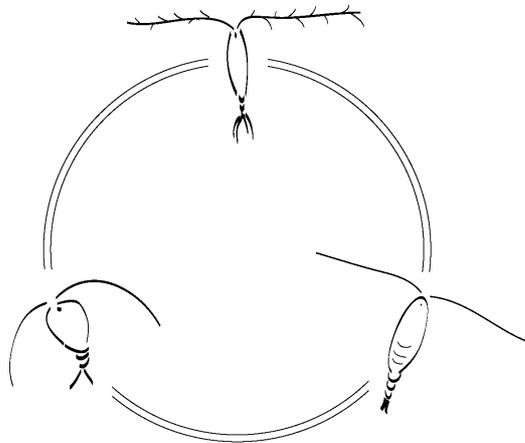


**Lipids in key copepod species of the Baltic Sea and North Sea  
– implications for life cycles, trophodynamics and food quality**

Janna Peters



Dissertation zur Erlangung des akademischen Grades eines  
Doktors der Naturwissenschaften (Dr. rer. nat)

Marine Zoologie  
Fachbereich Biologie / Chemie  
Universität Bremen

Juli 2006



What we know is but a drop.

What we have yet to discover is an ocean.

*Sir Isaac Newton*



## CONTENTS

|   |            |
|---|------------|
| <b>Abbreviations</b> .....  | <b>vii</b> |
| <b>Abstract</b> .....   | <b>ix</b>  |
| <b>Zusammenfassung</b> .....  | <b>xi</b>  |
| <b>Overview of publications</b> .....   | <b>xiv</b> |
| <br>  |            |
| <b>1. Scientific background and objectives</b> .....                          | <b>1</b>   |
| <b>2. General introduction</b> .....  | <b>6</b>   |
| 2.1 Lipids in relation to the life-cycles of marine copepods .....            | 7          |
| 2.2 Fatty acids as trophic markers .....                                      | 10         |
| 2.3 Food quality .....  | 11         |
| <b>3. Materials and methods</b> .....   | <b>13</b>  |
| 3.1 Field work .....  | 13         |
| 3.2 Analytical work .....   | 17         |
| 3.3 Statistics .....  | 18         |
| <b>4. Results and synoptic discussion</b> .....                               | <b>19</b>  |
| 4.1 Life-cycle patterns of copepods in the Baltic Sea .....                   | 19         |
| 4.2 Trophodynamics .....  | 25         |
| 4.2.1. Validation of fatty acid markers .....                                 | 25         |
| 4.2.2. Feeding ecology .....  | 27         |
| 4.3 Food quality .....  | 32         |
| 4.3.1 Influence of food quality on the reproductive success of copepods ..... | 32         |
| 4.3.2 Nutritional value of copepods .....                                     | 38         |
| <b>5. Perspectives</b> .....  | <b>43</b>  |
| <b>6. References</b> .....  | <b>45</b>  |

**Publications**

**Chapter I** ..... **59**

Trophodynamics and seasonal cycle of the copepods *Pseudocalanus acuspes* in the Central Baltic Sea (Bornholm Basin): evidence from lipid composition.

**Chapter II** ..... **81**

Life-cycle of *Pseudocalanus acuspes* Giesbrecht (Copepoda, Calanoida) in the Central Baltic Sea: II. Reproduction, growth and secondary production

**Chapter III** ..... **101**

Trophodynamics and condition of the copepods *Temora longicornis* and *Acartia longiremis* in the Bornholm Basin (Baltic Sea) – indications from lipid composition

**Chapter IV** ..... **121**

Impact of food quantity and quality on the reproductive success of the copepod *Temora longicornis* in the North Sea – the role of essential fatty acids

**Chapter V** ..... **141**

Trophic upgrading of nutrient limited algae by heterotrophic protists: effects on the reproduction of *Acartia longiremis*

**Presentations** ..... **155**

**Acknowledgements** ..... **157**

**Erklärung** ..... **159**

## **ABBREVIATIONS**

|              |  |
|--------------|--|
| ANOVA        | analysis of variance                   |
| ARA          | arachidonic acid, 20:4(n-6)            |
| C            | carbon                                 |
| chl <i>a</i> | chlorophyll <i>a</i>                   |
| CIV          | copepodite stage IV                    |
| CV           | copepodite stage V                     |
| DHA          | docosahexaenoic acid, 22:6(n-3)        |
| EPA          | eicosapentaenoic acid, 20:5(n-3)       |
| FAME         | fatty acid methyl ester                |
| GC-MS        | gas chromatography – mass spectrometry |
| KCl          | potassium chloride                     |
| n            | number of individuals                  |
| N            | nitrogen                               |
| PC           | principal component                    |
| PCA          | principal component analyses           |
| SiOH         | silica gel                             |
| TAG          | triacylglycerol                        |
| WE           | wax ester                              |



## ABSTRACT

The pelagic system of the Baltic Sea has experienced a drastic shift, over the last two decades, in the phyto- and zooplankton assemblage as well as in the pelagic fish community due to climate-induced hydrographical changes. While *Pseudocalanus acuspes* abundance declined, *Acartia* spp. and *Temora longicornis* populations increased strongly. The collapse of cod populations in combination with changing environmental conditions resulted in a strong increase of sprat stock size with a simultaneous decrease in sprat condition. The GLOBEC project, within which this thesis was embedded, assesses the mechanisms driving these changes by evaluating the influence of trophic interactions and physical forcing. This thesis elucidates the life-cycles and trophodynamics of the predominant copepod species *P. acuspes*, *T. longicornis* and *A. longiremis* in the Bornholm Basin and investigates the role of food quality for the reproductive success of copepods and higher trophic levels. A comprehensive data set on the lipid dynamics of copepods is presented and the data are discussed on the background of copepod distribution and stage structure that were collected by the GLOBEC project during the intensive monthly field work in 2002 and 2003.

Seasonal changes in lipid content and storage lipid composition revealed important aspects in the life-cycles and in particular in overwintering strategies of the copepods. Lipid reserves of *P. acuspes* consisted of wax esters and triacylglycerols, while *T. longicornis* and *A. longiremis* stored almost exclusively triacylglycerols. Lipid accumulation for overwintering started in early summer for *P. acuspes* in copepodite stage IV and V with high amounts of wax esters, whereas *T. longicornis* females did not switch from growth to lipid anabolism before late autumn. This arrest in development and reproduction apparently represented an “active diapause” of both species. Lipid stores of *A. longiremis* females were not elevated during winter, suggesting that most females may not survive the winter season and resting eggs might be an important source for population recruitment in the following year.

According to fatty acid markers in the storage lipids of the copepods distinct interspecific and interseasonal differences could be identified, reflecting different feeding strategies and a seasonal variation in food composition. Lipids of *T. longicornis* contained indicators for herbivory, such as 20:5(n-3) and 22:6(n-3), as well as 16:1(n-7) and 18:4(n-3). Diatom- and dinoflagellate-signals increased in times of high egg production and lipid accumulation. *A. longiremis* was characterised by high levels of polyunsaturated C18-fatty acids and selected strongly against diatoms at all times, which was most likely a consequence of ambush feeding on large motile prey. 18:1(n-9) was the major component in the storage lipids of *P. acuspes* and in particular an important part of the wax esters. High concentrations

of 18:1(n-9) in the seston coincided with peaks in ciliate biomass, thus elevated amounts of 18:1(n-9) in copepods might be interpreted as a trophic signal of ciliates. Other food sources of *P. acuspes* varied over the year, suggesting an opportunistic feeding behaviour. The spring period was characterized by an increase in typical diatom and dinoflagellate markers, whereas other sources, potentially cyanobacteria, became more important during summer.

To elucidate the limitation potential of essential fatty acids for egg production and egg viability in the field, the transfer of dietary fatty acids into the eggs of *T. longicornis* was studied on two cruises to the southern German Bight in May and July 2005. The lipid content of the eggs was very low (~2.5% of dry mass), indicating their reduced importance as energy reserves. The fatty acid composition of eggs depended strongly on the seston composition. Thus females showed low feeding selectivity as well as a low maternal regulation in the transfer of fatty acids into the eggs. The hatching success was high and independent of essential fatty acids in the eggs, while a positive correlation was observed with dietary 22:6(n-3) vs. 20:5(n-3) ratios. Essential fatty acids limited neither egg production nor hatching success, yet a positive correlation was observed between the relative content of 18:2(n-6) and egg production. The role of diatoms in the reproduction of copepods is a subject of ongoing debate and was therefore another focus of this field study on *T. longicornis*. Diatoms provide an important food source for *T. longicornis* in the North Sea as indicated by fatty acid markers in the storage lipids of the females and the lipids of the eggs. Egg production increased significantly with the increasing diatom marker 16:1(n-7) in the eggs, while hatching success was negatively correlated. However, the positive effect on the egg production over-compensated the only slightly reduced hatching success. Thus, rather food quantity than quality determined the reproductive success of *T. longicornis* at that time.

Changes in the condition of copepods were discussed in light of their relevance for higher trophic levels. Their nutritional quality in terms of 22:6(n-3) and 20:5(n-3) concentration was generally high, although below suggested optimal values for larval fish growth. Extremely low levels of arachidonic acid in copepods suggest that this fatty acid may be a critical dietary component for larval fish development in the Baltic Sea. Moreover, the switch in summer-feeding of sprat from lipid-rich *P. acuspes* copepodids (mean lipid content 32% of dry mass) towards lipid-poor *T. longicornis* (8% of dry mass) may deteriorate the overall food quality for sprat and explain the recently observed decrease in sprat condition. In conclusion, the present study contributes to our understanding of trophic interactions and their implications for life-cycle patterns and reproductive success of copepods in the Baltic Sea.

## ZUSAMMENFASSUNG

Aufgrund der sich ändernden Klimabedingungen haben sich im Laufe der letzten 20 Jahre im pelagischen System der Ostsee drastische Veränderungen vollzogen, welche sich sowohl in den Phyto- und Zooplankton-, als auch in den Fisch-Gemeinschaften widerspiegeln. Der starken Abnahme von *Pseudocalanus acuspes* steht dabei ein Anstieg in der Abundanz von *Acartia* spp. und *Temora longicornis* gegenüber. Das Zusammenbrechen der Dorschbestände und die sich ändernden Umweltbedingungen führten zu einem enormen Populationszuwachs in den Sprottenbeständen, bei zeitgleicher Abnahme ihrer Kondition. Ziel des GLOBEC-Projektes ist es, die diesen Veränderungen zu Grunde liegenden Prozesse aufzuschlüsseln, um ein besseres Verständnis für die Interaktion physikalischer und trophischer Einflüsse zu erlangen. Die vorliegende Arbeit, als Teil des Projektes, beleuchtet die Lebenszyklen und Trophodynamik der im Bornholm Becken sehr abundanten Copepodenarten *P. acuspes*, *T. longicornis* und *A. longiremis* und untersucht zudem den Einfluss von Nahrungsqualität auf den Reproduktionserfolg von Copepoden sowie auf deren Kondition. Ein umfassender Datensatz zur Lipiddynamik der Copepoden wird vor dem Hintergrund von Copepodenverteilung und Stadienzusammensetzung, die in intensiver monatlicher Feldarbeit in den Jahren 2002 und 2003 vom GLOBEC Projekt erhoben wurden, diskutiert.

Auf der Grundlage saisonaler Veränderungen im Lipidgehalt und in der Speicherlipid-Zusammensetzung wurden wichtige Aspekte der Lebenszyklen und insbesondere der Überwinterungsstrategien herausgearbeitet. Während *P. acuspes* sowohl Wachsester als auch Triacylglycerin speichert, bestehen die Speicherreserven von *A. longiremis* und *T. longicornis* fast ausschliesslich aus Triacylglycerin. Die Copepodite IV und V von *P. acuspes* begannen bereits im Frühsommer mit der verstärkten Einlagerung von Speicherreserven, hauptsächlich in Form von Wachsestern, während in *T. longicornis* der Übergang von Reproduktion zu Lipidakkumulation für die Überwinterung erst im späten Herbst stattfand. Diese Stagnation in Wachstum und Reproduktion ist ein Hinweis, dass beide Arten offenbar eine aktive Diapause durchlaufen. *A. longiremis* zeigt, im Gegensatz zu den beiden anderen Arten, keinen verstärkten Aufbau von Speicherlipid für die Überwinterung. Möglicherweise übersteht die Mehrheit der Weibchen den Winter nicht und es kommt zu einem Aufbau der Population im nächsten Jahr durch im Sediment lagernde Dauereier.

Die Fettsäuremarker in den Copepoden zeigen deutliche saisonale und artspezifische Unterschiede und spiegeln so die unterschiedlichen Ernährungsstrategien der Arten, aber auch eine saisonale Sukzession in der Nahrungszusammensetzung wider. Im Fettsäuremuster von *T. longicornis* wurden deutliche Anzeichen für eine vorwiegend herbivore Ernährung

gefunden, d.h. hohe Werte von 20:5(n-3), 22:6(n-3), 16:1(n-7) und 18:4(n-3). Diese Diatomeen- und Dinoflagellatenmarker waren besonders in Zeiten hoher Eiproduktion und starkem Speicherlipidaufbau erhöht. *A. longiremis* war durch verstärkte Einlagerung von C-18 mehrfach ungesättigten Fettsäuren und durch eine negative Selektion von Diatomeen gekennzeichnet. Letzteres ist eine mögliche Folge von „ambush feeding“ (lauerndes Fraßverhalten) von größerer mobiler Beute. Die Fettsäure 18:1(n-9) stellte eine wesentliche Komponente der Speicherlipide von *P. acuspes*, insbesondere der Wachsester, dar. Der Gehalt dieser Fettsäure im Seston und die Ciliaten-Biomasse waren zeitgleich erhöht, weshalb 18:1(n-9) in den Copepoden möglicherweise als ein Indikator für das Fressen von Ciliaten interpretiert werden kann. Andere Nahrungsquellen von *P. acuspes* änderten sich über das Jahr und spiegeln somit ein omnivores Fraßverhalten dieser Art wider. Das Frühjahr war durch einen Anstieg typischer Diatomeen- und Dinoflagellatenmarker gekennzeichnet, während andere Nahrungsquellen, vermutlich Cyanobakterien, im Sommer an Bedeutung gewannen.

Zur Abschätzung des Limitationspotentials essentieller Fettsäuren für die Eiproduktion und den Schlupferfolg, wurden auf zwei Reisen in die südliche Deutsche Bucht im Mai und Juli 2005, der Transfer von Fettsäuren aus der Nahrung in die Eier von *T. longicornis* näher untersucht. Der Lipidgehalt der Eier war mit 2,5% der Trockenmasse sehr gering und spielt damit für die Eier eine untergeordnete Rolle als Energiespeicher. Die Fettsäurezusammensetzung der Eier zeigte hohe Ähnlichkeiten mit der Zusammensetzung des Sestons, d.h. sowohl die Nahrungsselektivität der Weibchen als auch die Regulation des Fettsäuretransfers in die Eier war gering. Der Schlupferfolg war sehr hoch und unabhängig vom Gehalt essentieller Fettsäuren in den Eiern. Ein positiver Zusammenhang zeigte sich dagegen zwischen dem Schlupferfolg und dem 20:5(n-3) vs. 22:6(n-3)-Verhältnis der Nahrung. Essentielle Fettsäuren limitierten offensichtlich weder die Eiproduktion noch den Schlupferfolg. Es bestand jedoch ein positiver Zusammenhang zwischen der Eiproduktion und der Fettsäure 18:2(n-6) in den Eiern.

Die Bedeutung von Diatomeen für den Reproduktionserfolg von Copepoden ist ein Brennpunkt der gegenwärtigen Forschung und daher auch ein Hauptaugenmerk dieser Arbeit. Diatomeen stellten, angezeigt durch hohe Gehalte des Diatomeenmarkers 16:1(n-7) in den Weibchen und den Eiern, eine wichtige Nahrungsquelle für *T. longicornis* in der Nordsee dar. Die Eiproduktion stieg signifikant mit ansteigenden Diatomeenmarkern in den Eiern, während der Schlupferfolg zeitgleich sank. Der positive Effekt von Diatomeen auf die Eiproduktion kompensierte den nur leicht abfallenden Schlupferfolg bei weitem. Demnach

wurde der Reproduktionserfolg von *T. longicornis* eher durch die Quantität als durch die Qualität der Nahrung bestimmt.

Veränderungen in der Kondition von Copepoden werden vor dem Hintergrund ihrer Bedeutung für höhere trophische Ebenen diskutiert. Ihre Qualität war bezogen auf die Konzentrationen von 22:6(n-3) und 20:5(n-3) generell hoch, obwohl sie unter dem für die larvale Fischentwicklung als optimal beschriebenen Bereich lag. Sehr niedrige Gehalte von Arachidonsäure deuten darauf hin, dass diese Fettsäure eine kritische Nahrungskomponente für die Larvalentwicklung von Fischen in der Ostsee darstellt. Bei Sprotten trägt der Übergang von einer lipidreichen, aus Copepoditen von *P. acuspes* bestehenden Nahrung im Sommer (durchschnittlicher Lipidgehalt von 32% der Trockenmasse), zu einer lipidärmeren, von *T. longicornis* dominierten Nahrung (8% der Trockenmasse) zu einer Verschlechterung der Nahrungsqualität bei, welche den Abfall in der Sprotten-Kondition erklären könnte. Diese Arbeit trägt zu unserem Verständnis der trophischen Interaktionen und deren Bedeutung für die Lebenszyklen sowie dem Reproduktionserfolg von Copepoden der Ostsee bei.

## **OUTLINE OF PUBLICATIONS**

The following overview outlines the five publications included in this thesis and my contribution to the respective chapters. The overall objectives of this study were derived from the GLOBEC science plan.

### **CHAPTER I**

Peters J, Renz J, van Beusekom J, Boersma M, Hagen W

#### **Trophodynamics and seasonal cycle of the copepod *Pseudocalanus acuspes* in the Central Baltic Sea (Bornholm Basin): evidence from lipid composition.**

The experimental and analytical design of this study as well as the realisation was performed by myself, including the methodological developments for lipid class separation and the establishment of a refined analytical method for gas-chromatography. I wrote the manuscript with scientific and editorial advice by W Hagen. J Renz provided important ideas to the manuscript as well as the egg production data. The phytoplankton data was provided by J v Beusekom and M Boersma. The manuscript is published in *Marine Biology* (2006) DOI 10.1007/s00227-006-0290-8.

### **CHAPTER II**

Renz J, Peters J, Hirche HJ

#### **Life-cycle of *Pseudocalanus acuspes* Giesbrecht (Copepoda, Calanoida) in the Central Baltic Sea: II. Reproduction, growth and secondary production**

I shared the field work with the first author and was involved in writing the manuscript. The manuscript was submitted to *Marine Biology*.

### CHAPTER III

Peters J, Dutz J, Hagen W

#### **Trophodynamics and condition of the copepods *Temora longicornis* and *Acartia longiremis* in the Bornholm Basin (Baltic Sea) – indications from lipid composition**

I developed the concept of this study and performed the sampling and analyses. The manuscript was written by myself. W. Hagen contributed to writing the manuscript. Egg production data was provided by J Dutz and he was involved in writing the manuscript. The manuscript will shortly be submitted to Journal of Plankton Research.

### CHAPTER IV

Peters J, Dutz J, Hagen W

#### **Impact of food quantity and quality on the reproductive success of the copepod *Temora longicornis* in the North Sea – the role of essential fatty acids**

I developed the idea of this study. The experimental work was performed by myself in collaboration with J Dutz. I carried out all lipid analyses, evaluated the results and wrote the manuscript with advice of W Hagen. J Dutz provided valuable ideas for the interpretation of the data and was involved in writing the manuscript. The manuscript was submitted to Marine Ecology Progress Series.

### CHAPTER V

Augustin C, Schilling M, Peters J, Boersma M

#### **Trophic upgrading of nutrient limited algae by heterotrophic protists: effects on the reproduction of *Acartia longiremis***

I performed all lipid analyses and evaluated the data. I was involved in writing the manuscript. The manuscript will be submitted to Journal of Plankton Research.



