoms (Nichols et al.
1993, Dunstan et al. 1994, Skerrat et al. 1997), and the poly-unsaturated FA (PUFA) 18:4(n-3) as a marker for a dinoflagellate-based diet (Sargent et al. 1987, Graeve et al. 1994). A detailed description of the total fatty acid composition is given in Kreibich et al. (submitted), who conducted a parallel study with *T. longicornis* at Helgoland Roads throughout the same period.

**Taxonomic composition and cell numbers**

For the determination of phytoplankton composition and abundance, a 100 ml sub-sample of the water sample was preserved in Lugol’s solution on each sampling day. Depending on chl a content in the water, between 20 and 50 ml of the samples were analysed in Utermöhl chambers according to the procedures applied within the Helgoland time series of the Biologische Anstalt Helgoland (Utermöhl 1958, Hickel et al. 1993, Wiltshire & Manly 2004, Wiltshire et al. in review). Chambers were counted completely for >30 μm cells; 4 rows were counted for medium-sized cells and 2 rows for small cells. Algae and ciliates were identified to the lowest possible taxonomic level. All cells were categorized into three size-classes (70 – 30 μm, 12.5 – 30 μm, and <12.5 μm cell diameter, respectively). For better comparison of the results, diatom and dinoflagellate species were grouped into centric and pennate diatoms, and athecate and thecate dinoflagellates, respectively. Diatom cells in chains and colonies were counted and treated as individual cells in the calculations. Ciliate species, due to low abundances, were grouped, as well as flagellates, which cannot be determined to lower taxonomic level without larger methodological effort. Rare algae species (predominantly *Dictyocha* spp. and *Pediastrum* spp.) not belonging to the mentioned groups, were pooled as "Other algae". Other colonial algae (predominantly *Phaeocystis* sp. and Cyanophyceae) were not considered in the calculations because of generally low abundances in the samples.

**Analysis of feeding of Temora longicornis**

**Stable isotope signatures**

To detect changes in the trophic position, triplicates of 20 to 30 live and intact female *T. longicornis* were collected weekly from the net samples and transferred into acrylic cylinders with net bottoms placed in beakers filled with GF/C pre-filtered seawater. At ambient temperature, the females were allowed to defecate for at least 4 h. Particles, faecal pellets and eggs fell through the meshes, so that feeding on these was not possible. For analysis, copepods were individually washed in distilled water to remove any particles and salt, before they were transferred into pre-weighed Zn cartridges. 20 to 30 copepods were placed in one cartridge in order to have sufficient biomass for later analysis. Filled cartridges were immediately frozen at −80 °C. For analysis, the copepod samples were treated as the PM samples (see above). Prior to analysis, the Zn cartridges containing copepods were closed to
avoid loss of material. We did not treat the copepod samples with HCl, as is often recommended (e.g. Jacob et al. 2005), because the sample sizes were too small. Treatment would have caused high losses of biomass, making measurements impossible. Lipids were also not removed owing to the same reasons.

**Carbon and nitrogen content of *T. longicornis* females**

Triplicates of 10 alive field caught *T. longicornis* females were rinsed in distilled water, placed in pre-weighed Zn cartridges and frozen at –80 °C. These samples were analysed as the PM samples for carbon and nitrogen content.

**Fatty acid biomarkers in *Temora longicornis* females**

Triplicates of 10 alive field-caught *T. longicornis* females were individually rinsed in distilled water, placed in glass vials and immediately frozen at –80 °C. A detailed description of the method and the total fatty acid composition of the females is given by Kreibich et al. (submitted). Here, we focussed on the two trophic marker FA 16:1(n-7) for diatoms and 18:4(n-3) for dinoflagellates.

**Gut content and nauplius abundance**

It is known that *T. longicornis* may feed on eggs or copepod nauplii (Marshall & Orr 1966, Kleppel et al. 1991), and the latter can be detected in the guts of the copepods. Therefore, the gut content of 6 to 12 *T. longicornis* females from each sampling day were analysed qualitatively using a Zeiss Axioskop microscope. The guts were removed from the copepods and gently squeezed and embedded in polyvinylactopelhol on object slides. The gut contents were stained with Ligninpink for better identification of the contents. To estimate the possible influence of predation on copepod population dynamics, the abundance of copepod nauplii (not determined to species level) on the sampling days was determined from the Nansen net samples. These samples were split in a Folsom plankton splitter. Depending on abundance of nauplii in the original sample, samples were split down to 1/128th of the original sample. A minimum of 36 nauplii was counted from the samples on each experimental day.

**Feeding experiments**

A total of 14 feeding experiments were conducted during the investigation period from March to May to estimate grazing and faecal pellet production rates and to detect selective feeding. Throughout the time series, experiments lasted 24 h. The first results of HPLC pigment analysis showed that the phytoplankton standing stock <70 μm in March and April was very low. Therefore, we assumed fast depletion of food particles, thus food limitation, due to grazing in the 24 h incubation experiments. For that reason, four additional incubation
experiments, each lasting 7 h, were conducted from the end of April to the end of the time series in May.

For each experiment, healthy and intact looking *Temora longicornis* females were sorted alive on ice under cold light using a wide-opening glass pipette. 3 x 35 individuals were transferred into GF/C filtered seawater at ambient temperature, where they were kept for a maximum of 2 h until the experiments started.

Three incubation and three control bottles were filled with seawater screened over 70 μm mesh. F/2 medium was added to exclude an effect of copepod excretion on phytoplankton growth. Thirty-five copepods were added to each incubation bottle, and the bottles were gently filled rim-tight with seawater to avoid air bubbles in the bottles. All bottles were mounted on a plankton wheel at 0.8 rpm. At the end of each experiment, the copepods were gently poured out of the incubation bottles. Twelve (March 8) or 24 individuals per incubation bottle were individually placed in cell wells filled with GF/C filtered seawater in order to determine faecal pellet production. This was monitored in 15 min intervals for up to 3 h.

Faecal pellet production usually peaked 30 min after the transfer of the females from the bottles into the cell wells. Thereafter, it decreased to almost zero within one hour, indicating that most of the ingested food must have passed the gut within this period. Faecal pellet production was monitored further for up to 3 h and latest after 2 h, the majority of pellets were much smaller and lighter in colour than the initial ones. This was probably due to food limitation, since the females were kept in GF/C-filtered water to monitor faecal pellet production. Therefore, only the first 45 min were considered for calculations of daily faecal pellet production, as we assume that this gives a better estimate of the egestion rates during the incubation.

The water from the incubation bottles was sampled to determine pigment concentration and to analyse taxonomic composition and abundance of cells, as described above. The pigment concentration in the incubation water was too low for sufficient analysis of pigment grazing rates throughout the time series. Therefore, grazing rates were determined according to Frost (1972), based on Utermöhl sample counts. Jacobs Selectivity index (Jacobs 1974) was used to find any selectivity of *T. longicornis* for algal size classes and for algal groups.

**Results**

**Hydrography**

From March 8 to May 17 2005, seawater temperature increased continuously over time from 3.2 °C to 9.9 °C.
Salinity increased from 31.3 (8 March) to 33.7 (5 April). Throughout April to mid-May, salinity ranged between 32.4 (12 May) and 33.6 (6 April). Salinity dropped to 30.9 on 13 May (Fig. 1a). This variability indicates that water masses changed during the investigation period, with more coastal influence in early March and from mid-May on and with more central North Sea origin in between. This is in agreement with the computed water circulation patterns using the DRIFT model. Water masses in early March originated from the Elbe and Weser estuary, while in April, water with higher salinity originated from west to northwest of Helgoland. According to the model, the salinity drop in May was caused by the influence from the Weser estuary.