## 1. SCIENTIFIC BACKGROUND AND OBJECTIVES

The pelagic system of the Baltic Sea has experienced extreme changes over the last decades, affecting almost all trophic levels. While cod (*Gadus morhua*) populations collapsed during the 1990s most likely due to overfishing and unfavourable environmental conditions, the Baltic sprat (*Sprattus sprattus*) stock increased strongly (Köster and Möllmann 2000, Köster et al. 2003). At the same time a shift in the copepod composition occurred, with a strong decrease of *Pseudocalanus acuspes* and an increase in *Acartia* spp. and *Temora longicornis* biomass (Möllmann et al. 2000, 2003). Diatom blooms failed to appear in the Baltic Sea since the late 1980s, while dinoflagellates gained in importance (Wasmund et al. 1998, Wasmund and Uhlig 2003). The driving forces behind these changes are complex and involve not only physical forcing but also trophic top-down and bottom-up processes.

The Baltic Sea is a brackish water, semi-enclosed system, consisting of a series of deep basins, which are separated by shallow sills. It is characterised by a strong vertical stratification with a seasonal thermocline in summer and a permanent halocline in the deep basins. Two climate-induced changes in hydrography were observed over the last decades: a continuous decrease in salinity since the late 1970s and a stepwise increase in water temperature since the end of the 1980s (Möllmann et al. 2005).

The hydrography of the Baltic Sea is driven by three major factors: (i) atmosphere – sea surface interactions, (ii) inflow events of saline water from the North Sea and (iii) fresh water run-off from the rivers (Stigebrandt and Gustaffson 2003). The sea surface temperature of the Baltic Sea is strongly related to the North Atlantic Oscillation, with an increase in temperature in times of stronger-than-average westerlies that have been prevailing since the late 1980s, and colder temperatures during periods of easterly cold continental winds (Hurrell and Dickson 2004, Alheit et al. 2005). While the temperature regime in the upper water layer is mainly driven by interactions with the atmosphere, temperatures of the halocline are strongly dependent on the inflow of saline North Sea water. The shallow transition zone between the Baltic Sea and the North Sea hinders an intensive inflow of the dense oxygen-rich water from the North Sea. In times of reduced wind mixing in summer, the inflowing warm water is of higher salinity, thus reaching the halocline in the deep basins (Feistel et al. 2003a). Renewal processes of the deep water in the Baltic Sea depend, however, on major intrusion events of North Sea water, which are related to certain wind

conditions (Hänninen et al. 2000, Feistel et al. 2003b). Only two major inflow events have been reported over the last decade (Feistel et al. 2003b), leading to a decrease in salinity that was further amplified by a simultaneous increase in river run-off (Matthäus and Schinke 1994)

These hydrographical changes have strong consequences for the pelagic food web. Higher temperatures in winter reduce convective mixing in spring leading to an earlier stabilization of the water column (Fennel 1999). This lack of deep-reaching circulation was proposed to be one reason for the suppression of the non-motile diatoms, while dinoflagellate biomass increased (Wasmund et al. 1998).

The population dynamics of copepods in the Bornholm Basin seem to be directly influenced by physical changes. *P. acuspes* shows an ontogenetic vertical distribution in the Bornholm Basin, with younger stages in the upper water column, while older copepodids and females concentrate within the halocline probably due to stage-specific physiological requirements (Renz and Hirche 2006). An increased mortality of nauplii and eggs was proposed during stagnation periods as a consequence of lower salinities and oxygen concentrations (Möllmann et al. 2005), while inflow situations yielded in a higher nauplii abundance per female (Schmidt et al. 2003). Thus unfavourable conditions in the Bornholm Basin may be a driving force behind the observed decline in *P. acuspes* populations (Renz and Hirche 2006). Multivariate analyses on a long-term data series revealed a correlation between the strong increase in *Acartia* spp. and *T. longicornis* biomass and increasing temperatures (Möllmann et al. 2000, 2005). Spring reproduction of those species seems to be favoured by higher water temperatures (Dippner et al. 2000). Furthermore, a temperature dependent increase in hatching of resting eggs of *Acartia* spp. from the sediment (Dutz et al. 2004) was suggested as one factor driving the rise in *Acartia* spp. abundance (Alheit et al. 2005). However, the combined analyses of *A. bifilosa*, *A. longiremis* and *A. tonsa* can lead to severe problems in interpreting their reaction on changing environmental conditions, since these species apparently differ in their physiological requirements (e.g. Chinnery and Williams 2004, Holste and Peck 2006).

Sprat stocks also seem to have favoured from the prevailing conditions in the Bornholm Basin, e.g. due to an improvement in egg development by higher temperatures (Nissling 2004), high abundances of *Acartia* spp. as major food for larval growth (Voss et al. 2003, 2006) and low predation pressure by decreased cod populations (Köster et al. 2003). However, a substantial decrease of individual mass of herring and sprat at high stock size

was documented in the Baltic Sea and may be explained by food limitation (Flinkman et al. 1998, Cardinale et al. 2002). The German GLOBEC project, within which this thesis was embedded, assesses the mechanisms driving these changes by evaluating the influence of trophic interactions and physical forcing. Simultaneously to the changes in the Baltic Sea, a regime shift in the North Sea occurred in the late 1980s (Reid et al. 2001, Beaugrand 2003) and a major focus of the GLOBEC project was to study top-down and bottom-up processes comparatively in both ecosystems.

**OBJECTIVE 1** - Essential for the interpretation of changes in the Baltic Sea is a detailed knowledge on the life-cycle strategies of the predominant copepods. Resting eggs were proposed to play a central role, although overwintering strategies of *A. longiremis* and *T. longicornis* in the Baltic Sea are still unknown. *T. longicornis* produces resting eggs in the North Sea (Lindley 1990, Engel and Hirche 2004), while no hatching from sediments was reported in the Baltic Sea (Madhupratap et al. 1996). A population recruitment based on resting eggs has not been proved for *A. longiremis*, although within this genus resting egg production is common (Uye 1982, Viitasalo 1992, Belmonte and Puce 1994). The first objective of this thesis was therefore to elucidate the life-cycle patterns and, in particular, the overwintering strategies of copepods in the Baltic Sea (chapters I, II and III).

**OBJECTIVE 2** - Population dynamics of copepods may also be influenced by food composition. The shift from a generally diatom- to a dinoflagellate-dominated food web as well as the stronger stabilization of the water column might have severe consequences for the condition of copepods. Since older stages of *P. acuspes* are mainly restricted to greater depths, they strongly depend on sedimentation events from the euphotic zone. Knowledge on the diet of *P. acuspes* in the Baltic Sea is scarce (e.g. Schnack 1975) and no information is available on how strongly *P. acuspes* is coupled to sinking phytoplankton in the deep basins. Females of *T. longicornis* and *A. longiremis* are, in contrast to *P. acuspes*, more concentrated in the upper water column, although they mainly occur below the thermocline in summer (Hernroth and Ackefors 1979, unpublished GLOBEC data). Hence, a more direct link to primary production might be expected. While there are numerous studies on the feeding preferences of *T. longicornis* (De Mott 1988, Vincent and Hartmann 2001, Guisande et al. 2002, Koski et al. 2005), less is known on the dietary spectrum of *A. longiremis*. The latter was found to feed on heterotrophic protists (Levinsen et al. 2000), while no grazing on ciliates was observed during strong diatom bloom events (Fessenden and Cowels 1994). To

improve our understanding of the bottom-up control of copepods in the Bornholm Basin, the second objective of this thesis was to describe the respective trophic niches of the three copepod species and to reveal seasonal changes in their diet composition (chapters I and III).

**OBJECTIVE 3** - A further aspect that potentially influences the food-web structure in the Baltic Sea is food quality and its dependence on the changing phytoplankton composition. Several processes can be involved here, such as changes in the production of essential compounds for higher trophic levels or variations in the energy flux due to a different condition of prey organisms. Alheit and co-authors (2005) hypothesised that the reproductive success of *Acartia* spp. and *T. longicornis* in the Baltic Sea benefits from the decrease in diatom abundance, since diatoms can reduce the hatching success of copepods (Poulet et al. 1995, Ban et al. 2000, Miralto et al. 2003, Arendt et al. 2005). The biochemical composition of phytoplankton varies strongly between different taxa (Daalsgard et al. 2003) and might thus determine the reproductive success of copepods (Jónasdóttir 1994, Jónasdóttir et al. 2002).

Dietary changes will also alter the nutritional value of copepods for higher trophic levels. Induced by altering phytoplankton assemblages, variances in essential fatty acids were proposed to influence the reorganization of fish communities during regime shifts (Litzow et al. 2006). A depletion of arachidonic acid (ARA) in the Baltic Sea food web, probably caused by decreasing diatom abundances, was related to the reduced recruitment success of cod (Pickova et al. 1997) and salmon (*Salmo salar*) (Ahlgren et al. 2005). Thus climate-mediated shifts in essential fatty acids might be a further factor in the bottom-up control of the food web in the Bornholm Basin.

Regime shifts in shelf sea communities are often associated with a transition from lipid-rich to lipid-poor fish faunas (Litzow et al. 2006). Similarly, the copepod assemblage in the North Sea changed, with a stepwise decrease of the lipid-rich *Calanus finmarchicus* and an increase in lipid-poor *C. helgolandicus* abundance (Beaugrand 2003). These changes in prey condition might have strong consequences for the energy transfer between trophic levels. Bottom-up processes, i.e. a decrease of *P. acuspes* copepodids and females as major food source for sprat and herring may have caused a decrease in sprat and herring condition (Möllmann et al. 2004). Sprat compensates the reduced availability of *P. acuspes* by feeding on *T. longicornis* and *Acartia* spp. over summer (Möllmann et al. 2005). Thus differences in lipid content between copepod species would have strong consequences for the condition of sprat.

In conclusion, bottom-up processes are not only driven by food availability. Quality aspects also play a role. Thus, the third objective of this thesis was to assess the influence of food quality on the reproductive success of copepods, and a special emphasis was given to the relevance of essential fatty acids. Since changes in diatom abundance in the Bornholm Basin may influence the population dynamics of copepods, the respective role of diatoms in the diet of *T. longicornis* in the Baltic Sea and North Sea was assessed. Furthermore, implications of the condition of copepods for higher trophic levels are discussed. (chapters I, III, IV and V).

The main objectives of this thesis were to elucidate the (i) life-cycles and (ii) trophodynamics of the copepods *P. acuspes*, *T. longicornis* and *A. longiremis* in the Bornholm Basin and to evaluate the role of (iii) food quality for the reproductive success of copepods and higher trophic levels. Lipid analyses are a useful investigative tool to address these questions. Storage lipids play an essential role in the life-cycles of the copepods (section 2.1), fatty acids can be used as trophic markers (section 2.2) and both are important variables in evaluating food quality (section 2.3).

## **2. GENERAL INTRODUCTION**

Lipids are a structurally very diverse group of compounds, characterised by their solubility in organic solvents, and fulfil a magnitude of different metabolic functions. Glycerophospholipids are the essential structural element in biomembranes and form the largest group within the polar lipids in organisms. While their levels are relatively constant, their composition is subject to turnover, since some of the fatty acids, e.g. the polyunsaturated fatty acids 20:5(n-3) and 22:6(n-3) are of dietary origin (Sargent and Whittle 1981). However, due to structural requirements, the fatty acid composition of membranes is generally conservative (Falk-Petersen et al. 2000).

Neutral lipids, such as triacylglycerols (TAG) and wax esters (WE), provide, due to their high energy content and hydrophobic nature, metabolic energy reserves (Sargent and Henderson 1986). Lipid storage can be of high importance for many marine copepods, e.g. during overwintering, and an overview on their role in different life-cycle patterns of copepods will be given in section 2.1. The levels of neutral lipids in animals are basically determined by assimilation, anabolism and catabolism, with the last two processes constituting the phenomenon of turnover (Sargent and Whittle 1981). Thus, fatty acids in neutral lipids strongly depend on diet composition and can be used as trophic markers to describe the feeding history of copepods (section 2.2).

Sterols, and in particular cholesterol, play an important role in modulating membrane fluidity. They serve as precursors for steroidal hormones, and the activity of membrane proteins was supposed to be regulated by interactions with sterols (Yeagle 1989). Recent studies emphasise a high importance of dietary sterols for the reproductive success of marine copepods (see section 2.3). Besides the need of essential fatty acids for the integrity of cell membranes (Sargent et al. 1999a) and their high energetic value, long-chain polyunsaturated fatty acids can serve as precursors for biological active compounds, such as eicosanoids (Harrison 1990) that are involved in hormonal regulations in marine invertebrates (Rowley et al. 2005). Hence, essential fatty acids in the diet might have a strong effect on the structural functionality and hormonal processes of the consumer, and thus the potential to influence the condition of copepods and fish larvae (section 2.3).

## 2.1 LIPIDS IN RELATION TO THE LIFE-CYCLES OF MARINE COPEPODS

Calanoid copepods have conquered all regions of the oceans and their life-cycle strategies are adapted to manifold environmental conditions. Life histories with short-lived overlapping generations are commonly observed in small temperate copepods, while life-cycles tend to be long-standing in truly arctic species (Mauchline 1998 and references therein). Although less severe than in polar regions, copepod populations in the Baltic and North Seas can experience a strong seasonality in food supply. Mean primary production (1990-2001) in the western Baltic Sea ranged between less than 50 mg C m<sup>-2</sup> day<sup>-1</sup> and 1000 mg C m<sup>-2</sup> day<sup>-1</sup>, although much higher levels can be reached in particular cases (HELCOM 2003).

With an increase in latitude, life-cycle pattern of copepods tend to include a dormant phase, often associated with lipid accumulation to endure unfavourable environmental conditions with minimized energetical costs. Dormancy is commonly defined as a state of suppressed development that involves structural and physiological changes and represents either diapause or quiescence (Danks 1987, Dahms 1995). While quiescence describes a hypometabolic state that will disappear when unfavourable conditions change, diapause is characterised by a refractory phase, from which development cannot resume until the phase is passed (Elgmork and Nielssen 1978). Diapause responds to a predictable cyclic change and is usually expressed in a definite ontogenetic stage (Elgmork 1980), but can also include several stages (Dahms 1995). In a wider sense, diapause describes not only torpidity and metabolic inactivity, but also an arrest in development or reproduction of still active organisms (Elgmork 1996). This “active” form of diapause was proposed to be an evolutionary step to dormancy and inactivity (Elgmork 1980). Different triggers for the onset of diapause are suggested and might well be species-dependent. Potential triggers include photoperiod, temperature or food supply, the nutritional state of the copepod or further internal conditions (Dahms 1995 and references therein).

Three different life-cycle stages in calanoid copepods are generally observed to pass through a dormant or diapausing phase: (i) eggs, e.g. *Acartia* spp. (Viitasalo 1992) and *T. longicornis* (Castellani and Lucas 2003) (ii) older copepodite stages, e.g. *Pseudocalanus* spp. (Conover and Huntley 1991), and (iii) adults, e.g. *A. longiremis* (Davis 1976). Nauplii or young copepodids are rarely found as overwintering stages (Mauchline 1998 and references therein). This might be associated with the unfavourable relationship between energy consumption and storage space for lipid accumulation, since these stages do not usually accumulate lipids even during intensive feeding (Lee et al. 2006).

Energy can be stored in different lipid classes in marine zooplankton, i.e. TAGs, WEs, diacylglycerol-ethers and phospholipids (Lee et al. 2006), although only the first two are of high relevance for lipid accumulation in marine copepods (Lee 1975). While TAGs can be rapidly hydrolysed and provide fast usable energy, WEs generally serve as long term storage, especially in times of food scarcity (Sargent and Falk-Petersen 1988). Processes during lipid catabolism can regulate these different metabolic rates, with a hormonal-sensitive WE lipase (Sargent and Henderson 1986) allowing a slow conversion of WEs into TAGs (Lee et al. 2006). Lipid accumulation in turn was proposed to start with a storage of TAG droplets and WEs are only stored as a second step (Miller et al. 1998). While lipids serve as an important energy buffer against starvation in times of low food amount, they also play an essential role during reproduction and fuel egg production of some copepod species before the development of phytoplankton blooms (Lee et al. 2006). During oogenesis WEs and TAGs are converted into phospholipids that are used as structural component for lipovitellin (Lee et al. 2006). Lipid levels in small copepods, like those studied here, are generally low compared to the large mainly herbivorous copepods of polar regions, e.g. *Calanus* spp. (Båmstedt et al. 1990, Norrbin et al. 1990). However, they can play a significant role in the life-cycle of those copepods and an overview including the distribution and life-cycle patterns of *P. acuspes*, *T. longicornis* and *A. longiremis* will be given below.

#### *Pseudocalanus acuspes*

The taxonomy of the genus *Pseudocalanus* was subject to several revisions over the last decades. Earlier publications on Baltic Sea zooplankton referred to the Baltic species as *P. elongatus*, while recent genetic studies identified it as *P. acuspes* (Bucklin et al. 2003). *P. acuspes* mainly inhabits high latitudes (Frost 1989, Runge and Ingram 1991, Siferd and Conover 1992, Norrbin 1996). Due to its absence in the adjacent North Sea (Bucklin et al. 2003) and wide distribution in the Arctic, it is most likely a member of the Baltic glacial relict fauna. Life-cycles within this genus are diverse, with several generations per year in temperate regions (Marshall 1949, Digby 1950). Biennial (Cairns 1967) and annual cycles (Davis 1976, Conover and Siferd 1993, Lischka and Hagen 2005) up to cycles with two or more generations per year (Pertsova 1981, McLaren et al. 1989, Norrbin 1992) occur in high latitudinal habitats. An annual life-cycle was proposed in the Baltic Sea, potentially with a second smaller generation developing in summer (Renz and Hirche 2006). *P. acuspes* overwinters as copepodite stage III to V with reduced development, although without passing through an inactive diapause (Norrbin et al. 1990, Norrbin 1992). Overwintering stages of *Pseudocalanus* spp. accumulate lipid in oil sacs (McLaren et al. 1989), mainly

consisting of WEs with values up to 70% of total lipids (Båmstedt et al. 1990). TAGs reach 13% of total lipids in times of depleted lipid levels (Norrbin et al. 1990). No data is available on the lipid class and fatty acid composition of *P. acuspes* females.

#### *Temora longicornis*

*T. longicornis* is a temperate, neritic and euryhaline species (Krause et al. 1995 and references therein) with high abundances in the Baltic Sea (Hernroth and Ackefors 1979) as well as in the central and south-eastern areas of the North Sea (Fransz et al. 1991, Krause et al. 1995). Its distribution reaches from the Portuguese coast (Halsband-Lenk et al. 2002) up to higher latitudinal habitats, e.g. the Barents (Klekowski and Weslawski 1990) and White Seas (Pertzova 1990 as cited by Lukashin et al. 2003, Chikin et al. 2003). *T. longicornis* is a multi-voltine species, with 2-6 generations per year (Digby 1950, Petersen and Kimmerer 1994, Halsband-Lenk et al. 2004) and resting egg production (Castellani and Lucas 2003). TAG is the predominant storage lipid in *T. longicornis* and maximal values in the North Sea (approximately 50% of total lipids) are reached after the spring bloom in May (Kattner et al. 1981). In contrast, WE levels are generally low, never exceeding 5% of total lipids (Kattner et al. 1981, Fraser et al. 1989). Lipid levels of *T. longicornis* in the North Sea do not indicate that this species accumulates large lipid reserves before winter, however no stage resolved storage lipid data are available.

#### *Acartia longiremis*

*A. longiremis* is described as a euryhaline, arctic-neritic species with a circumpolar distribution (Krause et al. 1995 and references therein), and was reported from polar habitats, such as the Laptev (Kosobokova et al. 1998), Barents (Kwasniewski et al. 2003) and White Seas (Lukashin et al. 2003, Shaporenko et al. 2005). Its distribution extends far southwards, with high abundances along the western and southern Norwegian coasts as well as in the Skagerrak and in the Baltic Sea (Hernroth and Ackefors 1979, Krause et al. 1995). Life-cycle patterns of *A. longiremis* are not fully understood and overwintering strategies of this species might vary between populations (Norrbin 1992). In temperate habitats, eggs of *A. longiremis* hatch from sediments (Marcus 1990), although it is uncertain, whether the eggs represent a true diapausing stage. Females in high latitudinal regions survive the winter season with premature gonads (Davis 1976, Norrbin 1994) and elevated lipid reserves (Norrbin et al. 1990). These lipid stores consist of TAGs (up to 55% of total lipids) and to a lesser degree of WEs (4-12% of total lipids), with TAG levels strongly declining during winter (Norrbin et

al. 1990). In early winter the lipid-body is large and in close proximity to the ovary (Norrbin 2001). Its strong depletion before the onset of reproduction indicates that large amounts of TAGs are utilised for the development of primary oocytes, while lipids for vitellogenesis and final maturation are most likely derived from other sources, such as ingested food or WE stores (Norrbin 2001). Hence, a successful recruitment of *A. longiremis* in high latitudinal regions seems to strongly depend on a combination of lipid accumulation and food availability.

## 2.2 FATTY ACIDS AS TROPHIC MARKERS

The application of specific fatty acids as dietary markers in marine zooplankton is well established (Lee et al. 1971, Sargent and Whittle 1981, Graeve et al. 1994, Iverson et al. 2004) and was reviewed intensively in recent years (Daalsgard et al. 2003, Bergé and Barnathan 2005, Lee et al. 2006). Hence, this chapter will only briefly outline the general concept of the biomarker approach and more specific information on the here applied trophic markers will be given in section 4.2.

Primary producers provide the basic fatty acid pattern in marine food webs and are the main source of polyunsaturated fatty acids such as 18:2(n-6), 18:3(n-3) and their derivatives 20:5(n-3) and 22:6(n-3) (Bergé and Barnathan 2005). Several fatty acids are characteristic for specific taxonomic groups and are generally assimilated and transferred into the storage lipids by the consumer without further modification (Daalsgard et al. 2003 and references therein). Thus, dietary influences will be primarily mirrored in the storage lipids (Sargent and Henderson 1986). However, food derived fatty acids can be masked by the presence of high amounts of structural lipids, since the latter contain generally stable fatty acid profiles (Sargent et al. 1987). Hence, it will be a rigorous improvement, especially for organisms with low storage lipid amounts, to analyse trophic markers in the neutral lipid fraction solely, as done within this thesis.

Diatoms are known to be rich in 16:1(n-7) and 20:5(n-3) (Nichols et al. 1993, Dunstan et al. 1994), while dinoflagellates produce generally higher amounts of 18:4(n-3) and 22:6(n-3) (Sargent et al. 1987, Graeve et al. 1994). Ratios of these fatty acids are commonly used as dietary indicator, such as 16:1(n-7) vs. 16:0 or 20:5(n-3) vs. 22:6(n-3) (St John and Lund 1996, Phleger et al. 2002). However, recent evaluations suggest a limited suitability of ratios as feeding indicator, at least when applied to total lipid composition (Reuss and Poulsen 2002, Stübing and Hagen 2003). Thus, within this thesis a stronger emphasis was placed on the interpretation of the fatty acid pattern as a whole, rather than on specific ratios.

Another common fatty acid in pelagic systems is 18:1(n-9), which is often enriched in secondary producers (Falk-Petersen et al. 1999) and thus commonly interpreted as marker for heterotrophic feeding (Graeve et al. 1997). However, it can also be synthesised by copepods *de novo* and therefore only be used as dietary marker with special care (Falk-Petersen et al. 1990). Comparisons between the taxonomic and fatty acid composition of seston, as performed within this thesis, are a suitable tool to validate the interpretation of such unspecific markers.

### 2.3 FOOD QUALITY

Extensive laboratory and field work in recent years demonstrated that not only the quantity of food has a significant influence on the physiology of marine copepods, but that quality aspects are also involved. A variety of life history components can be affected, such as somatic growth rates, egg production, hatching success, fertility and survivorship (Sterner and Schulz 1998). An evaluation, whether a specific food source provides sufficient or insufficient quality for the reproductive success, can be performed quite easily by laboratory experiments. More difficulties arise in the attempt to characterise or even predict this quality, since egg production and hatching success can be affected differently and various factors may interact. The nutritional value of a specific food source is determined by its size, digestibility, energy content, toxicity as well as mineral and biochemical composition (Kiørboe 1989, Dam and Peterson 1991, Jónasdóttir et al. 1995, Sterner and Schulz 1998, Ianora et al. 2003). Essential fatty acids were for a long time the main focus in the effort to determine food quality. They play a major role, since they are needed for the structural integrity of cell membranes, are of high energetic value and precursors for metabolic active compounds (Harrison 1990, see also section 2.1).

Several indications for the high importance of dietary polyunsaturated fatty acids were derived from feeding experiments as well as field studies. However, they provide rather inconclusive results. Low growth rates, a strong decline in egg production, hatching success and even an irreversible atresia of gonads were observed under 20:5(n-3) (EPA) and 22:6(n-3) (DHA) lacking diet (Lacoste et al. 2001, Klein-Breteler et al. 2004, Tang and Taal 2005, Arendt et al. 2005). Egg production rates showed a positive, in some cases significant, relationship with dietary amounts of 18:2(n-6), 18:4(n-3), EPA or DHA as well as with the ratios DHA:EPA and (n-3):(n-6) (Hazzard and Kleppel 2003, Kleppel et al. 1998, Arendt et al. 2005, Shin et al. 2003, Jónasdóttir 1994, Jónasdóttir et al. 1995). In contrast, also negative correlations were observed with dietary levels of DHA as well as with the ratios DHA:EPA

and (n-3):(n-6) (Arendt et al. 2005). Other studies found no clear relationship between egg production and the quantity of ingested polyunsaturated fatty acids (Støttrup and Jensen 1990).

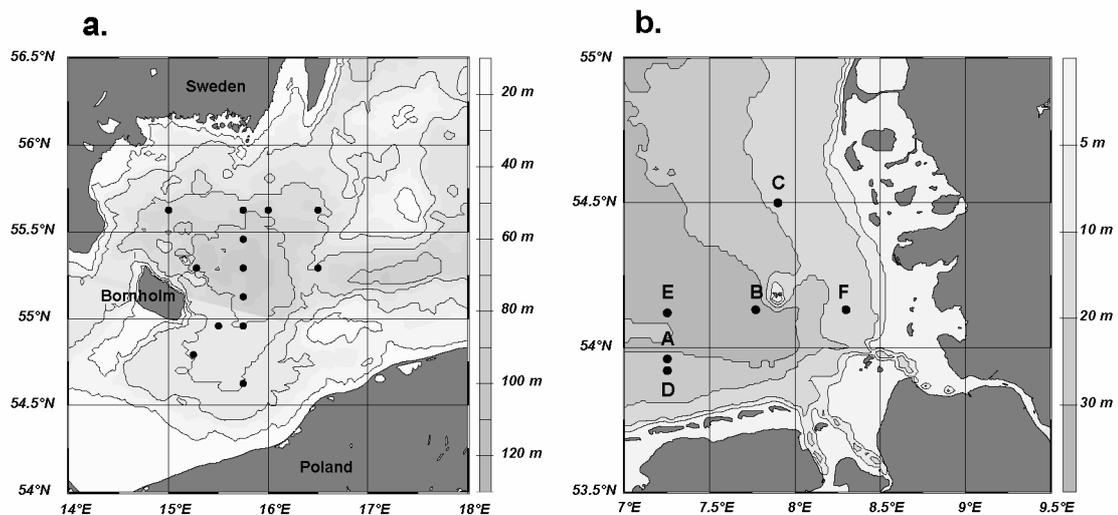
An interpretation of those correlations remains intricate. It has been suggested that they reflect a limitation of egg production by the specific compound (Hazzard and Kleppel 2003). However, there are two main assumptions involved: (i) the correlation is not a reflection of food availability or its energy content and (ii) the female is able to regulate the transfer of lipids into the eggs on the level of single fatty acids or this fatty acids containing compounds.

The idea that females strongly regulate the fatty acid composition of the eggs, and therefore reduce egg production in favour of increased egg quality, contrasts the observed relationships of dietary fatty acids with egg viability. Hatching success was found to be highly dependant on fatty acid ratios in the diet (Jónasdóttir and Kjørboe 1996, Arendt et al. 2005) or in the eggs (Shin et al. 2003) as well as with the sum of some ingested polyunsaturated fatty acids (Broglia et al. 2003) and the amount of 18:2(n-6) in the eggs (Pond et al. 1996). On the other hand, no difference in fatty acid composition of hatching or non-hatching eggs of *Calanus helgolandicus* was observed in experiments with DHA and EPA sufficient and lacking food (Lacoste et al. 2001). Apparently, lipid storing females such as *C. helgolandicus* are able to compensate at least to some degree dietary deficiencies with internal reserves, and are thus able to regulate the fatty acid composition of their eggs. It remains doubtful, if this is the case for small copepods since their egg production is mainly based on freshly ingested material, and the egg composition in *Acartia* spp. was found to strongly depend on dietary composition (Ederington et al. 1995, Støttrup et al. 1999). However, so far there is little knowledge on the regulation mechanisms of fatty acid transfer into the eggs.

Further information about the involved regulation processes during oogenesis might be derived from other lipid classes that are also essential for a successful egg development. Recent results from sterol-supplement experiments with diatoms emphasise that egg production of *Acartia* spp. can be sterol limited, since egg production rates increased after a certain level of cholesterol was added to the diet (Hasset 2004). This gives evidence that females controlled the egg composition, however only to a certain degree, since hatching success was low with unsupplemented diet, but strongly increased in sterol-rich diet. If those results can be transferred to the regulation of fatty acid composition of eggs will be a subject of further studies. An important issue of this thesis was to further elucidate the relationship between dietary amounts of essential fatty acids and the reproductive success of copepods in the field.

### 3. MATERIALS AND METHODS

This chapter outlines the applied methods within this thesis, but is restricted to the methods that were performed by myself. The methodologies used by my co-authors are presented in the respective chapters.



**Figure 1.** Study area, maps were created with ODV (Schlitzer 2002) **a.** Sampling grid Bornholm Basin (Baltic Sea), **b.** German Bight (North Sea), stations A-C May 2005, D-F July 2005.

#### 3.1. FIELD WORK

##### 3.1.1 BALTIC SEA - SEASONAL DATA SET (chapters I and III)

###### *Zooplankton*

Zooplankton samples were collected in approximately monthly intervals from March 2002 to March 2003 (except for October and December) on eleven cruises in the Bornholm Basin (Fig.1). To provide representative data for the whole basin, stations in central and marginal areas were sampled on each cruise and data were combined in average values for each month. Zooplankton was sampled using a WP-2 net with a 10L bucket end (vertically towed with  $0.2 \text{ m s}^{-1}$ , mesh size  $200 \mu\text{m}$ ,  $0.26 \text{ m}^2$  opening). Sampling depths were adjusted to hydrography covering the water column from the lower halocline up to the surface. Copepods were sorted on board under ambient temperature conditions into  $-80^\circ\text{C}$  precooled

glass vials. Depending on availability each sample consisted of 20 to 200 adult females of *Acartia longiremis*, *Temora longicornis* and *Pseudocalanus acuspes*, respectively. Additionally, females of *A. bifilosa* were sampled in May 2002 as well as copepodite stages V of *P. acuspes* in May, June, September, November and January.

For *P. acuspes* prosoma lengths of 30 females were measured using formalin preserved samples (4% in seawater) on three stations of each cruise. Prosoma lengths of *A. longiremis* and *T. longicornis* length were determined on living copepods (35-130 individuals per month) using a digital imaging system or a direct measurement on board.

Sprat larvae were sampled on monthly intervals from March to July 2002 using a bongo-net (mesh size 335  $\mu\text{m}$  and 500  $\mu\text{m}$ ) in double-oblique hauls covering the entire water column. Immediately after retrieval and during the whole sorting procedure, sprat larvae were stored on ice and larvae were sorted into pre-cooled ( $-80^{\circ}\text{C}$ ) caps. Sorting was completed within 30 min after the catch and samples were stored at  $-80^{\circ}\text{C}$  until further analyses. Prior to extraction the length of sprat larvae was determined on ice-cooled dishes using a digital imaging system, which was connected to the binocular. Within the presented thesis, only sprat larval values from larvae  $< 10$  mm were included.

### *Seston*

At all examined zooplankton stations, seston samples from five depths were taken with 10 l water sampler bottles. Vertical resolution was adapted to the hydrographic structure of the water column, with samples taken from the upper water layer (5 m), from above the thermocline (10 m), from the midwater layer, from above the halocline and in the halocline. Depending on seston concentrations two to six litres of water were filtered with low pressure on precombusted (12 h at  $400^{\circ}\text{C}$ ) GF/C filters. All zooplankton organisms were carefully removed under the stereomicroscope immediately after filtration and prior to freezing, so that they did not bias the seston data. The filters were permanently stored at  $-80^{\circ}\text{C}$  until further analysis.

For analyses of taxonomic seston composition aliquots of 100 ml were taken from water sampler bottles, preserved with 2% acid Lugol's solution and stored cool and dark until further investigation. Samples were analysed using Uthermöhl microscopy and phytoplankton as well as protozooplankton cell size was converted to biomass according to Edler (1979) and Putt and Stoecker (1989), respectively.

### *Egg production measurements*

For measurement of *in situ* egg production of *P. acuspes*, 30 females were sorted directly after the catch under ambient temperature conditions and individually incubated under *in situ* temperature for 24 h in 15 ml cell wells using 50 µm prefiltered water from the upper halocline. Clutch size and number of reproducing females were recorded. Daily egg production of 30 females per station of *A. longiremis* and *T. longicornis* were measured in spawning chambers with an inner compartment equipped with a false bottom made of net gauze of 100 µm mesh, filled with 48 µm pre-screened water (usually equally mixed from 5 and 10 m depths) as group (1 l beaker) or single (250 ml beaker) incubations. After 24 h, incubations were stopped by removing the inner compartment and the eggs were gently concentrated on a submerged sieve and counted immediately.

### 3.1.2 TRANSFER OF DIETARY FATTY ACIDS INTO EGGS (chapter IV)

Experiments were performed during two cruises to the southern German Bight, North Sea (17-28 May, 1-8 July 2005) on six stations with no or only low vertical stratification (Fig.1). Zooplankton samples were taken using a WP-2 net equipped with a non-filtering cod end from 20 m depth to the surface (mesh size 100 µm, vertically towed with 0.2 m s<sup>-1</sup>) or with an undulating towed multi-net (mesh size 335 µm, towed with 2.5 knots and 0.2 m s<sup>-1</sup>). Immediately on retrieval, the catch was carefully diluted with ambient seawater and kept in a walk-in cooling chamber. Seawater for egg production experiments was collected with 10 l Niskin bottles at 5 m and 10 m or, at vertically stratified stations, at the depth of the thermocline. The water was sieved through a submerged 48 µm net, mixed equally and filled into three to four spawning chambers with an inner compartment equipped with a false bottom made of net gauze of 100 µm mesh size. Within 10-20 min after capture 7-10 actively swimming females of *T. longicornis* were transferred to the spawning chambers and incubated shaded and at *in situ* temperature (8-9°C in May, 14°C in July). After 24 h, incubations were stopped by removing the inner compartment. The prosome length of females retained on the mesh bottom was measured under a dissecting microscope (Wild M 9.5, 60 x 16 magnification). Eggs collected in the outer compartment were gently concentrated on a submerged sieve of 20 µm, transferred to small Petri dishes and immediately counted under a stereo-microscope. After counting, eggs from each replicate were transferred to 325 ml bottles containing GF/F filtered ambient seawater and incubated for about 72 h on a rolling apparatus at 1 r.p.m. Eggs collected from supplementary experiments were incubated in dishes at the same temperature and monitored daily to match the incubation

time with hatching of eggs. At the end, bottle contents were concentrated on a 20 µm mesh and fixed with Lugol's solution (2% final concentration). In the laboratory, unhatched eggs, nauplii and empty egg shells were counted under the microscope. Cannibalized eggs accounted for less than 5% of the eggs produced. The egg size was determined in supplementary incubations at each station, at least 30-40 eggs were measured. Carbon-specific egg production rates were obtained by using carbon-size relationships for eggs and females of *T. longicornis* provided by Dam and Lopes (2003). The body carbon mass of females was calculated from an estimated dry mass assuming carbon to be 40% of dry mass.

For lipid analyses females were sorted on net gauze and shortly dried by removing the water below the gauze to minimize salt-crysts. Three samples containing 40-50 females of *T. longicornis* each were immediately frozen at -80°C at each station. To collect sufficient eggs for lipid analyses, approximately 300 females were incubated in 15 (3 x 5 replicates) 1 l containers with a 150 µm screened partition in 48 µm pre-screened water. Every 12 h in May and 8 h in July the animals were carefully transferred into new containers and the incubation water was filtered through a submerged sieve of 20 µm to harvest the eggs. The eggs of each series were rinsed into 20 ml glass vials and stored at 2-4°C until further processing to slow down development. After 24 h the eggs were transferred under the stereomicroscope into small vials with GF/F-filtered seawater using an apical thinned-out glass Pasteur pipette. This cleaning procedure was repeated to remove faecal pellets and algal detritus. The purified eggs were then sorted on precombusted (400°C for 12 h) GF/C-filters, which were filmed using a digital camera connected to a computer. The images were used to count the eggs in each sample with an average of 800 eggs per filter. After sorting, the samples were immediately frozen and stored at -80°C.

Seston samples were taken with 10 l water sampler bottles and 40-60 l of water from 5 m and 10 m depths, as well as 15 m depths at deep stations (st. B, st. E) were mixed in equal ratios to receive more representative data for the water column. During all subsequent preparation the samples were kept shaded under ambient temperature condition. Size fractions were produced by using a slow flow-through system of interleaved submerged sieves of 100 µm, 30 µm, 20 µm and 10 µm mesh size to prevent destruction of fragile organisms. After separation the fractions were carefully resuspended in 1-2 l of GF/F filtrated seawater. Aliquots were filtered with low pressure (<100 mbar) on precombusted (400°C for 12 h) GF/C filters for carbon, nitrogen and fatty acid analyses with three replicates each. The filters were immediately frozen and stored at -80°C.

### 3.2 Analytical work

After lyophilisation dry mass of copepods was determined using a Sartorius micro-balance ( $\pm 2 \mu\text{g}$ ). During weighing procedure, samples were temporarily stored in a vacuum desiccator to prevent unequal condensation on the tissue. Lipid extraction was performed with minor modifications as described in Folch et al. (1957) using ultrasonic disruption in dichloromethane:methanol (2:1/v:v) and a washing procedure with aqueous KCl solution (0.88%). For quantification of fatty acids, tricosanoic acid was added as an internal standard prior to extraction. An additional centrifugation step was carried out prior to the washing procedure for all samples on glass fibre filters to remove filter remains.

Lipid classes were separated by solid phase extraction, using 1 ml SiOH glass columns (CHROMABOND®, Macherey-Nagel) on a vacuum manifold. To remove residues the columns were washed with a solvent sequence of acetone, diethylether, and hexane:diethylether-mixtures, prior to sample load. After column conditioning with 4 ml of hexane, 4  $\mu\text{l}$  of lipid extract (lipid concentration approx.  $5 \mu\text{g} \mu\text{l}^{-1}$ ) were added. The neutral lipid fraction was washed out with 2.5 ml hexane:diethylether (95:5/v:v) and 2.5 ml hexane:diethylether (1:1/v:v).