**Particulate matter (PM) in the water column**

The Secchi depth, used as a proxy for turbidity, was low (ca. 2 m) during March, indicating high amounts of particulate matter in the water column. During April, it increased to a maximum of 11.8 m and after 4 May, it ranged between 6 and 8 m (Fig. 1a). The Secchi depth was not mirrored by the PM carbon content, suggesting that other components, possibly sediment, had strongly contributed to turbidity. Carbon content of the PM (<70 μm) was highest on 8 March with almost 500 μg C l\(^{-1}\). Thereafter, it varied between 250 and 400 μg l\(^{-1}\), except for 5 April (150 μg l\(^{-1}\)). N content varied between 50 and 190 μg l\(^{-1}\) with slightly higher contents toward the end of April and in May. As a consequence, the C/N ratio decreased over time from >10 in March to 6.5 at the end of May (Fig. 1b).

From March to the end of April, the \(\delta^{15}N\) of the PM ranged between 9.43‰ ± 0.58 (30 March) to 5.87‰ ± 0.82 (5 April), with a high short-term variability of more than 4‰ (Fig. 1c). In May (Fig. 1c) \(\delta^{15}N\) values were generally lower and variability decreased (4 May: 5.29‰ ± 0.13; 17 May: 17.29‰ ± 0.14). The \(\delta^{13}C\) in the PM ranged between -17.61‰ ± 0.19 (15 March) and -25.15‰ ± 0.39 (4 May) (Fig. 1d). It was enriched in March (approx. -18‰) and, within the first week in April it had decreased to -24‰. In May, the \(\delta^{13}C\) increased slightly to approx. -22‰. Thus, \(\delta^{13}C\) and Secchi depth were negatively correlated (\(r^2: 0.6; p: <0.0001; 95\% \text{ confidence interval (CI)}\)), indicating that the \(\delta^{13}C\) signatures are probably influenced by the resuspension of benthic material.

**Chlorophyll a concentration and taxonomic composition**

Chl \(a\) concentrations in the <70 μm size fraction of the PM ranged between 0.75 and 1.2 μg l\(^{-1}\) (Fig. 1b) in March, with higher concentrations in early than in late March. It remained low until mid-April but increased towards mid-May.

We had problems in detecting pigment levels on some sampling dates in March and April (Fig. 1b). Therefore, we compared our measurements with those of Wiltshire et al. (in review), who sampled on the same dates but did not screen the water over 70 μm (Fig. 1b). The results
agree from 8 to 22 March and on 12 April. On 31 March and 5 April, we could not detect chl a, while Wiltshire et al. (in review) did. The chl a concentration in April peaked at a maximum of 2.37 μg chl a l⁻¹ on 26 April in the <70 μm fraction, and at 7.12 μg l⁻¹ in the unscreened samples on 22 April according to Wiltshire et al. (in review). In May, chl a concentrations ranged between 1.29 μg chl a l⁻¹ (4 May) and 2.75 μg chl a l⁻¹ on 10 May in our measurements, while Wiltshire et al. (in review) measured concentrations between 8.3 μg l⁻¹ (10 May) and 3.3 μg l⁻¹ (17 May) (Fig. 1b). The higher concentrations determined by Wiltshire et al. (in review) in May can be attributed to a high abundance of the centric diatom Coscinodiscus spp. (www.bsh.de/de/Meeresdaten/Beobachtungen/MURSYS-Umweltreportsystem/MURSYS_031/seiten/noph2_01.jsp#2005).

Most of these cells were presumably excluded from our samples due to screening over 70 μm. Corresponding to changes in the chl a concentration, the number of phytoplankton cells varied throughout spring. Cell numbers were lowest at the end of March (124,700 cells l⁻¹), ranged around 300,000 cells l⁻¹ in late April and May and peaked at 467,000 cells l⁻¹ on 17 May (Fig. 2a). The size composition of the phytoplankton cells in our samples (<70 μm) showed that cells <12.5 μm were generally dominating, followed by the medium-sized cells between 12.5 to 30 μm diameter. Cells >30 μm occurred in low numbers only (Fig. 2b).

Throughout the time series flagellates prevailed, representing between 37% (15 March) and 90% (12 April) of all algae cells <70 μm (Fig. 2c). This is also reflected by the dominating size class of cells <12.5 μm. Diatom abundance was highest in the beginning of March (48%, 8 March) and decreased to a minimum in April before it increased slightly again in May (Fig. 2c). Centric diatoms were dominated by Melosira spp. in March (5,100 cells l⁻¹ on 15 March), followed by Thalassiosira spp. in late April and May (2,000 cells l⁻¹, 26 April). Cylindrotheca sp. and Thallassionema sp. were the most abundant species among pennate diatoms (<Cylindrotheca sp. 2,080 cells l⁻¹ on 31 March; Thallassionema sp. 1,050 cells l⁻¹ on 15 March). Dinoflagellates occurred throughout the time series but usually in low abundances. Their relative abundance increased from late April on, reaching a maximum on 4 May (thecate dinoflagellates: 43%, Fig. 2c). Dinoflagellates were usually not distinguished to genus level, except for Dinophysis sp., Ceratium sp. and Prorocentrum sp. among the thecate dinoflagellates. These species were never abundant (ranging between 50 cells l⁻¹ and 300 cells l⁻¹ on 10 and 17 May). Pediastrum sp. and Dictyocha sp. (grouped as "other algae") and colonial Cyanophycea and Phaeocystis sp. occurred throughout the time series but were also not abundant, except at the end of May. Ciliates occurred in low abundances and were most abundant in April, peaking on 12 April (3,220 ciliates l⁻¹). The dominating ciliates were Strombidium spp. and Lohmaniella spp., the latter being more abundant, when Strombidium abundance decreased. The low abundance of ciliates may be explained by the exclusion of cells >70 μm in this study.
Fatty acid concentration in the PM

In contrast to a high diatom abundance in March, the concentration of the diatom trophic marker fatty acid 16:1(n-7) in the PM remained near 1 μg l⁻¹ until the beginning of May, except on April 26 (Fig. 2d). In May, the concentration of 16:1(n-7) increased to values between 2 and 3 μg l⁻¹ and a peak near 5 μg l⁻¹ on May 17. Similarly, the concentration of the FA 18:4(n-3) in the PM remained low in March and showed a strong increase after 15 April, except on 4 May, peaking near 2 μg l⁻¹ on 17 May (Fig. 2d). The apparent mismatch between diatom cell counts and FA concentrations in the PM may be explained by higher synthesizing rates of the FA under conditions of nutrient limitation (Kattner et al. 1983), which was the case from mid-April on (Wiltshire, pers. comm.). The FA concentrations in the PM can thus not be considered as a quantitative measure for diatom abundance and therefore does not contradict the diatom cell counts.

Temora longicornis abundance

Abundance of T. longicornis females was low throughout March and April, but increased from mid-May on (12 to 23 ind m⁻³) (Fig. 3a). Abundance of males was similar to female abundance but increased strongly in May (Fig. 3a). C5 copepodids peaked on April 5th (362 ind m⁻³), C1 to C4 copepodids showed a small peak at 122 ind m⁻³ on 22 March, and were most abundant from the end of April on (Fig. 3b, c).

Carbon and nitrogen content in Temora longicornis females

Carbon and nitrogen contents in T. longicornis females were low throughout March to April 7 (C: 8.2 to 14.2 μg ind⁻¹; N: 2.3 to 3.1 μg ind⁻¹) and increased towards the end of May, peaking on 21 April at 21.6 μg C ind⁻¹ and 5.7 μg N ind⁻¹. C:N ratios increased from 3.5 in March to 4.4 in May (see also Kreibich et al., submitted).

Stable isotope signatures of Temora longicornis females

According to stable isotope analyses, the trophic position of T. longicornis females changed considerably during spring 2005. The δ¹⁵N data indicate a carnivorous feeding history in March to mid-April (Fig. 1c). During that period, δ¹⁵N ranged between 14.9‰ ± 0.13 (5 April) and 16.04‰ ± 0.27 (31 March). That is nearly 10‰ above the PM signature (e.g. 22 March). δ¹⁵N signatures decreased sharply after 5 April to values only slightly higher than the δ¹⁵N values measured in the PM throughout May (Fig. 1c). This indicates herbivorous feeding. δ¹⁵N signatures measured in T. longicornis were negatively correlated with increasing chl a concentration measured (r² = 0.59; p <0.0001, 95% CI for data by Wiltshire et al. (in review), Fig. 1a; r² = 0.32; p = 0.0003, 95% CI for our own chl a measurements, Fig. 1a). There was no
correlation between δ¹⁵N signatures and the abundance of any of the dominating algal groups and ciliates. δ¹³C signatures in *T. longicornis* ranged between –19.1‰ (8 March) and –20.6‰ (31 March) in March before they increased to –16.7‰ on 17 May (Fig. 1d). Compared to the δ¹³C signatures measured in the PM from 15 March to 31 March, δ¹³C signatures in *T. longicornis* were depleted, whereas they were enriched from April throughout May (Fig. 1d).

**Fatty acids in Temora longicornis**

The percentage of the biomarkers 16:1(n-7) for diatoms and 18:4(n-3) for dinoflagellates in % of total fatty acids (FA) in the females increased throughout the time series. Both markers showed similar trends. The monounsaturated FA (MUFA) 16:1(n-7) increased slightly during March until the second half of April from 0.9% FA to 3.2% of total FA (Fig. 4). From the end of April, the 16:1(n-7) content increased strongly and peaked on 17 May with 15.4% of total FA. The PUFA 18:4(n-3) in % of total FA increased from 1.1% FA (10 March) to the maximum of 6.8 % FA on 26 April (Fig. 4). This FA comprised between 6-7% of total FA throughout 10 May, before it decreased again (Fig. 4). The increase of both FA in the females reflected the increase of these FA in the PM, as is described in detail by Kreibich et al. (in prep). Coinciding with the increase in the MUFA 16:1(n-7) and the PUFA 18:4(n-3) dry mass in the copepods, the δ¹⁵N signatures measured in *T. longicornis* decreased. This correlated significantly for both FA (16:1(n-7) r² = 0.75, p < 0.0001, 95% CI; 18:4(n-3) r² = 0.92, p < 0.0001, 95% CI) (Fig. 5). A detailed description of total fatty acid composition in the copepods and the PM is given in Kreibich et al. (submitted).

**Gut content analysis**

The guts of *T. longicornis* females were filled to a greater extent in March compared to late April and May. The analyses confirmed that the females were feeding on nauplii, as remnants of extremities were found in their guts throughout the time series. However, feeding on nauplii seemed to be more frequent in March, as naupliar remnants were detected more frequently in *T. longicornis* guts at that time compared to late April and May. Gut contents furthermore revealed feeding on centric and pennate diatoms as well as on thecate dinoflagellates throughout the time series. Feeding on nauplii cannot be explained by high naupliar abundance in March, as this was low (between 62 and 352 nauplii m⁻³) compared to nauplii abundance throughout April and May (peak on 5 April with 2228 nauplii m⁻³) with a mean of 716 ± 292 nauplii m⁻³ between 12 April and 17 May (Fig. 3c). Accordingly, the δ¹⁵N signatures in *T. longicornis* were not significantly correlated with the abundance of copepod nauplii (linear regression).
Clearance and ingestion rates

*Temora longicornis* females were feeding in all experiments conducted throughout the time series. Clearance rates changed considerably over time with higher rates in March than in April and May (Fig. 6a). Ingestion rates were also higher in March than in April and May. Clearance rates from March 8 to March 22 ranged between 4 and 5 ml cop⁻¹ h⁻¹ (Fig. 5a). Throughout April to mid-May, the clearance rate ranged between 2.51 ± 0.27 ml cop⁻¹ h⁻¹ on 5 May and 1.55 ± 0.03 ml cop⁻¹ h⁻¹ on 10 May. The 7 h experiments showed similar clearance rates on 26 April (24 h: 2.54 ml cop⁻¹ h⁻¹ and 2.61 ml cop⁻¹ h⁻¹; 7 h: 2.56 ± 0.76 ml cop⁻¹ h⁻¹). On 4 May and 10 May, however, they were 2.6 and 3.7 times higher in the 7 h experiments (6.43 ± 0.35 ml cop⁻¹ h⁻¹ and 5.85 ± 0.91 ml cop⁻¹ h⁻¹ respectively) than in the corresponding 24 h experiments (Fig. 5b).

Ingestion rate peaked at 6,202 ± 266 cells cop⁻¹ d⁻¹ on 15 March. From 31 March to 10 May, ingestion rates ranged between 612 ± 141 cells cop⁻¹ d⁻¹ on 31 March and 2,176 ± 193 cells cop⁻¹ d⁻¹ on 10 May. Ingestion rates in the 24 h and 7 h experiments were similar on 4 May (24 h: 1,734 ± 611 cells cop⁻¹ d⁻¹; 7 h: 2,292 ± 1,326 cells cop⁻¹ d⁻¹) but a fourfold higher on 10 May (24 h: 2,176 ± 193 cells cop⁻¹ d⁻¹; 7h: 8,399 ± 2,302 cells cop⁻¹ d⁻¹). Specific ingestion rates for dominating algal groups are given in Table 3.

Size selectivity

*Temora longicornis* females were feeding on algae in the three size classes in the PM. Strongest selection was found for cells >30 μm diameter with one exception on 15 March (Fig. 6a). On that date, the centric diatom *Melosira* sp. comprised 40 % of all cells in that size class but it was not grazed upon. Smaller cells were ingested, but usually not preferentially. Cells within the 12.5 μm to 30 μm size class were preferred over the <12.5 μm size class in March and in May, while it was the opposite in April (Fig. 6a).

Selectivity by the females in the 7 h experiments was in accordance with the selectivity results in the 24 h experiments on 26 April (Fig. 6b). On May 4, selectivity was similar, except that the 12.5 – 30 μm cells were selected for in the 7 h experiments, while they were rather avoided in the 24 h experiments (Fig. 6b). On 10 May, 12.5 – 30 μm cells were preferred over >30 μm cells in the 7 h experiments, while this was not the case in the 24 h experiments (Fig. 6b).