For fatty acid analyses a subsample of total lipids as well as the total neutral lipid fraction were hydrolysed and fatty acids were converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulphuric acid at  $80^\circ\text{C}$  for 4 h (Kattner and Fricke 1986). After cooling, 2 ml of aqua bidest. were added, and FAMES were extracted three times with 1 ml hexane. Samples were analysed (modified from Kattner and Fricke 1986) using a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter,  $0.25 \mu\text{m}$  film thickness) operated with helium as carrier gas (constant flow  $0.8 \text{ ml min}^{-1}$ ) using the following temperature program:  $80^\circ\text{C}$  (5 min),  $30^\circ\text{C min}^{-1}$ ,  $165^\circ\text{C}$ ,  $4^\circ\text{C min}^{-1}$ ,  $240^\circ\text{C}$  (15 min). Samples were injected using a hot split/splitless inlet ( $250^\circ\text{C}$ , split mode 1:20) or a programmable temperature vaporizer injector (Gerstel® CIS3) with a baffled, deactivated siltek glass liner: solvent vent mode, injection volume  $10 \mu\text{l}$ , injection temperature  $25^\circ\text{C}$  (0.21 min,  $12^\circ\text{C min}^{-1}$ ,  $280^\circ\text{C}$ ), injection speed  $0.88 \mu\text{l min}^{-1}$ , purge flow  $125 \text{ ml min}^{-1}$ , purge time 0.2 min, split flow  $30 \text{ ml min}^{-1}$ , splitless time 2.5 min. The FAMES and fatty alcohols were detected by flame ionisation and identified by comparing retention times with those derived from standards of known composition. The accurate identification of the substances was checked for selected peaks using GC-MS.

Lipid class composition was analysed in triplicates by thin layer chromatography–flame ionisation detection on an Iatroscan Mk V according to Fraser et al. (1985). Single compound standards were used for calibration, with dipalmitoyl-phosphatidylcholine, cholesterol, triolein and oleic acid palmityl ester. Due to the adding of an internal FA standard prior to extraction, free fatty acids were excluded from calculations.

### **3.3 STATISTICS**

A general outline of the applied statistical processes will be presented here. More detailed information can be derived from the statistic sections of the specific chapters. All statistical analyses, including non-linear regression were performed using the software SPSS. For statistical operations that require normal distribution, percentage data (e.g. relative fatty acid composition) were transformed using an arc sine square root transformation. Normal distribution and homogeneity of variances were checked using the Shapiro-Wilk- and the Levene-test, respectively, according to sample size. For identification of coherences between variables principal component analyses (PCAs) were performed on the correlation matrix, extracting non-rotated components with eigenvalues  $>1$ . Relevant variables (i.e. length, biomass, total and storage lipid content) were analysed using one-way ANOVA followed by a Tukey's HSD or a Dunnet-T3 test for post-hoc comparisons with time as independent variable.

## 4. RESULTS AND SYNOPTIC DISCUSSION

The presented thesis focuses on the lipid dynamics of three predominant copepods in the Baltic Sea, *Pseudocalanus acuspes*, *Temora longicornis* and *Acartia longiremis*. A comprehensive year-round data set on seasonal changes in storage lipid content and fatty acid composition is provided to elucidate the life-cycle strategies of copepods in the Bornholm Basin (section 4.1) and to discuss their feeding ecology and respective trophic niches based on trophic marker comparisons (section 4.2).

Emphasis is also placed on the implications of essential fatty acids for the reproductive success of copepods. The relevance of food quality will be evaluated, with special regard to the role of diatoms in the diet of *Temora longicornis* in the Baltic Sea and North Sea (section 4.3). Since the copepod species provide the main food source for higher trophic levels, such as adult and larval fish, their nutritional values and potential dietary effects will be discussed in the last chapter (section 4.3)

### 4.1 LIFE-CYCLE PATTERNS OF COPEPODS IN THE BALTIC SEA

A major focus of this study was to elucidate the life-cycles of the copepods *P. acuspes*, *T. longicornis* and *A. longiremis* in the Baltic Sea and in particular to evaluate the respective importance of storage lipids during overwintering (chapters I and III). Lipid patterns, especially the seasonal progression in storage lipid content, are a useful indicator for life-cycle strategies. To describe the physiological state of a copepod not only its lipid content, i.e. stored energy, but also its reproductive activity, i.e. transferred energy, has to be considered. A good nutritional condition of copepods will be reflected in elevated lipid amounts and reduced reproductive effort, e.g. prior to overwintering, but also in depleted lipid stores coinciding with high reproductive activity. Reduced energy depots that are associated with low growth rates, will in turn indicate a physiological limitation, potentially due to poor food supply.

Hence, the following discussion not only compares lipid storing strategies with emphasis on overwintering, it also highlights characteristic aspects of reproduction and population structure of the three copepods.

*Pseudocalanus acuspes*

The life-cycle of *P. acuspes* in the Baltic Sea bears several resemblances to that of polar regions (Pertsova 1981, McLaren et al. 1989, Norrbin et al. 1990, Norrbin 1991, Conover and Siferd 1993): a basically annual life-cycle as indicated by the seasonal progression in stage composition (Renz and Hirche 2006), retarded development of the copepodite stages (C) IV and V suggested by their reduced moulting rates in summer (chapter II) and an onset of lipid accumulation in the overwintering stages in early summer, as indicated by elevated lipid levels in CV in comparison to the females (chapter I). The lipid-poor females found in summer most likely represented a still maturing but minor part of the population (chapter I), a phenomenon also reported from life-cycles of *P. acuspes* in other regions, with one or two main generations per year and some minor generations in summer (Pertsova 1981, McLaren et al. 1989, Lischka and Hagen 2005).

The formation of an overwintering stock by CIV and CV stages appears as a genus-specific characteristic, since similar stage structures were observed for *P. minutus* in high latitudinal regions (Lischka and Hagen 2005). Elevated abundances of CIV and CV in October, prolonged development of CV in August (Renz 2006) and basically similar WE levels (Kattner and Krause 1989) as observed for *P. acuspes* in the Bornholm Basin (chapter I) were reported for *P. elongatus* in the North Sea. This emphasises that even the more southerly occurring congener (Frost 1989) exhibits a life-cycle pattern, apparently suitable for overwintering in polar regions.

Despite the less pronounced seasonality as compared to high latitudinal regions and the generally longer periods of food supply, *P. acuspes* seems to retain Arctic life-cycle characteristics. However, living conditions for *P. acuspes* in the Baltic Sea might not be very different from those in polar regions. Due to the strong vertical stratification of the water column, older copepodite stages are mainly restricted to deeper water layers with higher salinity (Renz and Hirche 2006). Their habitat is characterised by constantly low temperatures, ranging from 3°C to 6.5°C at the weighted mean depth of females, with only a short increase to 9°C in late autumn (chapter II). Hence, extended generation times might be explained by unfavourable conditions, like cold temperatures, associated with high energy requirements due to osmotic stress. Strong seasonal changes in feeding conditions might be of even higher importance for the determination of life-cycle patterns. Food supply is strongly pulsed as a consequence of spring bloom sedimentation events, while during summer older stages of *P. acuspes* are largely decoupled from primary production above the thermocline, due to their general restriction to deeper water strata.

However, the trigger for the onset of overwintering is still unknown. Threshold lipid levels were suggested to induce an arrested development, with lipid-poor copepodids achieving maturation to produce potentially more successful offspring; whereas lipid-rich copepodids pass into an overwintering state, with continual feeding, suspended development and resting gonads (McLaren et al. 1989). Rather than a continuous process triggered by the physiological condition, Norrbin (Norrbin et al. 1990, Norrbin 1996) proposed a specific switching date, at which *P. acuspes* copepodids stop maturation but proceed to accumulate lipids. Changes in photoperiod or temperature are not very likely to have an influence on the copepods that early in the year in the Baltic Sea, whereas changes in food supply of the older copepodids might play a more important role. A strong decline of feeding conditions was suspected to induce a hormonal cessation of copepod development in favour of lipid storage (Klein Breteler and Gonzalez 1988).

#### *Temora longicornis*

While in *P. acuspes* the switch from development to lipid accumulation occurred quite early in the year, females of *T. longicornis* started lipid accumulation not until autumn (chapter III). Storage lipid levels began to rise in September, although energy was still transferred mainly into reproduction, as is evident from the second peak in egg production in October. The proportion of reproducing females declined from September (97%) to November (23%) (unpublished GLOBEC data), while their body size increased (chapter III). Hence, overwintering females most likely belonged to a new cohort or generation that proceeded development but stopped reproduction in favour of lipid accumulation. TAG levels reached a maximum in November and slowly decreased during winter, probably serving as an energy buffer against starvation. Reproductive activity remained low until the onset of the next year's spring bloom in March.

*T. longicornis* females stored almost exclusively TAGs as energy reserves, in accordance with previous publications (Kattner et al. 1981, Fraser et al. 1989). Since TAGs provide rapidly mobilised energy for short-term needs (Lee et al. 2006), they might be adequate for the endurance of only some months with reduced food supply. A slight increase in TAG levels in autumn was also observed in *T. longicornis* from the North Sea, although the lipid accumulation was less pronounced (Kattner et al. 1981). This might be a consequence of resting egg production in the North Sea. Although resting eggs contribute largely to the spring-population recruitment in other habitats (Lindley 1990, Castellani and Lucas 2003),

no hatching of *T. longicornis* eggs from sediments was observed in the Baltic Sea so far (Madhupratap et al. 1996, Dutz et al. in prep.).

Compared with *P. acuspes* and *A. longiremis*, *T. longicornis* females show a stronger association with primary production above the thermocline (chapter III). Even though they also prefer water masses below the summer-thermocline, weighted mean depths of females in summer were always higher than those of *P. acuspes* and *A. longiremis* females (unpublished GLOBEC data). Furthermore, *T. longicornis* was described to migrate diurnally above the thermocline in the Baltic proper (Hernroth and Ackefors 1979). Trophic markers in *T. longicornis* indicated a stronger tendency to herbivorous feeding than *P. acuspes* and *Acartia* spp. (section 4.2), and storage lipid amounts in November were at least partly built up by grazing on diatoms and dinoflagellates (chapter III). Apparently, *T. longicornis* intensively used the small bloom of diatoms and autotrophic dinoflagellates (van Beusekom subm.) that reached into deeper depths in October due to increased vertical mixing. The ability of *T. longicornis* to exploit phytoplankton in upper water layers might favour a strategy to endure the time of reduced food supply between autumn and spring bloom events by accumulating moderate lipid reserves.

#### *Acartia longiremis*

In contrast to the life-cycle pattern of *P. acuspes* in the Bornholm Basin, the overwintering strategy of *A. longiremis* differed from that found in polar regions. In high latitudinal habitats, females of *A. longiremis* endure winter with premature gonads and increased levels of TAGs and also WEs (Davis 1976, Norrbin et al. 1990), whereas females of *A. longiremis* in the Bornholm Basin showed no signs of increased lipid accumulation in winter (chapter III). There is no evidence for an overwintering of other copepodite stages, but high abundances of nauplii occurred during winter (unpublished GLOBEC data). Both, egg production and hatching success of *A. longiremis* were poor from January to April 2003 (Dutz et al. 2004). Sediment cores from the Bornholm Basin released large amounts of *Acartia* spp. nauplii after 6 to 8 weeks of incubation under ambient temperature conditions (Dutz et al. in prep.). Eggs of *A. longiremis* still hatched from sediments in other temperate regions (Marcus 1990). Hence, *A. longiremis* might, at least partly, recruit the first generation of the following year from the production of resting eggs and relies therefore less on the survival of last year's females throughout winter. This is consistent with their constantly low lipid levels and the investment of energy into reproduction rather than into storage depots.

Life-cycle strategies were found to be generally variable within this genus, with *Acartia* spp. females showing different duration of reduced egg production in winter (Davis 1976, Kjørboe and Nielsen 1994, Halsband and Hirche 2001) up to a continuous spawning (Ianora and Buttino 1990). Furthermore, resting eggs, copepodids or even nauplii were proposed as overwintering stages (Uye 1980, as cited by Mauchline 1998, Katajisto et al. 1998). *A. bifilosa* reproduces in the northern Baltic Sea in winter, and nauplii, which probably hatched from resting eggs, concentrated below the ice cover to a peak at ice-break up (Werner and Auel 2004). *A. bifilosa* copepodids showed, however, much higher levels in storage lipids, especially in WEs (Werner and Auel 2004), than *A. longiremis* females in the Bornholm Basin (chapter III). The absence of lipid accumulation in *A. longiremis* in the Baltic Sea may have several reasons: (i) limitation by food does not leave enough energy for an efficient storage lipid build-up, (ii) metabolic needs are increased due to unfavourable environmental conditions, (iii) an external trigger is absent in the Baltic Sea or (iv) temperate populations of *A. longiremis* generally exhibit a life-cycle different from that in high latitudinal habitats due to genetical differences.

The strategy for a continuous production of eggs in autumn and winter and the subsequent early onset of population recruitment bears several advantages, as also proposed for resting egg production of *A. bifilosa* in the Baltic Sea (Viitasalo 1992): a full utilization of the spring bloom, avoidance of high predation pressure on the nauplii and the ability to produce a strong first generation early in spring. This continuous recruitment may play an even more pivotal role for *A. longiremis*, since its egg production rates are generally low (Peterson et al. 1991, 2002, Gómez-Guitérrez and Peterson 1999), even under optimum food conditions (chapter V). Hence, in order to sustain the large standing stock in the Bornholm Basin a suitable strategy probably consists in the extension of the reproductive phase and a maximized exploitation of food sources to assure a fast ascent of nauplii in early spring. The strong tendency of *A. longiremis* to feed on microzooplankton in the Bornholm Basin probably provides a more constant energy supply (chapter III) but might not suffice for strong lipid accumulation in autumn.

#### *Overwintering strategies*

A classification of the above described overwintering strategies of the three copepod species proves to be difficult without further knowledge of the triggers for the onset and termination of their dormancy. Diapause is generally defined as a genetically compulsory response to changing environmental factors expressed as arrested development (Dahms 1995). An

important characteristic of diapause is the refractory phase, from which development cannot resume even after the return of favourable environmental conditions (Elgmork and Nielssen 1978, Danks 1987), and torpidity is then commonly observed (Hirche 1983, Elgmork 1996, Auel et al. 2003).

*P. acuspes* strongly reduces mobility and metabolism in high latitudinal regions over winter, but a complete shut down of activity does not occur (Norrbin 1992). Feeding most likely proceeded in *P. acuspes* in the Bornholm Basin, as is evident from changes of trophic markers in storage lipids of CV corresponding to seston composition (chapter I, Section 4.2). Hence, the retardation in development of copepodite stages can be classified as an “active diapause” (McLaren et al. 1989, Norrbin 1996), which was defined as an arrest in development and reproduction, but not in activity (Elgmork 1980).

This term probably also applies to the suspended reproduction of *T. longicornis* females in favour of lipid accumulation. Since copepodite stages still developed, as indicated by stage composition (unpublished GLOBEC data), the stoppage of development might only be restricted to reproductive activity. An unambiguous overwintering stage could not be identified for *A. longiremis* in the Bornholm Basin. Although a production of resting eggs was proposed, it remains unclear whether they meet the criteria of true diapausing, dormant eggs, or are normal eggs with longer development times due to cold temperatures.

## CONCLUSION

According to their general life-cycle characteristics the copepod species might be categorized into three overwintering types, with different emphasis of storage lipids for overwintering: (1) the “long-term-resting” strategy of *P. acuspes*, retaining adaptations to high latitudinal habitats, i.e. an early onset of overwintering as copepodite stages and strong lipid accumulation especially of WEs; (2) the “short-term-resting” strategy of *T. longicornis* with a late onset of overwintering as females, moderate storage depots of TAGs and reduced reproduction until the next spring bloom; (3) the “proceeding” strategy of *A. longiremis* with ongoing but limited egg production that leads to a continuous recruitment from sediments early in the year and involves no storage lipid accumulation.

These three different species-specific strategies for coping with environmental changes in the habitat all prove to be quite successful for the persistence of their populations in the Bornholm Basin and the respective patterns are, as described above, potentially related to their individual trophodynamical and distributional characteristics in the Baltic Sea.

## KEY RESULTS

---

- Lipid reserves of *Pseudocalanus acuspes* consisted of wax esters and triacylglycerols, while *Temora longicornis* and *Acartia longiremis* stored almost exclusively triacylglycerols.
  - Lipid accumulation for overwintering started in early summer for *P. acuspes* as copepodite stages IV and V with high amounts of wax esters, whereas *T. longicornis* females switched from growth to lipid anabolism not until late autumn. Lipid stores of *A. longiremis* females were not increased, suggesting that resting eggs might be an important source for population recruitment in the following year.
- 

## 4.2 TROPHODYNAMICS

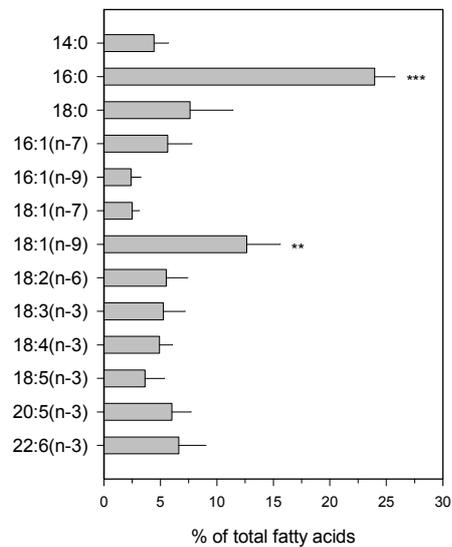
This chapter aims at describing seasonal changes in the diet of the dominant Baltic Sea copepod species and at identifying their respective trophic niches. For this, the concept of fatty acid markers was applied. The use of signature fatty acids to identify specific taxa in the diet is well established (Daalsgard et al. 2003). Nonetheless, it is essential to validate these results for the studied ecosystem by comparing fatty acid profiles of the seston with its taxonomic composition (section 4.2.1). In order to receive mostly unaltered signals of recent feeding that are not biased by the conservative composition of structural lipids, the analyses focused on the fatty acid dynamics in the storage lipids of the copepods (section 4.2.2).

### 4.2.1. VALIDATION OF FATTY ACID MARKERS

Annual means of fatty acids will provide basic information on general characteristics of the seston composition in the Bornholm Basin (Fig. 2). Significantly elevated and stable values of 16:0 were found in the seston at all times and quite similar levels were recorded in studies from several other temperate habitats (Kattner et al. 1983, Jónasdóttir et al. 1995). Seston was characterised by very high levels of 18:1(n-9), while all other trophic markers were on average equally abundant. This indicates a rather similar role of different food compounds in the pelagic system, although their seasonal importance varied strongly (chapters I and III).

**Figure 2.**

Fatty acids in the seston, unweighted annual means (based on monthly averages) of the most important fatty acids, Differences were tested by ANOVA-Dunnett T3, significance levels: \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$



A comprehensive data set on seasonally and vertically resolved seston compositions in terms of fatty acids was used to describe food abundance and seasonal variations in the offered diet compositions for the copepod species in the Bornholm Basin (chapters I and III). To validate the applicability of trophic markers, fatty acid patterns were compared, using multivariate analyses (chapter I), with the taxonomic seston composition derived from cell counts (van Beusekom et al. *subm.*).