Species selectivity

Dinoflagellates were selectively grazed upon throughout the time series. Thecate dinoflagellates were the most preferred cells on 15 March and in April, while athecate dinoflagellates were the most preferred cells on March 8th and May 10th (Table 1). Selectivity for diatoms was highest on 26 April and 4 May, when their abundance in the PM was low.
(Table 1). Centric diatoms were preferred over pennate diatoms except for March 8, 15 and on May 10. Pennate diatoms were not preferred in March (except 31 March) and on 12 April. Flagellates were not selected for in the majority of the experiments (exceptions were 15 March and 5 and 12 April). Ciliates were always selected for in all experiments except for 8 March. The 7 h incubations revealed similar selectivity patterns but showed different degrees of preference or avoidance (Table 2). On 26 April, positive selection was only found for centric diatoms and thecate dinoflagellates in the 7 h incubation, while only flagellates were not selected for in the 24 h incubation (Table 1 and 2). On 4 May, the avoidance of thecate dinoflagellates in the 24 h incubations was even more pronounced in the 7 h incubation (Table 2). On 17 May only dinoflagellates were not positively selected for, while strongest selection was found for centric diatoms.

Faecal pellet production
Throughout the time series, production of faecal pellets by *T. longicornis* females increased from 25 pellets cop\(^{-1}\) d\(^{-1}\) in March to 44 pellets cop\(^{-1}\) d\(^{-1}\) on 26 April (Fig. 7). Production in the 24 h experiments decreased again in May to 24 pellets cop\(^{-1}\) d\(^{-1}\) on 10 May. Faecal pellet production by the copepods in the 7 h experiments was slightly higher than in the 24 h experiments (Fig. 7). The increasing faecal pellet production correlated significantly with the increase in chl a content in the POM, however, the variance was high (\(r^2 = 0.47\), \(p = 0.04\), 0.95 % CI).

Discussion
Our combination of field data and laboratory experiments confirms previous studies that *T. longicornis* is an omnivorous calanoid copepod, preying on phytoplankton, microzooplankton and nauplii (Marshall & Orr 1966, Kleppel 1993, Dam & Lopes 2003). Clearance and ingestion rates during spring in the North Sea were similar to those reported by e.g. Dam (1986), Tackx et al. (1989) and Jansen et al. (2006), and, as has also been shown in other studies, were related to food concentration (e.g. Dam 1986). Despite the fact that *T. longicornis* is omnivorous, our results show that this species may feed selectively. In doing so, it is not only very flexible in adjusting its feeding behaviour to the food regimes, but it also has a strong impact on the development of certain algal groups.

Temporal pattern of feeding behaviour
From March through mid-April, when both chl a contents and phytoplankton cell numbers were still low in the water column, nauplii were frequently found in the guts of *T. longicornis* females. The two fatty acids we have focussed on meet the trophic biomarker concept in that they are synthesized *de novo* by diatoms (16:1(n-7)) and dinoflagellates (18:4(n-3)) and that they are no constituents of zooplankton membrane lipids (review in Dalsgaard et al. 2003). Hence, their
presence in the copepod tissues is the result of herbivorous feeding (e.g. Falk-Petersen et al. 1987, Fraser et al. 1989, Lischka & Hagen 2006). From March through mid-April, however, the percentages of these biomarkers, especially of 16:1(n-7), were relatively low, both in the PM and in the females. Also, the δ¹⁵N values of 15 ‰ point to a rather high trophic level for T. longicornis (e.g. Hobson & Welch 1992). All this suggests that T. longicornis relied to a large extent on food resources other than phytoplankton from March through mid April. In contrast, the δ¹⁵N signatures from mid-April to the end of May, when phytoplankton concentration had increased, ranged between 7 ‰ and 9 ‰. Apparently, in this period autotrophic organisms have contributed to a large extent to the food of T. longicornis. Concurrently, the amount of the FA 16:1(n-7) increased in late April, both in the PM and the females, suggesting feeding on diatoms as confirmed by the grazing experiments. In addition, T. longicornis fed on dinoflagellates, as indicated by increasing amounts of FA 18:4(n-3) from mid April trough the end of May, while the number of nauplii in the female guts decreased.

Summarizing, a clear picture emerges of T. longicornis females feeding carnivously in the beginning of spring and subsequently switching to herbivorous feeding. On the other hand, both grazing experiments and gut content analyses revealed that the females were feeding on algae also in March/April. Moreover, the low biomarker levels present in March/April do not necessarily indicate that the females have not been feeding on algae at that time. Algae synthesize these fatty acids especially during periods of nutrient limitation (Kattner et al. 1983), and this may explain, why the low concentrations of 16:1(n-7) in both PM and females contrast with a relatively high diatom abundance in the water column from March through mid April. The concentration of 16:1(n-7) in PM and subsequently in T. longicornis females increased only, when nutrients became depleted in mid April (Wiltshire, pers. comm.). Thus, the question arises, whether we can really attribute the extraordinary high δ¹⁵N signature in March/April to carnivorous feeding.

δ¹⁵N signatures near 15‰ are within the range reported for fishes, birds and other high-level consumers (e.g. Hobson & Welch 1992) and imply highly carnivorous feeding in T. longicornis. However, the physiological processes behind the fractionation processes are not yet fully understood (e.g., Burkhardt et al. 1999, Needoba et al. 2003, Aberle & Malzahn 2007). Isotope signatures in algae, which are the principal food component of many zooplankton taxa, depend on various factors such as CO₂ concentration, temperature, salinity, nutrient availability and cell size (Goering et al. 1990, Zohary et al. 1994, Leboulanger 1995, Montoya & McCarthy 1995, Rau et al. 1997, Burkhardt et al. 1999, Needoba et al. 2003, Savoye et al. 2003, Vuorio et al. 2006, Aberle & Malzahn 2007). Accordingly, a broad range of isotope enrichments in δ¹⁵N between as well as within phytoplankton taxa has been reported (Montoya & McCarthy 1995, Needoba et al. 2003, Vuorio et al. 2006). The isotopical signatures of algae can be highly variable between days (Aberle & Malzahn 2007) and accordingly, the δ¹⁵N enrichment between primary producers and consumers varies
considerably, between 0 ‰ and almost 6 ‰ (Checkley & Entzeroth 1985, Adams & Sterner 2000). Studies in lakes have shown that the δ¹⁵N values of animals from litoral, profundal or pelagic habitats differ significantly, even though these organisms feed upon the same kind of prey such as phytoplankton (Van der Zanden & Rasmussen 1999, Post 2002). The high variability in time and between sites points to the difficulty of drawing conclusions on feeding behaviour by comparison of isotope signatures, without considering additional information on nutrition, ecology and physiology (Gaye-Siessegger et al. 2004). Therefore, if no other data are available, such as ingestion rates and prey selectivity as well as the taxonomic composition of PM, interpretations based solely on isotope data can easily be misleading.

We suggest that the high δ¹⁵N signatures in March and April do not indicate feeding exclusively on heterotrophic organisms, but rather reflect omnivorous feeding, including ingestion of diatoms and dinoflagellates. Diatoms can have relatively high δ¹⁵N values compared to other phytoplankton taxa (Montoya & McCarthy 1995, Vuorio et al. 2006). Thus, the diatom cells ingested might have had higher δ¹⁵N signatures than the bulk PM and this, together with the heterotrophic prey ingested, may explain the strong enrichment in the females relative to the PM in March and the first half of April. In addition to the types of prey, i.e. diatoms and nauplii detected in the guts, it is likely that the females preyed upon copepod eggs and other heterotrophic prey, as has been reported for this species (Daan et al. 1988, Sell 2001, Dam & Lopes 2003). The females caught in March presumably hatched during the winter months (Wesche et al. 2006) and experienced low phytoplankton concentrations throughout their entire development (Hickel et al. 1993). Hence, the copepods have probably been feeding to a large extent on heterotrophic prey items at that time, resulting in a higher accumulation of δ¹⁵N in the tissue throughout ontogenesis.

With the increasing phytoplankton standing stock in spring, the δ¹⁵N signatures of the females decreased, nearly resembling PM signatures from late April onwards. The δ¹⁵N values of females ranged between 7 and 9 ‰ and these agree with previous studies on T. longicornis and other small calanoid copepods (see Table 4 for references). The importance of nauplii or other zooplankton as prey was less pronounced for these females, as they faced a higher availability of phytoplankton as well as ciliates as prey, the latter indicated by the higher ingestion rates for ciliates.

The picture emerging from the δ¹³C signatures in PM and females is different from the δ¹⁵N signatures. The computed coastal influence and the reduced Secchi depth in March indicate the import of terrigenous material and resuspension of benthic matter. This coincided with a period of increased δ¹³C signatures in PM in March relative to the females in March and to the PM in April and May. Coastal waters often have higher δ¹³C signatures than oceanic waters (Perry et al. 1999, Fredriksen 2003). This enrichment is due to the input of allochthonous material such as land-derived matter, as well as due to detritus and macroalgal fragments.
(Hobson & Welch 1992, Michener & Schell 1994, Pinnegar et al. 2000, Fredriksen 2003, Bode et al. 2006, Kaehler et al. 2006). It is likely that macroalgal fragments, originating in the vicinity of the island, had a strong influence on the PM $\delta^{13}$C signature and masked the $\delta^{13}$C signature of the food utilized by *T. longicornis* females, which explains the depletion in $\delta^{13}$C in the females relative to the PM in March. This also indicates that the imported and resuspended matter seems to be of low nutritional importance for the copepods.

The strong $\delta^{13}$C enrichment of on average 5.06 ‰ in *T. longicornis* relative to PM in April and May is in the upper range of what has been reported for this species (see Table 4 for references). These signatures indicate feeding on high trophic levels. This disagrees with the conclusions drawn from the $\delta^{15}$N signatures, the feeding experiments and the gut content analyses. We therefore assume that the $\delta^{13}$C signatures of the ingested cells again were masked in the bulk PM $\delta^{13}$C signatures.

**Selective feeding**

Establishing a relationship between food and consumer is generally difficult (Klein-Breteler et al. 2002), and this is certainly true for species selectively feeding on PM components. The analyses of the $\delta^{15}$N and $\delta^{13}$C of PM provide bulk isotopical signatures of particulate matter of a given size class only, in our case <70 μm. PM thus includes autotrophic and heterotrophic organisms as well as detritus and other particulate matter, all exhibiting potentially different isotopical signatures. Gut content analysis reveals only food items with hard structures, which can be found in the guts; soft-bodied organisms such as flagellates and ciliates cannot be detected by this method. To study selective feeding, we have therefore conducted grazing experiments.

*Temora longicornis* generally selects for large cells (e.g. Tackx et al. 1990, Vincent and Hartmann 2001). The retention efficiency in *T. longicornis* is 100% for cells >10 μm estimated spherical diameter (ESD), whereas it is only approximately 25 % for cells of 5 μm ESD (Ninivaggi 1979, cited in Dam 1986), and ingestion rates increase with increasing food size (O’Connors 1980). Accordingly, the females in our study selected for cells >12.5 μm over cells <12.5 μm. This included dinoflagellates, diatoms as well as ciliates. Flagellates, belonging to the <12.5 μm size class, were also ingested but only at low rates compared to their relative abundance. In contrast, Cottonec et al. (2001) found selection for cryptophytes (7 μm) by *T. longicornis* during a *Phaeocystis* sp. bloom. They concluded that the selection for these small cells was due to their nutritive value rather than their abundance, which was low (Cottonec et al. 2001).
Preference for larger cells can simply be a result of higher encounter rates, contrasting with encounter rates for smaller cells (Price et al. 1983). However, also within the >12.5 μm size classes the females selected for specific groups. Ciliates were always preferably ingested. Their contribution to the nutrition of *Temora longicornis* females was probably severely underestimated in our grazing experiments, due to screening the incubation water through 70 μm mesh in order to remove copepod nauplii and eggs. Dinoflagellates, generally known to contribute substantial components to copepod nutrition (Kleppel et al. 1991, reviewed in Kleppel 1993, Guisande et al. 2002, Jansen et al. 2006), were selected for especially in March. Other studies, in contrast, found no selection for the larger dinoflagellates even though these were of high nutritional value (Cottonec et al. 2001). The authors, however, did not rule out the presence of toxic dinoflagellates, which might have caused the avoidance (Cottonec et al. 2001). Also Vincent and Hartmann (2001) reported no selection for dinoflagellates by *T. longicornis* in winter in the Bay of Biscay.

Diatoms, in general, constitute a major fraction of copepod nutrition and their role in copepod nutrition is widely debated (e.g., reviewed in Kleppel 1993, Lincoln et al. 2001, reviewed in Ianora et al. 2003, Jónasdottir et al. 2005). In our study, diatoms were ingested in all experiments. Even though they were abundant and detected in the guts in March, they were not selected for then, as opposed to April and May, when their abundance in the phytoplankton was low in the <70 μm size class. Therefore, even though the selectivity index may indicate strong avoidance of specific algae as prey, it does not imply that these are not ingested at all. Indicated avoidance of diatoms matched the results on 15 March, when *T. longicornis* females clearly avoided the most abundant and chain-forming centric diatom *Melosira* sp. The consumption of such colonial and chain-forming diatoms may be limited, because problems can arise in dismembering the colonies (Cottonec et al. 2001). Paffenhöfer (1976) reported decreasing ingestion rates, when abundance of chain-forming algae increased. Handling problems alone are, however, not likely to be the only reason for the avoidance of *Melosira* sp. on 15 March, because the chain-forming diatom *Thalassiosira* sp. becoming abundant later in the season, was ingested at high rates. High grazing rates on *Thalassiosira* sp. were also found by Koski et al. (2005) during a four-week mesocosm study, which studied the influence of a *Phaeocystis globosa* bloom on selective grazing by *T. longicornis*. *Eurytemora affinis* and *Acartia tonsa* feeding on *Melosira jurgensii* have been reported from the Westerschelde estuary (Tackx et al. 1995). These authors reported the avoidance of single cell *M. jurgensii* by *A. tonsa*, in favour of larger-sized douplets (Tackx et al. 1995). The *Melosira* sp. we found were >30 μm diameter. Small size as limiting factor can thus be excluded. Based on the information we have, we cannot depict what caused the avoidance of *Melosira* sp. on 15 March, as handling problems as well as size are unlikely to have limited feeding on this diatom.
Feeding impact