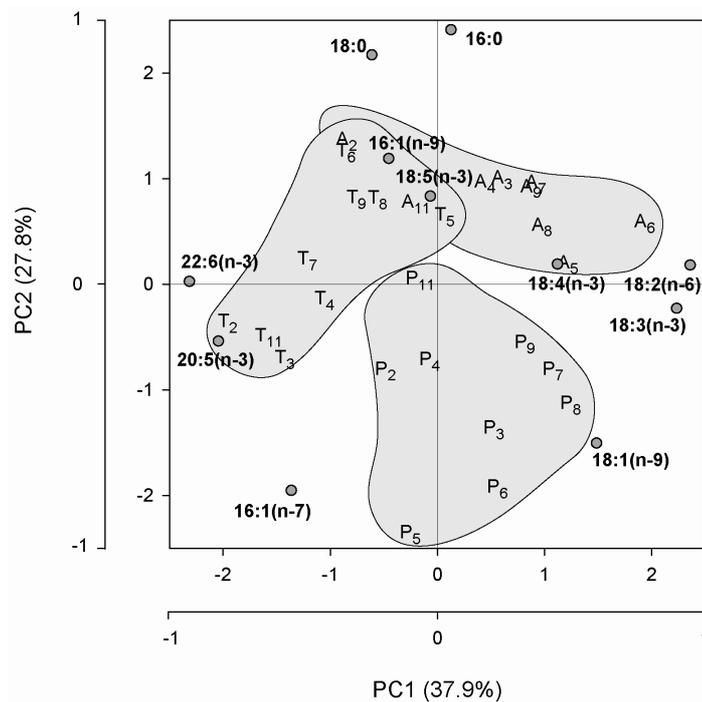
The assignment of some typical signature fatty acids were confirmed that were described to be unambiguous for specific groups, e.g. 16:1(n-7) and 20:5(n-3) originating from diatoms (Nichols et al. 1993, Dunstan et al. 1994) or 18:4(n-3) and 22:6(n-3) from dinoflagellates (Sargent et al. 1987, Graeve et al. 1994). In contrast, an application of 18:1(n-9), which was a major component in the seston for most of the time, and also very abundant in the storage lipids of *P. acuspes* and, to a lesser degree, of *A. longiremis* and *T. longicornis* (chapters I and III), proved to be difficult. Oleic acid is not only an indicator for carnivorous or detritivorous feeding in copepods (Sargent and Falk-Petersen 1981, Falk-Petersen et al. 1990), it can be also synthesised *de novo* (Pascal and Ackman 1976, Sargent and Henderson 1986, Kattner et al. 1994, Kattner and Hagen 1998). However, 18:1(n-9) levels in the seston were associated with ciliate abundance, thus strongly emphasising that 18:1(n-9) can be interpreted as a reflection of heterotrophic feeding, presumably on ciliates.

A characteristic of the Baltic Sea is the appearance of cyanobacteria blooms during summer, but since they are quite diverse and their fatty acid compositions can be very variable (Gugger et al. 2002), no clear trophic signals could be established. Many studies reported

16:1, 18:2(n-6) and 18:3(n-3) as characteristic fatty acids of cyanobacteria (e.g. Murata and Nishida 1987 and references therein, Vargas et al. 1998, Gugger et al. 2002) and due to coherences of 18:2(n-6) and 18:3(n-3) found with cyanobacteria abundance (chapter I), they were interpreted in this study as a marker for feeding on cyanobacteria. The above specified trophic markers were applied in the following chapter to describe feeding histories of the Baltic Sea copepods.

#### 4.2.2. FEEDING ECOLOGY

Interseasonal and interspecific differences in the fatty acid markers in storage lipids of *T. longicornis*, *A. longiremis* and *P. acuspes* were observed over the course of the year (chapters I and III), indicating a progression in diet composition and species-specific food selectivity. Based on the relative fatty acid compositions, the species were clearly distinguishable, with only marginal overlapping between *A. longiremis* and *T. longicornis* (Fig. 3).

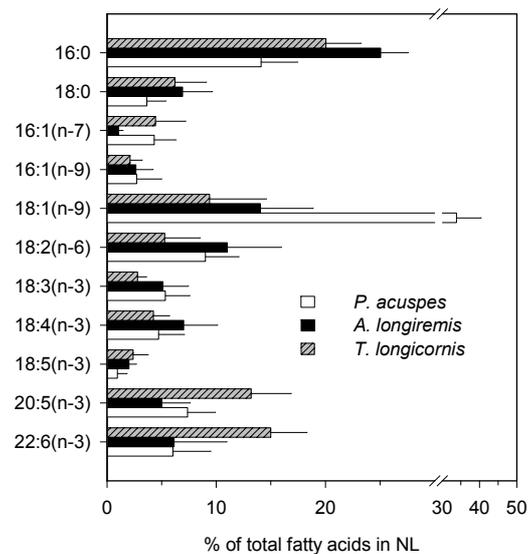


**Figure 3.** Principal component analysis based on the relative fatty acid composition of neutral lipids of adult females of *Pseudocalanus acuspes* (*P*), *Temora longicornis* (*T*) and *Acartia longiremis* (*A*), indices = sampled month; filled circles = fatty acids, scales were adjusted to combine plots: scales of principal components (PC) refer to sample plot, scale of variables ranges from -1 to +1 for both PCs

While *T. longicornis* was related to the polyunsaturated fatty acids 20:5(n-3), 22:6(n-3) and to a lesser degree to 16:1(n-7), *A. longiremis* was associated with elevated amounts of the C18-polyunsaturated fatty acids 18:4(n-3), 18:2(n-6) and 18:3(n-3), and with very low levels of 16:1(n-7). *P. acuspes*, in contrast, was characterised by extremely high year-round values of 18:1(n-9) (Fig. 4). While seasonal trends in markers within a species will provide relatively confirmed results on dietary variations, interspecific comparisons have to consider influences of species-specific metabolisms and will thus only allow tentative interpretations, unless further experimental calibrations are provided. However, fatty acid markers in *T. longicornis* from the Baltic Sea and North Sea varied significantly (chapters III and IV), and fatty acid compositions of *Acartia* sp. were recorded to correspond strongly to mono-algal diets (Veloza et al. 2005). Thus, it seems likely that interspecific varieties found for the species in the Bornholm Basin can be ascribed to actual differences in feeding history. These differences are most likely associated with their vertical distribution in the water column as well as species-specific feeding behaviour, reflecting different trophic niches of the three copepods.

**Figure 4.**

Annual means of the most important fatty acids in the storage lipids of females of *Pseudocalanus acuspes*, *Temora longicornis* and *Acartia longiremis*; error bars = standard deviation, NL = neutral lipids



*T. longicornis* females exhibited the most direct coupling to primary production in the upper water layers, with similar amounts of diatom and dinoflagellate markers during the spring bloom and a reflection of the second smaller diatom bloom observed in July (chapter III). Furthermore, lipids in November were built up mainly by feeding on dinoflagellates and diatoms. Implications of this relatively close coupling with phytoplankton in autumn on the life-cycle have already been discussed (section 4.1). As a typical suspension feeder (Tiselius

and Jonsson 1990, Jakobsen et al. 2005) diet selectivity of *T. longicornis* was described to be generally low, provided that the offered food is of suitable size (De Mott 1988, Koski et al. 2005, Kozłowsky-Suzuki et al. 2006). This is consistent with the strong similarities observed in fatty acid marker compositions between seston (1-30  $\mu\text{m}$ ) and storage lipids of females and, even more strongly, eggs of *T. longicornis* in the North Sea (chapter IV). However, several studies reported a selective feeding on ciliates (Vincent and Hartmann 2001, Anatjan 2004), cryptophytes (Breton et al. 1999, Cotonnec et al. 2001), diatoms or dinoflagellates (Antajan 2004, Guisande et al. 2002).

While *T. longicornis* showed a general tendency to feed herbivorously, fatty acid markers in *A. longiremis* emphasised a strongly reduced feeding on diatoms. This can be explained by the slightly deeper distributional range of *A. longiremis* during summer compared to *T. longicornis* and, even more likely, by their difference in feeding mode (chapter III). *Acartia* spp. was described to switch from a suspension to an ambush feeding mode in times of a low or moderate food supply or in the presence of large motile prey, such as large dinoflagellates and especially ciliates (Tiselius and Jonsson 1990, Kjørboe et al. 1996, Jakobsen et al. 2005). Thus, prevailing ratios between diatoms and mobile prey in the Baltic Sea probably favoured ambush feeding of *A. longiremis* for most of the time, leading to an increase in heterotrophic feeding or at least in feeding on non-diatom food items. This might change in times of diatom bloom events (Fessenden and Cowels 1994), since elevated diatom marker levels have been recorded in the fatty acid profiles of *A. longiremis* (Norrbin et al. 1990, Peters et al. 2004) and other *Acartia* species (Kattner et al. 1981, Cotonnec et al. 2001, Werner and Auel 2004).

In contrast to the distinct differences in diatom signals in *A. longiremis* and *T. longicornis*, the respective role of flagellates in the diet of both species is more difficult to interpret. The partially parallel progression of 18:4(n-3) and 22:6(n-3) in *T. longicornis* most likely reflected feeding on dinoflagellates (chapter I, section 4.2.1), while *A. longiremis* showed no increase in 22:6(n-3) levels, yet 18:4(n-3), 18:3(n-3) and 18:2(n-6) were strongly elevated. This finding can only be interpreted with caution. While 18:3(n-3) and 18:2(n-6) were found to be associated with cyanobacteria abundance (section 4.2.1, chapter I), C18-polyunsaturated fatty acids are also known to be characteristic markers in some cryptophytes (Daalsgard et al. 2003). However, the increase of these markers in all three species coincided with the occurrence of cyanobacteria during summer. This suggests that cyanobacteria provided an important food source during this time, when food was apparently limited, since lipid levels and production were low in the females of all species (chapters I and III).

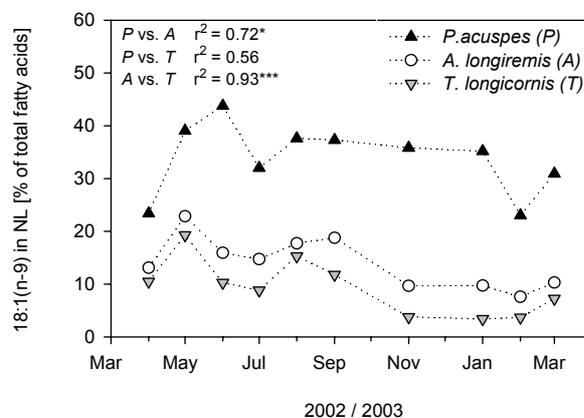
A general capability to utilise cyanobacteria was described at least for *T. longicornis* and *Acartia* spp. (Heerkloss et al. 1984, Schmidt and Jónasdóttir 1997).

The fatty acid profiles of *P. acuspes* resembled those of *T. longicornis* in several aspects. Dinoflagellate and diatom markers were occasionally elevated and feeding on cyanobacteria was reflected during summer (chapter I). However, relative amounts of polyunsaturated fatty acids were much lower in *P. acuspes* at all times. This is associated with the predominant deposition of WEs that contain extremely high amounts of 18:1(n-9), as indicated by strong correlations between the 18:1(n-9) and fatty alcohol levels in the females (unpublished data). Oleic acid is a major component of WEs in this genus (Fraser et al. 1989, Norrbin et al. 1990). At the beginning of the year phytoplankton signals in *P. acuspes* lagged behind those found in *T. longicornis* and behind the progression of phytoplankton abundance in the seston. This retardation probably derived from the generally higher lipid amounts in *P. acuspes* females. Low lipid levels reflect changes much faster, causing a more synchronous progression of fatty acid compositions of seston and storage lipids later in the season. In contrast to *T. longicornis*, *P. acuspes* females seem to be strongly decoupled from primary production above the thermocline, since the small peaks of diatoms in summer and of diatoms and dinoflagellates in October were not reflected in the fatty acid patterns of the females, albeit to some degree in the still lipid-accumulating CV.

A remarkable characteristic in the fatty acid patterns of all three copepods consisted, although on quite different levels, in the relatively parallel seasonal progression of 18:1(n-9), with a highly significant correlation between *A. longiremis* and *T. longicornis* (Fig. 5). The difficulties in the interpretation of the oleic acid signal have already been discussed (section 4.2.1).

**Figure 5.**

Seasonal progression of 18:1(n-9) in the storage lipids of females of *Pseudocalanus acuspes*, *Temora longicornis* and *Acartia longiremis*, NL = neutral lipids



The observed occasional increase in the proportion of 18:1(n-9) was most likely not only a phenomenon of variability in other markers, since high levels in 18:1(n-9) coincided, at least in May, with an increase of lipids in all three copepods and with high concentrations of oleic acid in the seston (chapters I and III). Hence, it is likely that 18:1(n-9) originated at least partly from the diet and was directly incorporated into the storage lipids, probably most efficiently in *P. acuspes*. In May, the degradation of the spring bloom proceeded and ciliates gained in biomass compared to other food taxa (chapter I). This may be a further indication of a high relevance of ciliates in the pelagic food web of the Baltic Sea. However, the correlation between the relative 18:1(n-9) levels in the different species remains enigmatic. Due to the variability in other markers, these strong similarities could not be expected, even if ciliates were ingested at similar rates.

## CONCLUSION

The results of this study proved that fatty acid markers, if analysed in the neutral lipid fraction, are a suitable tool for a general characterisation of trophic niches of the copepods in the Baltic Sea, although some severe problems arose in the attempt to describe their diet composition in more detail. An assignment of some markers like 18:1(n-9), 18:2(n-6) and 18:3(n-3) to specific food sources was tainted with some ambiguities, although their synchronous occurrence in the seston with ascertained food taxa suggested an origin of specific dietary sources, like ciliates and cyanobacteria. Further experimental studies are required to calibrate between ingested food and reflected dietary signals to account for the species-specific metabolism and to assess retention efficiencies of specific fatty acids during lipid anabolism and catabolism.

## KEY RESULTS

---

- Lipids in the pelagic system of the Bornholm Basin were generally characterised by the fatty acid 18:1(n-9), with high year-round values in seston lipids and a synchronous development of oleic acid levels in storage lipids of copepods. High concentrations of oleic acid in the seston coincided with peaks in ciliate biomass, thus elevated amounts of 18:1(n-9) in copepods might be interpreted as a trophic signal of ciliates.

- Signature fatty acids in storage lipids of *Temora longicornis*, *Acartia longiremis* and *Pseudocalanus acuspes* females showed distinct interseasonal differences, reflecting changes in food composition.
  - Lipids of *T. longicornis* contained strong indicators for herbivory, such as 20:5(n-3) and 22:6(n-3), as well as 16:1(n-7). Diatom- and dinoflagellate-signals increased in times of high egg production and lipid accumulation.
  - *A. longiremis* was characterised by high levels of polyunsaturated C18-fatty acids and selected strongly against diatoms at all times, which was most likely a consequence of ambush feeding on large motile prey.
  - 18:1(n-9) was the major component in the storage lipids of *P. acuspes* at all times, probably indicating a species-specific storage pattern as well as a ciliate-dominated diet.
- 

### 4.3 FOOD QUALITY

The first section of this chapter focuses on the role of essential fatty acids for the reproductive success of copepods and assesses the relationship of food quantity and quality in terms of fatty acids in the field. Special emphasis is given to the reproductive success of *T. longicornis* in the North Sea. Diatoms provided an important food source for *T. longicornis* and their role will be discussed into more detail in the second part of this section. Since copepods provide a major food source for the next trophic levels, such as fish larvae, aspects of their nutritional value will be presented in section 4.3.2.

#### 4.3.1 INFLUENCE OF FOOD QUALITY ON THE REPRODUCTIVE SUCCESS OF COPEPODS

##### *Role of essential fatty acids*

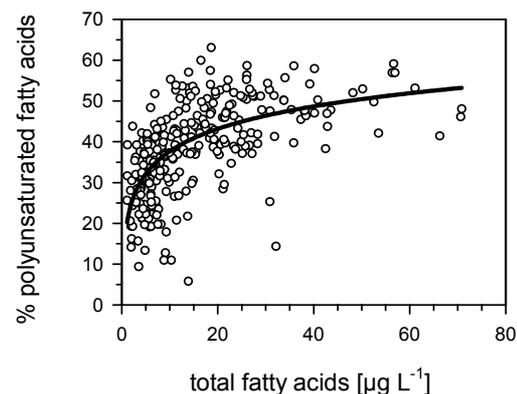
The idea that essential fatty acids are an important factor in the regulation of the reproductive success of marine copepods was and is subject of many studies (Jónasdóttir 1994, Jónasdóttir et al. 2002, Broglio et al. 2003, Shin et al. 2003). Reduced productivity and failures in egg development were often associated with diet deficiencies in essential fatty acids or with the ratios of specific fatty acids like DHA and EPA (Hazzard and Kleppel 2003, Tang and Taal 2005, Arendt et al. 2005). Still, most knowledge is derived from correlations of egg

production or hatching success with diet composition, while involved processes are yet not truly understood and causalities have not been established convincingly.

Whereas high egg production rates tend to result in a high hatching success, in times of low production, egg viability was found to be much more variable (Jónasdóttir and Kiørboe 1996, Jónasdóttir et al. 2002, Shin et al. 2003). If food amount is high enough, females might be able to compensate low quality by channelling limited compounds more efficiently into the eggs. On the other hand elevated levels of food supply often co-occur with high food quality. A concordant phenomenon was observed in the fatty acid patterns of seston in the Baltic Sea. At times of enhanced lipid concentrations in the seston, the relative content of polyunsaturated fatty acids in the seston was constantly high, whereas food quality became more variable with decreasing lipid levels (Fig. 6).

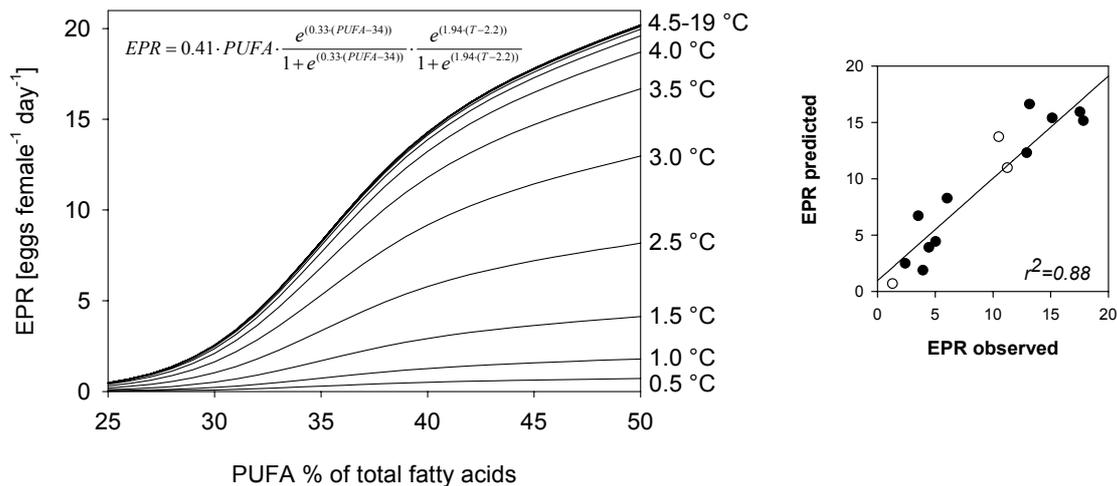
**Figure 6.**

Relationship between relative amount of polyunsaturated fatty acids and total lipid concentration in the seston of the Bornholm Basin



To assess the influence of essential fatty acids on the reproductive success of *Acartia bifilosa* in the Bornholm Basin, individual egg production rates were compared with levels of polyunsaturated fatty acids in the seston. The application of a non-linear regression of egg production versus temperature and polyunsaturated fatty acids explained 88% of the variations within *in situ* egg production (Fig. 7B, C). Egg production rates increased with increasing proportions of polyunsaturated fatty acids in the seston, although temperature had a significant negative effect below 4°C. Since this model is based on field data, it does not cover the complete array of temperature and fatty acid levels. A validation with empirical data from feeding experiments (Dutz and Peters in prep.) suggests that the model might be suitable to describe the general relation of *in situ* egg production with dietary fatty acids.

However, since polyunsaturated fatty acids increased more strongly than other fatty acids with increasing lipid amounts in the seston (Fig. 8), the effects of food quantity and quality cannot be separated by this model. Further studies on gross growth-efficiencies will be necessary to assess limitation potentials of specific dietary compounds.