Zooplankton impact on phytoplankton development such as during diatom blooms, may potentially be of great importance (e.g. Bathmann et al. 1990, Greve et al. 2004, Maar et al. 2004, Wiltshire & Manly 2004). We therefore calculated the grazing impact of *T. longicornis* females on the phytoplankton standing stock, based on the ingestion rates we determined (% ingested cells of standing stock). The grazing impact decreased with increasing phytoplankton-standing stock. On average, 7.2% of total available phytoplankton was ingested by *T. longicornis* according to the 24h incubations. The average impact was even 18.4%, if calculated from the 7h incubations only, since here 41% were ingested on 10 May. Grazing impact was highest on cells >30μm, the main food particle size. Between 9.5 and 26.5% of these were ingested, with one exception of 1.4% on March 31. The impact on the smaller cells was on average 8.2% (12.5 – 30 μm) and 6.86 (<12.5 μm). Also the impact on different algal groups differed. During maximum abundances of diatoms (March 8 to 22), *T. longicornis* females removed between 3.6 and 8.6% of the diatom standing stock. The impact was greater when diatom abundance was low, ranging between 34% (26 April) according to the 24h incubations and in excess of the standing stock according to the 7h incubations (17 May, 123%). Similarly, between 1.4% and all dinoflagellates were ingested by *T. longicornis*, with rates ranging between 26 and 35 % in March and minimum rates in April and May (24h incubations), when the dinoflagellate standing stock was high. Again, the 7h incubations yielded higher impacts on the standing stock (212% 10 May). Mean rates for flagellates and ciliates were 8.5% and 22.4%, respectively. Such calculations only represent very simple interactions between copepods and phytoplankton and exclude other potential phytoplankton grazers as well as other factors influencing phytoplankton growth and development. Therefore, such calculations have to be considered with care. However, the calculations show that the grazing impact of e.g. copepods on phytoplankton can be high and they are in line with other studies and assumptions (e.g. Bathmann et al. 1990, Greve et al. 2004, Maar et al. 2004, Wiltshire & Manly 2004), stating that copepods can have a substantial negative impact on stocks of certain phytoplankton groups that are considered as important food sources for zooplankton.

Conclusions

Kleppel (1993) focuses in his review of copepod diets on the importance of dietary diversity. Having a broader range of potential food is important under conditions when specific food sources are depleted. This is of special importance for copepods that are unable to store lipids as energy source. *T. longicornis*, as a representative of these copepods, having high metabolic requirements, a high biomass turnover and low energy reserves (Mayzaud et al. 1992) is dependent on a constant food supply. This species, as shown in our study, copes with this challenge by showing a broad and opportunistic feeding behaviour, which includes
feeding on other zooplankton, as well as on protists and phytoplankton of various size classes. Even though feeding on a broad dietary spectrum, feeding is selective and does not necessarily reflect the abundance of potential food organisms.

Based on the $\delta^{15}$N signatures, our study shows that the trophic level of *T. longicornis* females ranges from herbivory to omnivory with a large contribution of heterotrophic organisms. The feeding experiments and gut content analyses furthermore indicate a broad range of potential food items. The range in trophic levels also points to the difficulty in assigning one species to a trophic position in a food web, indicating the high complexity in linkages between trophic levels. This difficulty is even more pronounced, when a single species utilizes different food sources (Rolff 2000), such as *T. longicornis* did in our study. We therefore recommend long-term time series for studies in marine pelagic food webs in order to elucidate the trophic ranges of species.

**Acknowledgements**

We thank the crew of the R.V. Aade as well as the team at the Biologische Anstalt Helgoland/Alfred Wegener Institut für Polar- und Meeresforschung Bremerhaven for their continuous and strong support during our experimental phase as well as thereafter. We especially thank K. H. Wiltshire for providing chlorophyll data, K. Carstens for measuring pigment concentrations and S. Janisch for valuable information on the phytoplankton around Helgoland. We are grateful to C. von Waldthausen, S Hardenberg and S. Gehncke for counting and determining the phytoplankton and zooplankton. Finally, we would like to thank M. Boner and his team at AGROISOLAB GmbH for the stable isotope measurements and H. Auel for critical reading and valuable comments on the manuscript. This work was funded by the Helmholtz-Gemeinschaft as part of the Helmholtz-Hochschul-Nachwuchsgruppe "Trophische Interaktionen in pelagischen Ökosystemen: die Rolle des Zooplanktons" (VH-NG-058).

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### Tables

Table 1: Qualitative gut content analysis of *Temora longicornis* females caught in the field on selected experimental days throughout the time series from March to May conducted at Helgoland roads 2005. + indicates relative abundance among recognizable fragments: + low; ++ frequent; +++ very frequent.

<table>
<thead>
<tr>
<th>Day/month</th>
<th>n</th>
<th>Diatoms</th>
<th>Dinoflagellates</th>
<th>Naupliar remains</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3</td>
<td>8</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>15.3</td>
<td>8</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>26.4</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.5</td>
<td>10</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>10.5</td>
<td>11</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>17.5</td>
<td>9</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2: Jacobs selectivity index for main algal groups ingested in the 24h experiments. –1: no selection, +1 positive selection (Jacobs 1974). Mean values ± standard deviation, 26.4.: Selectivity index from the two bottles with indicated feeding.

<table>
<thead>
<tr>
<th>Day/month</th>
<th>8.3.</th>
<th>15.3.</th>
<th>22.3.</th>
<th>31.3.</th>
<th>5.4.</th>
<th>12.4.</th>
<th>26.4.</th>
<th>26.4.</th>
<th>4.5.</th>
<th>10.5.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrales</td>
<td>-0.47 ± 0.1</td>
<td>-0.25 ± 0.19</td>
<td>0.25 ± 0.42</td>
<td>0.5 ± 0.44</td>
<td>0.38 ± 0.28</td>
<td>0.32 ± 0.15</td>
<td>0.94</td>
<td>0.88</td>
<td>0.6 ± 0.17</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>Pennates</td>
<td>-0.03 ± 0.27</td>
<td>-0.32 ± 0.33</td>
<td>-0.48 ± 0.42</td>
<td>0.29 ± 0.54</td>
<td>0.11 ± 0.34</td>
<td>-0.41 ± 0.3</td>
<td>0.93</td>
<td>0.78</td>
<td>0.3 ± 0.19</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>Athecates</td>
<td>0.8 ± 0.03</td>
<td>0.26 ± 0.19</td>
<td>0.36 ± 0.35</td>
<td>0.62 ± 0.44</td>
<td>-0.14 ± 0.51</td>
<td>0.33 ± 0.08</td>
<td>-0.19</td>
<td>0.37</td>
<td>0.07 ± 0.3</td>
<td>0.95</td>
</tr>
<tr>
<td>Thecates</td>
<td>-1</td>
<td>0.43 ± 0.3</td>
<td>-0.52 ± 0.68</td>
<td>0.96 ± 0.02</td>
<td>1</td>
<td>0.99</td>
<td>0.98</td>
<td>0.94</td>
<td>-0.01 ± 0.19</td>
<td>0.38 ± 0.06</td>
</tr>
<tr>
<td>Flagellates</td>
<td>-0.31 ± 0.29</td>
<td>0.09 ± 0.06</td>
<td>-0.28 ± 0.46</td>
<td>-0.54 ± 0.41</td>
<td>0.02 ± 0.02</td>
<td>0.33 ± 0.47</td>
<td>-1</td>
<td>-0.084</td>
<td>-0.47 ± 0.41</td>
<td>-0.95 ± 0.04</td>
</tr>
<tr>
<td>Ciliates</td>
<td>-1</td>
<td>0.34 ± 0.33</td>
<td>0.3 ± 0.43</td>
<td>0.85 ± 0.05</td>
<td>0.47 ± 0.09</td>
<td>0</td>
<td>0.68</td>
<td>0.59</td>
<td>0.51 ± 0.06</td>
<td>0.45 ± 0.03</td>
</tr>
<tr>
<td>Other algae</td>
<td>-0.93 ± 0.01</td>
<td>0</td>
<td>-0.97 ± 0.03</td>
<td>0.82 ± 0.63</td>
<td>0.26 ± 0.14</td>
<td>-0.72 ± 0.39</td>
<td>0.87</td>
<td>0.78</td>
<td>0.27 ± 0.18</td>
<td>-1</td>
</tr>
</tbody>
</table>
Table 3: Jacobs selectivity index for main algal groups ingested in the 7h experiments. –1: no selection, +1 positive selection. Mean values ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Day/month</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26.4.</td>
</tr>
<tr>
<td>Centrales</td>
<td>0.85 ± 0.2</td>
</tr>
<tr>
<td>Pennates</td>
<td>-0.04 ± 0.16</td>
</tr>
<tr>
<td>Athecates</td>
<td>-0.99 ± 0.02</td>
</tr>
<tr>
<td>Thecates</td>
<td>0.18 ± 0.85</td>
</tr>
<tr>
<td>Flagellates</td>
<td>-0.61 ± 0.55</td>
</tr>
<tr>
<td>Ciliates</td>
<td>-0.67 ± 0.47</td>
</tr>
<tr>
<td>Other algae</td>
<td>-0.53 ± 0.66</td>
</tr>
</tbody>
</table>
Table 4: Ingested cells per *T. longicornis* female in the feeding experiments conducted at Helgoland from March to May 2005. Data represented for the dominating phytoplankton groups and ciliates.