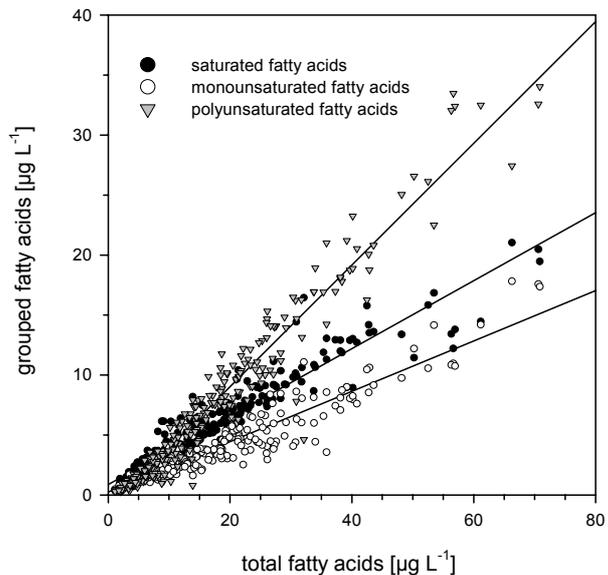
**Figure 7.** a. Model based on non linear regression: individual egg production rates of *Acartia biflosa* vs. polyunsaturated fatty acids (PUFA) in the seston under different *in situ* temperatures b. Predicted egg production rates (EPR) from the model vs. observed EPR, filled circles – *in situ* EPR, open circles – validation data from feeding experiments

To further elucidate the factors that determine egg production and hatching success in the field, a main emphasis was placed in this thesis on the coupling of food composition with egg quality. Thus, consequences of egg composition for the reproductive success of *T. longicornis* were examined during a field study in the German Bight (chapter IV) and in particular the role of essential fatty acids is discussed.

The lipid content of the eggs of *T. longicornis* was extremely low with an average of 2.5% lipids per dry mass. Hence, lipids do not serve as an important energy deposit in the eggs of this species, but take on mainly structural functions (chapter IV). This is consistent with the fast embryonic development, since it takes only a few days between the production of an egg and the onset of first feeding by the nauplii at the prevailing temperatures during the study period (Klein Breteler et al. 1990, Halsband-Lenk et al. 2002). Thus, this species strongly relies on a planktotrophic larval development and the need for long lasting energy reserves is reduced.

**Figure 8.**

Relationship of polyunsaturated, monounsaturated and saturated fatty acids with total fatty acids in the seston in the Bornholm Basin



The fatty acid composition of the eggs of *T. longicornis* varied significantly between stations and seasons, and depended strongly on the available food, as indicated by several correlations between fatty acid amounts in the seston (1-30  $\mu\text{m}$ ) and in the eggs. Hence, feeding selectivity of *T. longicornis* was relatively low and the females showed no or only low regulation in the transfer of fatty acids into the eggs at that time. A strong maternal regulation of egg composition should lead, in times of unbalanced food supply, to reduced egg production with enhanced egg quality, while a low regulation would entail a higher production of eggs with lower quality. If the observed low regulation is characteristic for this species and does not change even under limited feeding conditions, then the hatching success rather than the egg production should be affected more strongly in times of low availability of essential fatty acids.

The hatching success of *T. longicornis* was relatively stable, ranging from 77-94% (chapter IV). A significantly positive influence of dietary DHA levels and DHA:EPA ratios on the *in situ* egg viability of *T. longicornis* was observed in the North Sea (Arendt et al. 2005). Similarly, hatching success of *T. longicornis* in the present study correlated positively with the DHA:EPA ratio of the diet (chapter IV). No causal relationship with DHA:EPA ratios could be established, when egg quality itself was taken into account, although egg composition generally corresponded to dietary fatty acids. DHA:EPA ratios ranged from 1.0 to 3.0 in the eggs and from 0.5 to 1.9 in the seston (1-30  $\mu\text{m}$ ). At similar dietary ratios (DHA:EPA <2) hatching success of *T. longicornis* was reduced to less than 50%, in most cases (Arendt et al. 2005), which contrasts the high hatching success in the present study. Relationships between quality indicators, such as dietary DHA:EPA ratios, and the reproduc-

tive success are generally more variable in the field (Arendt et al. 2005), contrasting those found in mono-algal experiments (Jónasdóttir and Kiørboe 1996, Shin et al. 2003). Although dietary DHA:EPA ratios can have a significant metabolic influence on a consumer, as proved for example for fish larvae (see section 4.3.2), they also reflect specific feeding conditions in the field and are mainly determined by the proportions of diatoms in the diet. Since some diatoms were suggested to provide food of low quality (Støttrup and Jensen 1990, Jónasdóttir and Kiørboe 1996), the amount of non-diatom food available for the copepod might be of relevance for the reproductive success (Kleppel et al. 1991). In conclusion, even though the composition of eggs of *T. longicornis* was quite variable, the hatching success was independent from the essential fatty acid levels in the eggs (chapter IV). Thus, fatty acids were apparently transferred into the eggs in sufficient amounts.

While hatching success was probably not limited by essential fatty acids, egg production (corrected for females size and temperature difference) showed several positive relationships with dietary fatty acid levels. In particular, a significant correlation was found with EPA amounts in the seston and the eggs as well as with the relative amount of 18:2(n-6) in the eggs. A stoichiometric approach was used to assess the potential of essential fatty acids to limit egg production rates compared to the general food supply. Ratios between polyunsaturated fatty acids and nitrogen, as an indicator for food amount, in the eggs and seston revealed that polyunsaturated fatty acids abounded in seston compared to nitrogen. It seems likely that the low amounts of polyunsaturated fatty acids in the eggs can be easily covered at prevailing lipid concentrations in the seston. Hence, correlations of egg production with fatty acid levels reflected rather the influence of food availability in general than a limitation by fatty acids themselves.

#### *Role of diatoms*

As stated in the previous chapter, food quality in terms of essential fatty acids limited neither egg production nor hatching success of *T. longicornis* in May and July in the North Sea, but the quantity of food was a crucial factor for the reproductive success (chapter IV). As indicated by elevated levels of diatom markers in females and eggs, diatoms were an important food component for *T. longicornis* most of the time.

Egg viability negatively correlated with the relative amount of diatom markers in the eggs. Similarly, *in situ* hatching success of *T. longicornis* was found to be negatively related to dietary 16:1(n-7) levels in the North Sea (Arendt et al. 2005). In contrast, egg production significantly increased with the 16:1(n-7) content in the seston and the eggs (chapter IV).

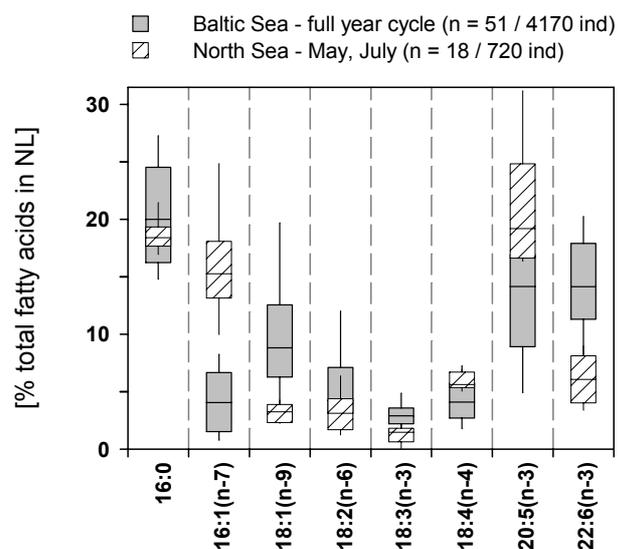
This positive effect by diatoms on egg production outweighed the reduced hatching success by far, leading to an increased reproductive success with increasing diatom abundance in the water.

The role of diatoms for the reproductive success of marine copepods continues to be a subject of intense discussions (Ban et al. 1997, Jónasdóttir et al. 1998, Miralto et al. 1999, Irigoien et al. 2002, Ianora et al. 2004). Field and experimental studies indicate that diatom diets can result in a significant decrease of egg production (Poulet et al. 2006) or hatching success and nauplii viability (Poulet et al. 1995, Uye 1996, Ban et al. 2000, Miralto et al. 2003), albeit the underlying processes are still not conclusively resolved. Reduced egg viability might be induced by toxic effects, e.g. by transfer of antimetabolic compounds into the eggs during vitellogenesis (Poulet et al. 1994, Ianora et al. 1995, Pohnert et al. 2002) or by nutritional insufficiencies of diatoms (Støttrup and Jensen 1990, Kleppel et al. 1991, Jónasdóttir and Kiørboe 1996, Crockett and Hasset 2005, Jones and Flynn 2005). Other observations, in contrast, revealed no relation between diatom abundance and reduced *in situ* hatching success (Pond et al. 1996, Irigoien et al. 2002). Experimental studies suggest that deleterious effects of diatoms strongly depend on the copepod species as well as the specific diatom species or strain (Starr et al. 1999, Ceballos and Ianora 2003, Ianora et al. 2003).

In spite of the observed negative influence of diatoms on the egg viability of *T. longicornis* (chapter IV), the hatching success remained generally high. Hence, it may be assumed that the detected effects were, if at all, most likely based on minor nutritional deficiencies in non-fatty acid components, rather than on toxic effects.

**Figure 9.**

Relative fatty acid composition of storage lipids of *Temora longicornis* females in the Bornholm Basin and the German Bight, NL = neutral lipids



While diatoms provided, at least at times, the main food source for *T. longicornis* in the North Sea, they were significantly less available for this species in the Baltic Sea. The diatom signal, particularly 16:1(n-7), was much lower in females from the Bornholm Basin at all times (chapter III and IV, Fig. 9). However, diatoms seem to occasionally inhaled an important role in the nutrition of *T. longicornis* in the Bornholm Basin. Elevated levels of diatoms markers in *T. longicornis* in spring and autumn coincided with important aspects in the life-cycle of the copepod (chapter III): (i) the individual egg production peaked, when diatom marker levels were high in April, and (ii) the elevated storage lipids in November were at least partly built up by grazing on diatoms. So despite the much lower abundance of diatoms in the Bornholm Basin, they obviously fuelled crucial periods of the life-cycle.

## CONCLUSION

Food quality is a burning issue in the debate on the role of diatoms for the reproductive success of copepods and dietary deficiencies contrast deleterious effects by toxins. No indication for a decrease in reproductive success of *T. longicornis* by diatoms was found here, since egg production significantly increased with increasing diatom abundance in the water, while hatching success decreased only slightly. No deficiency of essential fatty acids in the eggs was evident, thus copepods compensated a possible nutritional insufficiency in fatty acids of diatoms rather by heterogeneous feeding than by physiological regulation. The requirements of essential fatty acids for the production of eggs by *T. longicornis* were low. However, in times of strongly depleted dietary fatty acids levels, their limitation potential might be well increased. *In situ* food quantity and quality are no independent variables. Their separation is problematic, although essential, to understand the nutritional impact on the reproductive success. Further studies on the mechanisms regulating the transfer of fatty acids into the eggs will be needed and a special emphasis has to be placed on the determination of minimum nutritional requirements for a successful egg development, also including non-fatty acid compounds.

### 4.3.2 NUTRITIONAL VALUE OF COPEPODS

Since *P. acuspes*, *T. longicornis* and *A. longiremis* provide the major food source for sprat, herring and juvenile cod in the Baltic Sea, their condition may strongly influence fish recruitment in the Bornholm Basin. Hence, the nutritional values of the different copepods for higher trophic levels will be compared in this chapter. Beside the pivotal need of fish

larvae to receive enough suitable food in terms of size at the right time to meet energetic concerns (Cushing 1972, 1990), food quality in terms of fatty acids might have an influence on the successful recruitment of fish stocks.

Dietary DHA levels were found to correlate with DHA proportions in marine fish larvae (Evjemo et al. 2003) and high levels of di-DHA molecules were detected in the eyes as well as brains of cod (Bell and Dick 1991) and herring larvae (Bell et al. 1995). Diet deficiencies in DHA change the fatty acid composition of neural tissue in juvenile herring, associated with a decrease in efficiency to capture prey at low light intensities (Bell et al. 1995). Recent aquaculture studies emphasise that not only the amount of polyunsaturated fatty acids but also their relative composition, especially of DHA, EPA and ARA play a major role in larval requirements. This is attributed to the competitive interaction between DHA and EPA for enzymes that esterify these fatty acids onto the glycerophospho-base structure of polar lipids (Sargent et al. 1999a).

The interaction of dietary EPA and ARA levels on the other hand most likely originate from the formation of eicosanoids, which bear various physiological roles in fish metabolism. Both fatty acids can be used as precursors for eicosanoids, but they probably result in molecules with different biological activity. Larval fish size and survival were found to correlate with the ratio of DHA and EPA in the diet (Copeman et al. 2002) and despite species-specific requirements an optimal ratio of DHA:EPA:ARA was suggested to be around 10:5:1 (Sargent et al. 1999b).

**Table 1.** Fatty acid quality indicators of *Temora longicornis*, *Acartis longiremis* and *Pseudocalanus acuspes*; F = females, CV = copepodite stage V, mean = annual mean of monthly means, min = lowest monthly mean, max = highest monthly mean, FA = fatty acids, DHA = 22:6(n-3), EPA = 20:5(n-3), ARA = 20:4(n-6), PUFA = polyunsaturated fatty acids, HUFA = highly polyunsaturated fatty acids

|                                      | <i>T. longicornis</i> F |       |      | <i>A. longiremis</i> F |       |      | <i>P. acuspes</i> F |       |       | <i>P. acuspes</i> CV |       |       |
|--------------------------------------|-------------------------|-------|------|------------------------|-------|------|---------------------|-------|-------|----------------------|-------|-------|
|                                      | mean                    | min   | max  | mean                   | min   | max  | mean                | min   | max   | mean                 | min   | max   |
| Total FAs [ $\mu\text{g ind}^{-1}$ ] | 0.7                     | 0.3   | 1.3  | 0.3                    | 0.2   | 0.5  | 1.1                 | 0.9   | 1.8   | 3.5                  | 1.8   | 4.7   |
| Total FAs [% DM]                     | 7.9                     | 5.1   | 12.3 | 9.9                    | 6.2   | 14.2 | 11.2                | 9.3   | 14.3  | 32.0                 | 23.8  | 38.6  |
| DHA [% of tFAs]                      | 28.2                    | 10.9  | 35.8 | 18.9                   | 11.8  | 26.6 | 22.2                | 16.6  | 26.2  | 15.2                 | 10.6  | 20.5  |
| EPA [% of tFAs]                      | 18.1                    | 8.0   | 21.8 | 13.7                   | 9.2   | 18.8 | 15.2                | 12.4  | 19.5  | 12.0                 | 9.8   | 14.3  |
| ARA [% of tFAs]                      | -                       | < 0.5 | 1.9  | -                      | < 0.5 | 0.5  | -                   | < 0.5 | < 0.5 | -                    | < 0.5 | < 0.5 |
| DHA:EPA                              | 1.6                     | 1.1   | 2.2  | 1.4                    | 1.0   | 1.8  | 1.4                 | 1.2   | 1.7   | 1.2                  | 1.1   | 1.4   |
| PUFAs [% of tFAs]                    | 59.4                    | 26.8  | 66.7 | 54.8                   | 49.9  | 57.3 | 50.8                | 42.4  | 58.3  | 48.4                 | 45.2  | 51.1  |
| HUFAs [% of tFAs]                    | 47.2                    | 21.7  | 53.4 | 33.6                   | 21.3  | 42.7 | 37.5                | 29.5  | 44.2  | 27.2                 | 20.4  | 34.9  |

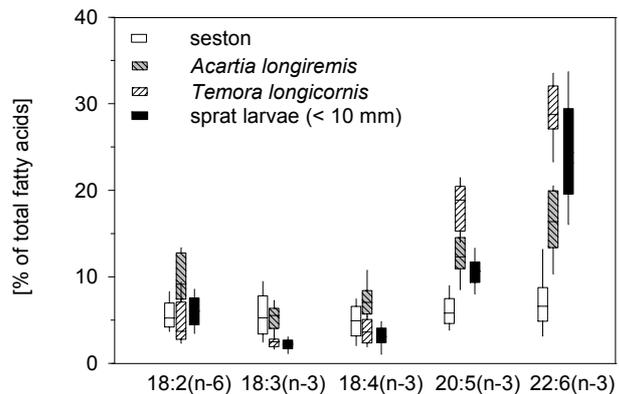
This ratio was never reached in the copepods of the Bornholm Basin (Tab. 1), although DHA levels were generally high and the DHA:EPA ratio always exceeded 1. Quality indicators of *A. longiremis* females tended to be lower than those of *T. longicornis*, especially during the important months of sprat larval development (May-July), however DHA:EPA ratios in *A. longiremis* were relatively high, ranging from 1.3 to 1.6 at that time.

A strong enrichment in relative DHA levels was observed in the Bornholm Basin from seston via females of *A. longiremis* to sprat larvae (Fig. 10). Small first-feeding larvae (<10 mm) mainly feed on microzooplankton (Dickmann 2006) and younger copepodite stages of *Acartia* spp., while larger larvae (>10 mm) select older copepodite stages (Voss et al. 2003). Hence, the quality indicators of copepod females can only serve as estimates, since they do not necessarily reflect the fatty acid composition of the younger copepodite stages or nauplii, which were most likely the major food components for the sprat larvae studied (length <10 mm). However, the apparent enrichment of DHA emphasises that sprat larvae might at least partly cover their high DHA requirements by a selective assimilation, incorporation or retention of DHA or by feeding selectively on DHA-rich food particles.

Low ARA levels in the copepods, which usually never exceeded 0.5% of total fatty acids, may be crucial for the survival or development of sprat larvae in the Bornholm Basin. Due to the lack in delta-5 desaturase activity, marine fish are not able to convert 18:2(n-6) into ARA, hence it must be provided with the diet (Sargent et al. 1997). Values of ARA in sprat larvae were generally low, never exceeding 1.3% with a mean of 0.6% of total fatty acids. An imbalance in fatty acid composition, reflected by high DHA:ARA ratios in Baltic Sea copepods, was proposed to be a reason for reproductive disturbances in Atlantic salmon (Ahlgren et al. 2005). Furthermore, significantly reduced ARA levels were found in the polar lipids of Baltic cod, when compared to oceanic stocks, and the ARA content as well as the DHA:EPA ratio in the polar lipid fraction were supposed to be important factors influencing egg viability (Pickova et al. 1997). Due to their inability to sufficiently synthesize polar lipids *de novo*, fish larvae have a strong requirement for pre-formed phospholipids (Teshima et al. 1987, as cited by Bell et al. 2003) and TAGs and phospholipids are only slightly modified during digestion and assimilation. Cod larvae in the Bornholm Basin specifically selected older copepodite stages and females of *P. acuspes* (Voss et al. 2003). The nutritional value of *P. acuspes* was relatively high, in terms of high DHA:EPA ratios in the polar lipids (mean 1.8) and elevated lipid contents of copepodids. However, ARA levels were low in both stages of *P. acuspes* and especially the concentration in the polar lipid fraction never exceeded 0.5% of fatty acids and might therefore be a crucial factor for a successful larval development.

**Figure 10.**

Relative amount of the major polyunsaturated fatty acids of seston, females of *Acartia longiremis* and *Temora longicornis* and sprat larvae (<10mm)



Beside the quality aspects of fatty acids, lipid levels of copepods, i.e. energy supply might be one factor causing the decrease in sprat condition in the Bornholm Basin (Möllmann et al. 2004). While in spring (May) the lipid content of all three copepod species was generally high, their condition strongly declined over summer as is evident from the low lipid levels of females coinciding with low egg production (chapters I and III). At that time *P. acuspes* copepodids still provided food of high energy, since they started lipid accumulation early in the year (Tab. 1). Although no data are available on the lipid content of copepodite stages of *A. longiremis* and *T. longicornis*, no strong lipid accumulation is to be expected, since they still developed and reproduced, i.e. they rather invested into growth than into energy storage (chapter III and unpublished GLOBEC data). The switch in summer-feeding of sprat from lipid-rich *P. acuspes* towards lipid-poor *T. longicornis*, in order to compensate for decreasing *P. acuspes* abundance, may deteriorate feeding conditions in addition to the decrease in food availability due to intra-specific competition (Möllmann et al. 2005).