<table>
<thead>
<tr>
<th>Day/month</th>
<th>Duration (h)</th>
<th>Centric diatoms</th>
<th>Pennate diatoms</th>
<th>Athecate dinoflagellates</th>
<th>Thecate dinoflagellates</th>
<th>Flagellates</th>
<th>Ciliates</th>
<th>Other Algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3.</td>
<td>24</td>
<td>44 ± 14</td>
<td>702 ± 102</td>
<td>653 ± 82</td>
<td>192</td>
<td>502 ± 264</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>15.3.</td>
<td>24</td>
<td>922 ± 135</td>
<td>275 ± 106</td>
<td>2039 ± 30</td>
<td>196</td>
<td>2665 ± 117</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>22.3.</td>
<td>24</td>
<td>229 ± 82</td>
<td>109 ± 39</td>
<td>899 ± 622</td>
<td>1 ± 2</td>
<td>1472 ± 1035</td>
<td>14 ± 2</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>31.3.</td>
<td>24</td>
<td>76 ± 48</td>
<td>51 ± 27</td>
<td>217 ± 26</td>
<td>10 ± 1</td>
<td>215 ± 155</td>
<td>35 ± 2</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>5.4.</td>
<td>24</td>
<td>14 ± 6</td>
<td>49 ± 17</td>
<td>178 ± 134</td>
<td>14 ± 10</td>
<td>470 ± 234</td>
<td>31 ± 2</td>
<td>2</td>
</tr>
<tr>
<td>13.4.</td>
<td>24</td>
<td>46 ± 12</td>
<td>22 ± 13</td>
<td>83 ± 13</td>
<td>10 ± 4</td>
<td>1195 ± 332</td>
<td>63 ± 2</td>
<td>607 ± 858</td>
</tr>
<tr>
<td>27.4.</td>
<td>24</td>
<td>272</td>
<td>222</td>
<td>55</td>
<td>108</td>
<td>0</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>27.4.</td>
<td>24</td>
<td>288</td>
<td>149</td>
<td>252</td>
<td>83</td>
<td>355</td>
<td>37</td>
<td>1</td>
</tr>
<tr>
<td>5.5.</td>
<td>24</td>
<td>255 ± 24</td>
<td>124 ± 18</td>
<td>182 ± 157</td>
<td>706 ± 248</td>
<td>488 ± 337</td>
<td>6 ± 1</td>
<td>3</td>
</tr>
<tr>
<td>10.5.</td>
<td>24</td>
<td>30 ± 1</td>
<td>261 ± 1</td>
<td>1344 ± 75</td>
<td>90 ± 17</td>
<td>60 ± 40</td>
<td>16 ± 2</td>
<td>0</td>
</tr>
<tr>
<td>26.4.</td>
<td>7</td>
<td>458 ± 99</td>
<td>88 ± 82</td>
<td>5 ± 8</td>
<td>69 ± 49</td>
<td>1959 ± 2770</td>
<td>18 ± 26</td>
<td>0</td>
</tr>
<tr>
<td>4.5.</td>
<td>7</td>
<td>551 ± 327</td>
<td>308 ± 226</td>
<td>384 ± 149</td>
<td>677 ± 321</td>
<td>338 ± 478</td>
<td>16 ± 7</td>
<td>28 ± 3</td>
</tr>
<tr>
<td>10.5.</td>
<td>7</td>
<td>92 ± 21</td>
<td>1259 ± 129</td>
<td>3750 ± 615</td>
<td>342 ± 133</td>
<td>2901 ± 1789</td>
<td>50 ± 13</td>
<td>0</td>
</tr>
<tr>
<td>17.5.</td>
<td>7</td>
<td>581 ± 137</td>
<td>13 ± 18</td>
<td>958 ± 1125</td>
<td>69 ± 25</td>
<td>2835 ± 1649</td>
<td>24 ± 17</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 5: Selected literature data on $\delta^{15}$N and $\delta^{13}$C reports for *Temora longicornis* and other calanoid copepods or zooplankton from the northern hemisphere.