## CONCLUSION

In the line of the “food-quality-limitation hypothesis” by Litzrow et al. (2006), a climate-induced shift in prey quality for pelagic fish apparently occurred in the Baltic Sea. Two general processes can be observed: (i) the change in the zooplankton assemblage led to a shift from a high-energy (*P. acuspes* copepodids) to a low-energy prey field (*T. longicornis* females) for adult sprat, while (ii) the altering phytoplankton assemblage potentially caused a decrease in food quality, in particular in ARA levels, with copepods functioning as mediator. The consequences for fish stock recruitment can only be assessed with difficulties, since this decrease in prey energy and quality contrasts an increase in food availability for larval fish (high *Acartia* spp. biomass) and in potentially favourable environmental conditions, thus leading to a high reproductive success of sprat.

## KEY RESULTS

---

- Food quantity (total lipid concentration) and quality (polyunsaturated fatty acids) in the seston were closely connected. Elevated lipid concentrations coincided with a high quality of food, while the variability of food quality increased with decreasing food amounts.
  - Low lipid levels in the eggs of *Temora longicornis* indicate their reduced importance as energy reserves. The fatty acid composition of eggs of *Temora longicornis* depended strongly on the seston, indicating low feeding selectivity by the females as well as a low maternal regulation in the transfer of fatty acids into the eggs.
  - The hatching success was high and independent from essential fatty acids in the eggs, while a positive correlation was observed with dietary DHA:EPA ratios. Essential fatty acids limited neither egg production nor hatching success, yet a positive correlation was observed between the relative content of 18:2(n-6) and egg production.
  - The hatching success was negatively correlated with the diatom marker 16:1(n-7) in the eggs. However, diatoms provided an important food source for *T. longicornis* in the North Sea, and the positive effect on the egg production over-compensated the only slightly reduced hatching success.
  - Diatoms were of high relevance as major food source for the production of *T. longicornis* in the North Sea. Although diatoms were less available for *T. longicornis* in the Baltic Sea, as indicated by constantly lower 16:1(n-7) levels, they served as important food components at the time of high egg production and lipid accumulation.
  - The nutritional quality of the copepods (in terms of DHA and EPA) for higher trophic levels was generally high, although below suggested optimal values for larval fish growth.
  - A significant enrichment of DHA in sprat larvae was observed.
  - Low levels of ARA in copepods emphasise that it might be a critical dietary component for development of fish larvae in the Baltic Sea.
  - The changes in the copepod assemblage led to a shift from a high-lipid prey field (*P. acuspes* dominated) for sprat to the lipid-poor prey field (dominated by *T. longicornis* and *A. longiremis*).
-

## 5. PERSPECTIVES

This thesis revealed several new aspects in the life-cycle strategies and feeding ecology of copepods in the Baltic Sea, and further knowledge on dietary factors influencing their reproductive success was gained. Yet, a lot of new questions emerged, which provide promising perspectives for future research. Their answers will be important for our understanding of food-web dynamics in the Baltic Sea. This chapter will shortly outline the most burning issues.

Different life-cycle patterns of copepods in the Bornholm Basin were revealed with an “active diapause” of *P. acuspes* copepodids early in the year, while *T. longicornis* females reduced reproduction in late autumn. In order to better understand the driving forces in population dynamics of those copepods, studies should be carried out addressing the physiological mechanisms that trigger the onset and, even more important, the termination of those resting phases. *In situ* experiments combining different food levels with abiotic factors, such as light and temperature, might be a promising approach to elucidate those triggers. This knowledge will be crucial to predict the ecological response on potentially changing climatic conditions. Lipid dynamics in *A. longiremis* did not indicate any dormancy strategy of females in winter. It will be subject to future research, whether recruitment in the following year is driven by resting egg production and whether those eggs pass through a true diapause.

Interspecific differences in the fatty acid marker composition indicated different trophic niches of copepods in the Bornholm Basin. However, those differences provide rather qualitative than quantitative information. Further calibrations will be needed to separate species-specific signatures from dietary uptake, and mono- and mixed algal feeding studies provide a suitable tool. During this thesis difficulties in interpreting some fatty acid markers arose, i.e. the denotation of the C18 polyunsaturated fatty acids in *A. longiremis* as well as the high 18:1(n-9) amounts in *P. acuspes* and the strong correlation of 18:1(n-9) levels between the species. Seasonal *in situ* grazing experiments as ground truthing for signature fatty acids will add further information on the feeding habits of those copepods in the Baltic Sea.

Insights into the role of food quality for the reproductive success of copepods probably evoke the most new questions within this thesis and will provide much potential for future studies. Our knowledge on the metabolic processes involved is still insufficient and the next

steps in evaluating the influence of specific dietary compounds will be to address assimilation efficiencies, metabolic pathways and, in particular, the physiological requirements of those compounds for successful embryogenesis, growth and reproduction. To identify mechanistic relationships, a major challenge lays in the manipulation of ideally one dietary component. Supplementation experiments, i.e. the addition of a single component to a food item, will be a first step to determine the limitation potential and the amounts needed for a successful reproduction. Adding stable isotope labelled compounds, such as triacylglycerols and phospholipids, will allow to trace the pathways of those compounds during digestion, assimilation, lipid anabolism and catabolism as well as vitellogenesis. Further insights into relevant processes in the field will be given by empirical multi-factorial approaches that include several aspects of food quality simultaneously, such as fatty acids, sterols, amino acids, C:N ratios but also vitamins as well as energy content. When combining those dietary measurements with egg composition, as performed within this thesis for fatty acids, some of the open questions may be solved.

Another topic, which could only be touched within this thesis, is the nutritional value of the different copepods for higher trophic levels. The significant enrichment of DHA as well as the low and potentially limiting ARA values indicate that limitation by food quality might be of relevance in the recruitment success of sprat. The next step will be to include larger sprat larvae into the analyses, since older stages of larvae were proposed to be a critical phase for survival in the Baltic Sea (Voss et al. 2006). To further address the hypothesis that the low condition of sprat is driven by food limitation, a next step might be to draw an energy balance within the system. Since strong differences in the lipid content between species and stages were observed, it will be of relevance to compare stage resolved energy contents of copepods with the ingested diet of sprat.

## 6. REFERENCES

- AHLGREN, G, VAN NIEUWERBURGH, L, WÄNSTRAND, I, PEDERSÉN, M, BOBERG, M, and SNOEIJIS, P (2005) Imbalance of fatty acids in the base of the Baltic Sea food web - a mesocosm study. *Canadian Journal of Fisheries and Aquatic Science* 62: 2240-2253.
- ALHEIT, J, MÖLLMANN, C, DUTZ, J, KORNILOVS, G, LOEWE, P, MOHRHOLZ, V, and WASMUND, N (2005) Synchronous ecological regime shifts in the central Baltic and North Sea in the late 1980s. *ICES Journal of Marine Science* 62: 1205-1215.
- ANTAJAN, E (2004) Responses of calanoid copepods to changes in phytoplankton dominance in the diatom - *Phaeocystis globosa* dominated Belgium coastal waters, Ph.D. thesis, Free University of Brussel. Brussel, pp. 142.
- ARENDRT, KE, JÓNASDÓTTIR, SH, HANSEN, PJ, and GÄRTNER, S (2005) Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. *Marine Biology* 146: 513-530.
- AUEL, H, KLAGES, M, and WERNER, I (2003) Respiration and lipid content of the Arctic copepod *Calanus hyperboreus* overwintering 1 m above the seafloor at 2,300 m water depth in the Fram Strait. *Marine Biology* 143: 275-282.
- BÅMSTEDT, U, HÅKANSON, JL, BRENNER-LARSEN, J, BJÖRNSEN, PK, GEERTZ-HANSEN, O, and TISELIUS, P (1990) Copepod nutritional condition and pelagic production during autumn in Kosterfjorden, western Sweden. *Marine Biology* 104: 197-208.
- BAN, S, BURNS, C, CASTEL, J, CHAUDRON, Y, CHRISTOU, E, ESCRIBANO, R, UMANI, SF, GASPARINI, S, RUIZ, FG, HOFFMEYER, M, IANORA, A, KANG, H-K, LAABIR, M, LACOSTE, A, MIRALTO, A, NING, X, POULET, S, RODRIGUEZ, V, RUNGE, J, SHI, J, STARR, M, UYE, S-I, and WANG, Y (1997) The paradox of diatom-copepod interactions. *Marine Ecology Progress Series* 157: 287-293.
- BAN, S, LEE, H-W, SHINADA, A, and TODA, T (2000) *In situ* egg production and hatching success of the marine copepod *Pseudocalanus newmani* in Funka Bay and adjacent waters off southwestern Hokkaido, Japan: associated to diatom bloom. *Journal of Plankton Research* 22: 907-922.
- BEAUGRAND, G (2003) Long-term changes in copepod abundance and diversity in the north-east Atlantic in relation to fluctuations in the hydroclimatic environment. *Fisheries Oceanography* 12: 270-283.
- BELL, MV, and DICK, JR (1991) Molecular species composition of the major diacylglycerophospholipids from muscle, liver, retina and brain of cod (*Gadus morhua*). *Lipids* 26: 565-573.
- BELL, MV, BATTY, RS, DICK, JR, FRETWELL, K, NAVARRO, JC, and SARGENT, JR (1995) Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids* 30: 443-449.
- BELL, JG, MCEVOY, LA, ESTEVEZ, A, SHIELDS, RJ, and SARGENT, JR (2003) Optimising lipid nutrition in first-feeding flatfish larvae. *Aquaculture* 227: 211-220.
- BELMONTE, G, and PUCE, M (1994) Morphological aspects of subitaneous and resting eggs from *Acartia josephinae* (Calanoida). *Hydrobiologia* 292/293: 131-135.
- BERGÉ, J-P, and BARNATHAN, G (2005) Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. *Advances in Biochemical Engineering / Biotechnology* 96: 49-125.

- BRETON, E, SAUTOUR, B, and BRYLINSKI, J-M (1999) No feeding on *Phaeocystis* sp. as solitary cells (post-bloom period) by the copepod *Temora longicornis* in the coastal waters of the English Channel. *Hydrobiologia* 414: 13-23.
- BROGLIO, E, JÓNASDÓTTIR, SH, CALBET, A, JAKOBSEN, HH, and SAIZ, E (2003) Effect of heterotrophic versus autotrophic food on feeding and reproduction of the calanoid copepod *Acartia tonsa*: relationship with prey fatty acid composition. *Aquatic Microbial Ecology* 31: 267-278.
- BUCKLIN, A, FROST, BW, BRADFORD-GRIEVE, J, ALLEN, LD, and COPLEY, NJ (2003) Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Marine Biology* 142: 333-343.
- CAIRNS, AA (1967) The zooplankton of Tanquary Fjord, Ellesmere Island, with special reference to the calanoid copepods. *Journal of Fisheries Research Board of Canada* 24: 555-568.
- CARDINALE, M, CASINI, M, and ARRHENIUS, F (2002) The influence of biotic and abiotic factors on the growth of sprat (*Sprattus sprattus*) in the Baltic Sea. *Aquatic Living Resources* 15: 273-281.
- CASTELLANI, C, and LUCAS, IAN (2003) Seasonal variation in egg morphology and hatching success of the calanoid copepods *Temora longicornis*, *Acartia clausi* and *Centropages hamatus*. *Journal of Plankton Research* 25: 527-536.
- CEBALLOS, S, and IANORA, A (2003) Different diatoms induce contrasting effects on the reproductive success of the copepod *Temora stylifera*. *Journal of Experimental Marine Biology and Ecology* 294: 189-202.
- CHIKIN, SM, TARASOVA, NA, SARALOV, AI, and BANNIKOVA, OM (2003) The distribution of bacterio- and mesozooplankton in the coastal waters of the White and Barents Seas. *Microbiology* 72: 213-220.
- CHINNERY, FE, and WILLIAMS, JA (2004) The influence of temperature and salinity on *Acartia* (Copepoda: Calanoida) nauplii survival. *Marine Biology* 145: 733-738.
- CONOVER, RJ, and HUNTLEY, M (1991) Copepods in ice-covered seas - distribution, adaptations to seasonally limited food, metabolism, growth patterns and life cycle strategies in polar seas. *Journal of Marine Systems* 2: 1-41.
- CONOVER, RJ, and SIFERD, TD (1993) Dark-season survival strategies of coastal zone zooplankton in the Canadian Arctic. *Arctic* 46: 303-311.
- COPEMAN, LA, PARRISH, CC, BROWN, JA, and HAREL, M (2002) Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. *Aquaculture* 210: 285-304.
- COTONNEC, G, BRUNET, C, SAUTOUR, N, and THOUMELIN, G (2001) Nutritive value and selection of food particles by copepods during a spring bloom of *Phaeocystis* sp. in the English Channel, as determined by pigment and fatty acid analyses. *Journal of Plankton Research* 23: 693-703.
- CROCKETT, EL, and HASSET, RP (2005) A cholesterol-enriched diet enhances egg production and egg viability without altering cholesterol content of biological membranes in the copepod *Acartia hudsonica*. *Physiological and Biochemical Zoology* 78: 424-433.
- CUSHING, DH (1972) The production cycle and the number of marine fish. *Symposia of the Zoological Society of London* 29: 213-232.

- CUSHING, DH (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in Marine Biology* 26: 249-293.
- DAALSGARD, J, ST. JOHN, M, KATTNER, G, MÜLLER-NAVARRA, D, and HAGEN, W (2003) Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* 46: 225-340.
- DAHMS, H-U (1995) Dormancy in the Copepoda - an overview. *Hydrobiologia* 306: 199-211.
- DAM, HG, and PETERSON, WT (1991) *In situ* feeding behaviour of the copepod *Temora longicornis*: effects of seasonal changes in chlorophyll size fractions and female size. *Marine Ecology Progress Series* 71: 113-123.
- DAM, HG, and LOPES, RM (2003) Omnivory in the calanoid copepod *Temora longicornis*: feeding, egg production and egg hatching rates. *Journal of Experimental Marine Biology and Ecology* 292: 119-137.
- DANKS, HV (1987) Insect dormancy: An ecological perspective. Biological Survey of Canada Monograph Series No. 1. Ottawa, pp. 439.
- DAVIS, CC (1976) Overwintering strategies of common planktic copepods in some north Norway fjords and sounds. *Astarte* 9: 37-42.
- DEMOTT, WR (1988) Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnology and Oceanography* 33: 397-408.
- DICKMANN, M (2006) Feeding ecology of sprat (*Sprattus sprattus* L.) and sardine (*Sardina pilchardus* W.) larvae in the Baltic Sea and in the North Sea, PhD thesis, Rostock University. Rostock, pp. 93.
- DIGBY, PSB (1950) The biology of the small planktonic copepods of Plymouth. *Journal of the Marine Biological Association of the United Kingdom* 29: 393-438.
- DIPPNER, JW, KORNILOVS, G, and SIDREVICIS, L (2000) Long-term variability of mesozooplankton in the central Baltic Sea. *Journal of Marine Systems* 25: 23-31.
- DUNSTAN, GA, VOLKMAN, JK, BARRETT, SM, LEROI, J-M, and JEFFREY, SW (1994) Essential polyunsaturated fatty acids from 14 species of diatoms (Bacillariophyceae). *Phytochemistry* 35: 155-161.
- DUTZ, J, MOHRHOLZ, V, PETERS, J, RENZ, J, and ALHEIT, J (2004) A strong impact of winter temperature on spring recruitment of a key copepod species in the Bornholm Basin: potential linkages to climate variability. *International GLOBEC Newsletter* 10.1: 9-10.
- EDERINGTON, MC, MCMANUS, GB, and HARVEY, HR (1995) Trophic transfer of fatty acids, sterols, and triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. *Limnology and Oceanography* 40: 860-867.
- EDLER, L (1979) Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. *The Baltic Marine Biologists Publication* 5: 1-38.
- ELGMORK, K (1980) Evolutionary aspects of diapause in freshwater copepods, 411-417. In W. C. Kerfoot (ed), Evolution and ecology of zooplankton communities. American Society of Limnology and Oceanography. Hanover.
- ELGMORK, K (1996) Variation in torpidity of diapause in freshwater cyclopoid copepods. *Hydrobiologia* 320: 63-70.
- ELGMORK, K, and NILSSEN, JP (1978) Equivalence of copepod and insect diapause. *Verhandlungen. Internationale Vereinigung für theoretische und angewandte Limnologie* 20: 2511-2517.

- ENGEL, M, and HIRCHE, HJ (2004) Seasonal variability and inter-specific differences in hatching of calanoid copepod resting eggs from sediments of the German Bight (North Sea). *Journal of Plankton Research* 26: 1083-1093.
- EVJEMO, JO, REITAN, KI, and OLSEN, Y (2003) Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossos* L.) with special emphasis on the nutritional value. *Aquaculture* 227: 191-210.
- FALK-PETERSEN, S, HOPKINS, CCE, and SARGENT, JR (1990) Trophic relationships in the pelagic, Arctic food web, 315-333. In M. Barnes and R. N. Gibson (eds), Trophic relationships in the marine environment - Proceedings of the 24th European Marine Biological Symposium. Aberdeen University Press. Aberdeen.
- FALK-PETERSEN, S, SARGENT, JR, LØNNE, OJ, and TIMOFEEV, S (1999) Functional biodiversity of lipids in Antarctic zooplankton: *Calanus acutus*, *Calanus propinquus*, *Thysanoessa macrura* and *Euphausia crystallorophias*. *Polar Biology* 21: 37-47.
- FALK-PETERSEN, S, HAGEN, W, KATTNER, G, CLARKE, A, and SARGENT, J (2000) Lipids, trophic relationships, and biodiversity in Arctic and Antarctic krill. *Canadian Journal of Fisheries and Aquatic Science* 57: 178-191.
- FEISTEL, R, NAUSCH, G, MOHRHOLZ, V, ŁYSIAK-PASTUSZAK, E, SEIFERT, T, MATTHÄUS, W, KRÜGER, S, and HANSEN, IS (2003a) Warm waters of summer 2002 in the deep Baltic Proper. *Oceanologia* 45: 571-592.
- FEISTEL, R, NAUSCH, G, MATTHÄUS, W, and HAGEN, E (2003b) Temporal and spatial evolution of the Baltic deep water renewal in spring 2003. *Oceanologica* 45: 623-642.
- FENNEL, K (1999) Convection and the timing of phytoplankton spring blooms in the western Baltic Sea. *Estuarine, Coastal and Shelf Science* 49: 113-128.
- FESSENDEN, L, and COWLES, TJ (1994) Copepod predation on phagotrophic ciliates in Oregon coastal waters. *Marine Ecology Progress Series* 107: 103-111.
- FLINKMAN, J, ARO, E, VUORINEN, I, and VIITASALO, M (1998) Changes in northern Baltic zooplankton and herring nutrition from 1980s to 1990s: top-down and bottom-up processes at work. *Marine Ecology Progress Series* 165: 127-136.
- FOLCH, J, LEES, M, and SLOANE-STANLEY, GH (1957) A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226: 497-509.
- FRANSZ, HG, COLEBROOK, JM, GAMBLE, JC, and KRAUSE, M (1991) The zooplankton of the North Sea. *Netherlands Journal of Sea Research* 28: 1-52.
- FRASER, AJ, TOCHER, DR, and SARGENT, JR (1985) Thin-layer chromatography - flame ionization detection and the quantification of marine neutral lipids and phospholipids. *Journal of Experimental Marine Biology and Ecology* 88: 91-99.
- FRASER, AJ, SARGENT, JR, and GAMBLE, JC (1989) Lipid class and fatty acid composition of *Calanus finmarchicus* (Gunnerus), *Pseudocalanus* sp. and *Temora longicornis* Muller from nutrient-enriched seawater enclosure. *Journal of Experimental Marine Biology and Ecology* 130: 81-92.
- FROST, BW (1989) A taxonomy of the marine calanoid copepod genus *Pseudocalanus*. *Canadian Journal of Zoology* 67: 525-551.
- GÓMEZ-GUITÉRREZ, J, and PETERSON, WT (1999) Egg production rates of eight calanoid copepod species during summer 1997 off Newport, Oregon, USA. *Journal of Plankton Research* 21: 637-657.