<table>
<thead>
<tr>
<th>Source</th>
<th>Area</th>
<th>Species</th>
<th>Zoo $\delta^{15}$N</th>
<th>Zoo $\delta^{13}$C</th>
<th>POM / Food type</th>
<th>POM $\delta^{15}$N</th>
<th>POM $\delta^{13}$C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Checkley &amp; Entzeroth (1985)</td>
<td>Gulf of Mexico</td>
<td><em>Temora longicornis</em> and <em>T. stylifera</em></td>
<td>08. Aug</td>
<td>-19.1</td>
<td>POM (&lt; 100 μm)</td>
<td>3.0</td>
<td>-21.9</td>
</tr>
<tr>
<td>Sommer et al. (2005)</td>
<td>Hopavågen (Norway)</td>
<td><em>T. longicornis</em></td>
<td>6.8 – 7.2</td>
<td><em>P. elongatus</em></td>
<td>7.7 – 8.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klein-Breteler et al. (2002)</td>
<td>Mesocosm (Laboratory)</td>
<td><em>T. longicornis</em></td>
<td>-12.6</td>
<td><em>Isochrysis galbana</em></td>
<td>-13.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamelander et al. (2006)</td>
<td>Barents Sea</td>
<td><em>C. hyperboreus</em> females</td>
<td>8.9 – 9</td>
<td><em>Oxyrrhis marina</em></td>
<td>4.2 – 5.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fry (1988)</td>
<td>Georges Bank (USA)</td>
<td>Copepods</td>
<td>7</td>
<td>-20.8</td>
<td>POM (&lt; 53μm)</td>
<td>05. Jan</td>
<td>-21.3</td>
</tr>
<tr>
<td>Rolff (2000)</td>
<td>Northern Baltic proper (Baltic Sea)</td>
<td>Copepods (adults)</td>
<td>6.4 – 9.2</td>
<td>-24.9 – -22.1</td>
<td>POM (5 – 100 μm)</td>
<td>0.5 – 8.2</td>
<td>-24.7 – -17.2</td>
</tr>
<tr>
<td>Gorokhova et al. (2005)</td>
<td>Baltic Sea</td>
<td>Mainly copepods</td>
<td>10. Apr</td>
<td>-22.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McConnaughey &amp; McRoy (1979)</td>
<td>Eastern Bering Sea</td>
<td>Zooplankton</td>
<td>-22.1</td>
<td>POM (phytoplankton mainly)</td>
<td>-24.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bode et al. (2004)</td>
<td>Bay of Biscay NE Atlantic (Spain)</td>
<td>Zooplankton (mostly copepods)</td>
<td>6 – 9.75</td>
<td>-18.75 – -17</td>
<td>POM (20 – 200 μm)</td>
<td>5 – 6</td>
<td>-19.5 – -17.75</td>
</tr>
<tr>
<td>Schwamborn et al. (1999)</td>
<td>North East –Brazilian Shelf waters</td>
<td>Zooplankton</td>
<td>06. Apr</td>
<td>-19.8 – 16.3</td>
<td>POM (28 - &gt;116 μm)</td>
<td>1.6 – 5.6</td>
<td>-25.8 – -21.3</td>
</tr>
<tr>
<td>Perry et al. (1999)</td>
<td>Shelf and slope off British Columbia (Canada)</td>
<td>Zooplankton (100 – 425 μm)</td>
<td>-18.9</td>
<td>POM (&lt; 28 - &gt;116 μm)</td>
<td>-20.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>shelf</td>
<td>-22.2</td>
<td>slope</td>
</tr>
</tbody>
</table>
Figure captions

Fig: 1: a: Secchi depth and salinity at Helgoland Roads; b: Chl a and C:N ratios in the PM, black bars (chl a in the < 70 μm size fraction), grey bars (total chl a content measured by Wiltshire et al. (2007, in review)); c: δ^{15}N signatures in the PM (black) and in T. longicornis females (grey); d: δ^{13}C signatures in the PM (black) and in T. longicornis females (grey). Error bars indicate standard deviation (CI: 0.95).

Fig. 2: Taxonomic composition and abundance of phytoplankton and ciliates (<70 μm) from March to May 2005, according to cells counts. a: Total cell numbers (cells l^-1); b: % abundance of the three size classes; c: taxonomic composition and % abundance of dominating phytoplankton groups and ciliates in the counts from the PM samples taken during the time series. Counts are based upon Utermöhl counts, given in cells l^-1; d: concentration of the FA 16:1(n-7) and 18:4(n-3) in μg l^-1 in the PM samples. Error bars indicate standard deviation (CI: 0.95).

Fig. 3: Temora longicornis and copepod nauplii abundance (ind m^-3) at Helgoland Roads from March to May 2005. a: adult T. longicornis; b: C5 T. longicornis; c: C I – IV T. longicornis and copepod nauplii.

Fig. 4: Concentration of the biomarker fatty acids 16:1(n-7) (diatoms) and 18:4(n-3) (dinoflagellates) in % of total FA content in T. longicornis females over time. Error bars indicate standard deviation (CI: 0.95).

Fig. 5: Clearance – and ingestion rates of Temora longicornins females in the 24 h (a) and 7 h (b) incubation experiments, determined as described by Frost (1972).

Fig. 6: Selectivity Index for algal size fractions by Temora longicornis throughout the time series according to Jacobs (1974) a: 24 h experiments; b: 7 h experiments.

Fig. 7: Mean daily individual faecal pellet production of Temora longicornis over time. Black: rates determined in the 24h incubation experiments, grey: 7h incubation experiments. Error bars indicate standard deviation (CI: 0.95).
Figure 1:

- **Figure 1a:** Depth (m) versus Salinity.
- **Figure 1b:** Secchi depth (m) and Salinity.
- **Figure 1c:** Chlorophyll-a concentration (μg l⁻¹) with δ¹⁵N PM and δ¹⁵N Ti.
- **Figure 1d:** Chlorophyll-a concentration (μg l⁻¹) with δ¹³C PM and δ¹³C Ti.

### Key Data

- **δ¹³C:**
  - March: 8, 15, 22, 31
  - April: 5, 12, 22, 26
  - May: 4, 10, 17, 24

- **δ¹⁵N:**
  - PM: 4, 6, 8, 10
  - Ti: 4, 6, 8, 10

- **C:N Ratio:**
  - 34.0, 33.5, 33.0, 32.5, 32.0, 31.5, 31.0, 30.5
Figure 2:

(a) Graph showing cells per liter (Cells l\(^{-1}\)) variation over March, April, and May.

(b) Bar chart representing size fractions of different taxa, categorized into > 30 μm, 12.5-30 μm, and < 12.5 μm.

(c) Bar chart illustrating the taxonomy percentage of different algal categories, including Centric diatoms, Pennate diatoms, Athecate dinoflagellates, Thecate dinoflagellates, Flagellates, Ciliates, and Other algae.

(d) Graph showing changes in the concentration of 16:1(n-7) and 18:4(n-3) μg l\(^{-1}\) from March to May.
Figure 3:

(a) Ind m⁻³

- T. longicornis females
- Males

(b) C5

(c) C1-4
- Nauplii unspecified

March | April | May
---|---|---
8 | 15 | 22 | 31 | 5 | 12 | 26 | 4 | 10 | 17 | 24

81 5 2 2 31 5 12 26 41 0 17 24
Figure 4:

![Graph showing the percent total FA of 16:1 (n-7) and 18:4 (n-3) over months March to May. The graph includes error bars indicating variability. The x-axis represents the days of the month, with specific days labeled for March (8, 15, 22, 31) and April and May. The y-axes represent the percent total FA, with values ranging from 0 to 18 and 0 to 8 respectively. The graph shows an increase in both 16:1 (n-7) and 18:4 (n-3) over time, with a peak around the end of April.]

- **16:1 (n-7)**: Starts at approximately 1% in March, increases steadily to around 17% by May.
- **18:4 (n-3)**: Begins at around 3% in March, peaks at about 7% in April, and stabilizes around 6% in May.

The graph provides a visual representation of the changes in these fatty acids over the course of the months.
Figure 5:

(a) Ingestion rate (cells cop⁻¹ d⁻¹) and Clearance rate (ml cop⁻¹ h⁻¹)

(b) March, April, May data points
Figure 6:

![Graph showing preference avoidance of cells of different sizes over March, April, and May.

Figure 7:

![Graph showing faecal pellet amounts over time with 24h and 7h conditions.

March 8, 15, 22, 31, April 5, 12, 26, May 4, 10, 17]
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Eidesstattliche Erklärung
(Gem. § 6(5) Nr. 1-3 PromO)

Hiermit versichere ich, dass ich die vorliegende Arbeit
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