- GRAEVE, M, KATTNER, G, and HAGEN, W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. *Journal of Experimental Marine Biology and Ecology* 182: 97-110.
- GRAEVE, M, KATTNER, G, and PIEPENBURG, D (1997) Lipids in Arctic benthos: does fatty acid and alcohol composition reflect feeding and trophic interactions? *Polar Biology* 18: 53-61.
- GUGGER, M, LYRA, C, SUOMINEN, I, TSITKO, I, HUMBERT, J-F, SALKINOJA-SALONEN, MS, and SIVONEN, K (2002) Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (Cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology* 52: 1007-1015.
- GUISANDE, C, MANEIRO, I, RIVEIRO, I, BARREIRO, A, and PAZOS, Y (2002) Estimation of copepod trophic niche in the field using amino acids and marker pigments. *Marine Ecology Progress Series* 239: 147-156.
- HALSBAND, C, and HIRCHE, HJ (2001) Reproductive cycles of dominant calanoid copepods in the North Sea. *Marine Ecology Progress Series* 209: 219-229.
- HALSBAND-LENK, C, HIRCHE, H-J, and CARLOTTI, F (2002) Temperature impact on reproduction and development of congener copepod populations. *Journal of Experimental Marine Biology and Ecology* 271: 121-153.
- HALSBAND-LENK, C, CARLOTTI, F, and GREVE, W (2004) Life-history strategies of calanoid congeners under two different climate regimes: a comparison. *ICES Journal of Marine Science* 61: 709-720.
- HÄNNINEN, J, VUORINEN, I, and HJELT, P (2000) Climatic factors in the Atlantic control the oceanographic and ecological changes in the Baltic Sea. *Limnology and Oceanography* 45: 703-710.
- HARRISON, KE (1990) The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. *Journal of Shellfish Research* 9: 1-28.
- HASSET, RP (2004) Supplementation of a diatom diet with cholesterol can enhance copepod egg production rates. *Limnology and Oceanography* 49: 488-494.
- HAZZARD, SE, and KLEPPEL, GS (2003) Egg production of the copepod *Acartia tonsa* in Florida Bay: role of fatty acids in the nutritional composition of the food environment. *Marine Ecology Progress Series* 252: 199-206.
- HEERKLOSS, R, ARNDT, H, HELWIG, J, VIETINGHOFF, U, GEORDGI, F, and WESSEL, B (1984) Consumption and assimilation by zooplankton related to primary production in the Baltic coastal inlet Barther Bodden. *Limnologica* 15: 387-394.
- HELCOM (2003) The 2002 oxygen depletion event in the Kattegat, Belt Sea and Western Baltic. *Baltic Sea environment proceedings* 90: pp 65.
- HERNROTH, L, and ACKEFORS, H (1979) The zooplankton of the Baltic Proper: a long-term investigation of the fauna, its biology and ecology. *Report, Fishery Board of Sweden, Institute for Marine Research* 2: 1-160.
- HIRCHE, H-J (1983) Overwintering of *Calanus finmarchicus* and *Calanus helgolandicus*. *Marine Ecology Progress Series* 11: 281-290.
- HOLSTE, L, and PECK, MA (2006) The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): a laboratory investigation. *Marine Biology* 148: 1061-1070.

- HURRELL, JW, and DICKSON, RR (2004) Climate variability over the North Atlantic, 15-31. In N. C. Stenseth, O. G., J. W. Hurrell and A. Belgrado (eds), Marine ecosystems and climate variation - the North Atlantic. Oxford University Press. Oxford.
- IANORA, A, and BUTTINO, I (1990) Seasonal cycles in population abundances and egg production rates in the planktonic copepods *Centropages typicus* and *Acartia clausi*. *Journal of Plankton Research* 12: 473-481.
- IANORA, A, POULET, SA, and MIRALTO, A (1995) A comparative study of the inhibitory effect of diatoms on the reproductive biology of the copepod *Temora stylifera*. *Marine Biology* 121: 533-539.
- IANORA, A, POULET, SA, and MIRALTO, A (2003) The effects of diatoms on copepod reproduction: a review. *Phycologia* 42: 351-363.
- IANORA, A, MIRALTO, A, POULET, SA, CAROTENUTO, Y, BUTTINO, I, ROMANO, G, CASOTTI, R, POHNERT, G, WICHARD, T, COLUCCI-D'AMATO, L, TERRAZZANO, G, and SMETACEK, V (2004) Aldehyde suppression of copepod recruitment in blooms of a ubiquitous planktonic diatom. *Nature* 429: 403-407.
- IRIGOIEN, X, HARRIS, RP, VERHEYE, HM, JOLY, P, RUNGE, J, STARR, M, POND, D, CAMPBELL, R, SHREEVE, R, WARD, P, SMITH, AN, DAM, HG, PETERSON, W, TIRELLI, V, KOSKI, M, SMITH, T, HARBOUR, D, and DAVIDSON, R (2002) Copepod hatching success in marine ecosystems with high diatom concentrations. *Nature* 419: 387-389.
- IVERSON, SJ, FIELD, C, BOWEN, WD, and BLANCHARD, W (2004) Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs* 74: 211-235.
- JAKOBSEN, HH, HALVORSEN, E, HANSEN, BW, and VISSER, AW (2005) Effect of prey motility and concentration on feeding in *Acartia tonsa* and *Temora longicornis*: the importance of feeding modes. *Journal of Plankton Research* 27: 775-785.
- JÓNASDÓTTIR, SH (1994) Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. *Marine Biology* 121: 67-81.
- JÓNASDÓTTIR, SH, and KIØRBOE, T (1996) Copepod recruitment and food composition: do diatoms affect hatching success? *Marine Biology* 125: 743-750.
- JÓNASDÓTTIR, SH, FIELDS, D, and PANTOJA, S (1995) Copepod egg production in Long Island Sound, USA, as a function of chemical composition of seston. *Marine Ecology Progress Series* 119: 87-98.
- JÓNASDÓTTIR, SH, KIØRBOE, T, TANG, KW, ST. JOHN, M, VISSER, AW, SAIZ, E, and DAM, HG (1998) Role of diatoms in copepod production: good, harmless or toxic? *Marine Ecology Progress Series* 172: 305-308.
- JÓNASDÓTTIR, SH, GUDFINNSSON, HG, GISLASON, A, and ASTTHORSSON, OS (2002) Diet composition and quality for *Calanus finmarchicus* egg production and hatching success off south-west Iceland. *Marine Biology* 140: 1195-1206.
- JONES, RH, and FLYNN, KJ (2005) Nutritional status and diet composition affect the value of diatoms as copepod prey. *Science* 307: 1457-1459.
- KATAJISTO, T, VIITASALO, M, and KOSKI, M (1998) Seasonal occurrence and hatching of calanoid eggs in sediments of the northern Baltic Sea. *Marine Ecology Progress Series* 163: 133-143.

- KATTNER, G, and FRICKE, HSG (1986) Simple gas-liquid chromatography method for simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *Journal of Chromatography* 361: 263-268.
- KATTNER, G, and HAGEN, W (1998) Lipid metabolism of the Antarctic euphausiid *Euphausia crystallorophias* and its ecological implications. *Marine Ecology Progress Series* 170: 203-212.
- KATTNER, G, and KRAUSE, M (1989) Seasonal variations of lipids (wax esters, fatty acids and alcohols) in calanoid copepods from the North Sea. *Marine Chemistry* 26: 261-275.
- KATTNER, G, KRAUSE, M, and TRAHMS, J (1981) Lipid composition of some typical North Sea copepods. *Marine Ecology Progress Series* 4: 69-74.
- KATTNER, G, GERCKEN, G, and EBERLEIN, K (1983) Development of lipids during a spring plankton bloom in the northern North Sea I. Particulate fatty acids. *Marine Chemistry* 14: 149-162.
- KATTNER, G, GRAEVE, M, and HAGEN, W (1994) Ontogenetic and seasonal changes in lipid and fatty acid/alcohol compositions of the dominant Antarctic copepods *Calanus propinquus*, *Calanoides acutus* and *Rhincalanus gigas*. *Marine Biology* 118: 637-644.
- KIØRBOE, T (1989) Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. *Marine Ecology Progress Series* 55: 229-234.
- KIØRBOE, T, and NIELSEN, TG (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 1. Copepods. *Limnology and Oceanography* 39: 493-507.
- KIØRBOE, T, SAIZ, E, and VIITASALO, M (1996) Prey switching behaviour in the planktonic copepod *Acartia tonsa*. *Marine Ecology Progress Series* 143: 65-75.
- KLEIN BRETELER, WCM, and GONZALEZ, SR (1988) Influence of temperature and food concentration on body size, weight and lipid content of two calanoid copepod species. *Hydrobiologia* 167/168: 201-210.
- KLEIN BRETELER, WCM, SCHOGT, N, and GONZALEZ, SR (1990) On the role of food quality in grazing and development of life stages, and genetic change of body size during cultivation of pelagic copepods. *Journal of Experimental Marine Biology and Ecology* 135: 177-189.
- KLEIN BRETELER, WCM, KOSKI, M, and RAMPEN, S (2004) Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate. *Marine Ecology Progress Series* 274: 199-208.
- KLEKOWSKI, RZ, and WESLAWSKI, JM (1990) Atlas of the marine fauna of southern Spitsbergen. Invertebrates part 1, vol.II, pp. 97-103.
- KLEPPEL, GS, HOLLIDAY, DV, and PIEPER, RE (1991) Trophic interactions between copepods and microplankton: a question about the role of diatoms. *Limnology and Oceanography* 36: 172-178.
- KLEPPEL, GS, BURKART, CA, and HOUCHIN, L (1998) Nutrition and regulation of egg production in the calanoid copepod *Acartia tonsa*. *Limnology and Oceanography* 43: 1000-1007.
- KOSKI, M, DUTZ, J, and KLEIN BRETELER, WCM (2005) Selective grazing of *Temora longicornis* in different stages of a *Phaeocystis globosa* bloom - a mesocosm study. *Harmful Algae* 4: 915-927.

- KOSOBOKOVA, KN, HANSEN, H, HIRCHE, H-J, and KNICKMEIER, K (1998) Composition and distribution of zooplankton in the Laptev Sea and adjacent Nansen Basin during summer, 1993. *Polar Biology* 19: 63-76.
- KÖSTER, FW, and MÖLLMANN, C (2000) Trophodynamic control by clupeid predators on recruitment success in Baltic cod? *ICES Journal of Marine Science* 57: 310-323.
- KÖSTER, FW, HINRICHSSEN, HH, SCHNACK, D, ST. JOHN, MA, MACKENZIE, BR, TOMKIEWICZ, J, MÖLLMANN, C, KRAUS, G, PLIKSHS, M, MAKARCHOUK, A, and ARO, E (2003) Recruitment of Baltic cod and sprat stocks: identification of critical life stages and incorporation of environmental variability into stock-recruitment relationships. *Scientia marina* 67: 129-154.
- KOZLOWSKY-SUZUKI, B, CARLSSON, P, RÜHL, A, and GRANÉLI, E (2006) Food selectivity and grazing impact on toxic *Dinophysis* spp. by copepods feeding on natural plankton assemblages. *Harmful Algae* 5: 57-88.
- KRAUSE, M, DIPPNER, JW, and BEIL, J (1995) A review of hydrographic controls on the distribution of zooplankton biomass and species in the North Sea with particular reference to a survey conducted in January-March 1987. *Progress in Oceanography* 35: 81-152.
- KWASNIEWSKI, S, HOP, H, FALK-PETERSEN, S, and PEDERSEN, G (2003) Distribution of *Calanus* species in Kongsfjorden, a glacial fjord in Svalbard. *Journal of Plankton Research* 25: 1-20.
- LACOSTE, A, POULET, SA, CUEFF, A, KATTNER, G, IANORA, A, and LAABIR, M (2001) New evidence of the copepod maternal food effects on reproduction. *Journal of Experimental Marine Biology and Ecology* 259: 85-107.
- LEE, RF (1975) Lipids of Arctic zooplankton. *Comparative Biochemistry Physiology* 51B: 263-266.
- LEE, RF, NEVENZEL, JC, and PAFFENHÖFER, G-A (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. *Marine Biology* 9: 99-108.
- LEE, RF, HAGEN, W, and KATTNER, G (2006) Lipid storage in marine zooplankton. *Marine Ecology Progress Series* 307: 273-306.
- LEVINSEN, H, TURNER, JT, NIELSEN, TG, and HANSEN, BW (2000) On the trophic coupling between protists and copepods in Arctic marine ecosystems. *Marine Ecology Progress Series* 204: 65-77.
- LINDLEY, JA (1990) Distribution of overwintering calanoid copepod eggs in sea-bed sediments around southern Britain. *Marine Biology* 104: 209-217.
- LISCHKA, S, and HAGEN, W (2005) Life histories of the copepods *Pseudocalanus minutus*, *P. acuspes* (Calanoida) and *Oithona similis* (Cyclopoida) in the Arctic Kongsfjorden (Svalbard). *Polar Biology* 28: 910-921.
- LITZOW, MA, BAILEY, KM, PRAHL, FG, and HEINTZ, R (2006) Climate regime shifts and reorganization of fish communities: the essential fatty acid limitation hypothesis. *Marine Ecology Progress Series* 315: 1-11.
- LUKASHIN, VN, KOSOBOKOVA, KN, SHEVCHENKO, VP, SHAPIRO, GI, PANTYULIN, AN, PERTZOVA, NM, DEEV, MG, KLYUVITKIN, AA, NOVIGATSKII, AN, SOLOV'EV, KA, PREGO, R, and LATSHE, L (2003) Results of multidisciplinary oceanographic studies in the White Sea in June 2000. *Oceanology* 43: 224-239.

- MADHUPRATAP, M, NEHRING, S, and LENZ, J (1996) Resting eggs of zooplankton (Copepoda and Cladocera) from the Kiel Bay and adjacent waters (southwestern Baltic). *Marine Biology* 125: 77-87.
- MARCUS, NH (1990) Calanoid copepod, cladoceran, and rotifer eggs in sea-bottom sediments of northern Californian coastal waters: identification, occurrence and hatching. *Marine Biology* 105: 413-418.
- MARSHALL, SM (1949) On the biology of the small copepods in Loch Striven. *Journal of the Marine Biological Association of the United Kingdom* 28: 45-122.
- MATTHÄUS, W, and SCHINKE, H (1994) Mean atmospheric circulation patterns associated with major Baltic inflows. *Deutsche hydrographische Zeitschrift* 46: 321-339.
- MAUCLINE, J (1998) The biology of calanoid copepods. *Advances in Marine Biology* 33: 1-710.
- MCLAREN, IA, LABERGE, E, CORKETT, CJ, and SÉVIGNY, J-M (1989) Life cycles of four species of *Pseudocalanus* in Nova Scotia. *Canadian Journal of Zoology* 67: 552-558.
- MILLER, CB, MORGAN, CA, PRAHL, FG, and SPARROW, MA (1998) Storage lipids of the copepod *Calanus finmarchicus* from Georges Bank and the Gulf of Maine. *Limnology and Oceanography* 43: 488-497.
- MIRALTO, A, BARONE, G, ROMANO, G, POULET, SA, IANORA, A, RUSSO, GL, BUTTINO, I, MAZZARELLA, G, LAABIR, M, CABRINI, M, and GIACOBBE, MG (1999) The insidious effect of diatoms on copepod reproduction. *Nature* 402: 173-176.
- MIRALTO, A, GUGLIELMO, L, ZAGAMI, G, BUTTINO, I, GRANATA, A, and IANORA, A (2003) Inhibition of population growth in the copepods *Acartia clausi* and *Calanus helgolandicus* during diatom blooms. *Marine Ecology Progress Series* 254: 253-268.
- MÖLLMANN, C, KORNILOVS, G, and SIDREVICS, L (2000) Long-term dynamics of main mesozooplankton species in the central Baltic Sea. *Journal of Plankton Research* 22: 2015-2038.
- MÖLLMANN, C, KORNILOVS, G, FETTER, M, KÖSTER, FW, and HINRICHSEN, H-H (2003) The marine copepod *Pseudocalanus elongatus* as a mediator between climate variability and fisheries in the Central Baltic Sea. *Fisheries Oceanography* 12: 360-368.
- MÖLLMANN, C, KORNILOVS, G, FETTER, M, and KÖSTER, FW (2004) Feeding ecology of central Baltic Sea herring and sprat. *Journal of Fish Biology* 65: 1563-1581.
- MÖLLMANN, C, KORNILOVS, G, FETTER, M, and KÖSTER, FW (2005) Climate, zooplankton, and pelagic fish growth in the central Baltic Sea. *ICES Journal of Marine Science* 62: 1270-1280.
- MURATA, N, and NISHIDA, I (1987) Lipids of blue-green algae (Cyanobacteria), 315-347. In P. K. Stumpf (ed), *The biochemistry of plants*. Academic Press. New York.
- NICHOLS, DS, NICHOLS, PD, and SULLIVAN, CW (1993) Fatty acid, sterol and hydrocarbon composition of Antarctic sea ice diatom communities during the spring bloom in McMurdo Sound. *Antarctic Science* 5: 271-278.
- NISSLING, A (2004) Effects of temperature on egg and larval survival of cod (*Gadus morhua*) and sprat (*Sprattus sprattus*) in the Baltic Sea - implications for stock development. *Hydrobiologia* 514: 115-123.
- NORRIN, MF (1991) Gonad maturation as an indication of seasonal cycles for several species of small copepods in the Barents Sea. In E. Sakshaug, C. C. E. Hopkins and N. A.

- Øritsland (eds), Proceedings of the Pro Mare Symposium on Polar Marine Ecology, Trondheim, 12-16 May 1990. *Polar Research* 10 (2): 421-432.
- NORRBIN, F (1992) Overwintering strategies of small copepods in high latitude marine environments, Dissertation, University of Gothenburg. Gothenburg, pp. 129.
- NORRBIN, MF (1994) Seasonal patterns in gonad maturation, sex ratio and size in some small, high-latitude copepods: implications for overwintering tactics. *Journal of Plankton Research* 16: 115-131.
- NORRBIN, MF (1996) Timing of diapause in relation to the onset of winter in the high-latitude copepods *Pseudocalanus acuspes* and *Acartia longiremis*. *Marine Ecology Progress Series* 142: 99-109.
- NORRBIN, F (2001) Ultra-structural changes in the reproductive system of overwintering females of *Acartia longiremis*. *Marine Biology* 139: 697-704.
- NORRBIN, MF, OLSEN, R-E, and TANDE, KS (1990) Seasonal variation in lipid class and fatty acid composition of two small copepods in Balsfjorden, northern Norway. *Marine Biology* 105: 205-211.
- PASCAL, JC, and ACKMAN, RG (1976) Long chain monoethylenic alcohol and acid isomers in lipids of copepods and capelin. *Chemistry and Physics of Lipids* 16: 219-223.
- PERTSOVA, NM (1981) Number of generations and their span in *Pseudocalanus elongatus* (Copepoda, Calanoida) in the White Sea (in Russian). *Journal of Zoology* 60: 673-684.
- PERTSOVA, NM (1990) On the ecology of the boreal copepods *Centropages hamatus* and *Temora longicornis* in the White Sea and within its habitats (in Russian). *Biologicheskije resursy Belogo morya (Biological Resources of the White Sea)*: 80-92.
- PETERS, J, TUSCHLING, K, and BRANDT, A (2004) Zooplankton in the arctic Laptev Sea—feeding ecology as indicated by fatty acid composition. *Journal of Plankton Research* 26: 227-234.
- PETERSON, WT, and KIMMERER, WJ (1994) Processes controlling recruitment of the marine calanoid copepod *Temora longicornis* in Long Island Sound: egg production, egg mortality, and cohort survival rates. *Limnology and Oceanography* 39: 1594-1605.
- PETERSON, WT, TISELIUS, P, and KIØRBOE, T (1991) Copepod egg production, moulting and growth rates, and secondary production, in the Skagerrak in August 1988. *Journal of Plankton Research* 13: 131-154.
- PETERSON, WT, GÓMEZ-GUITÉRREZ, J, and MORGAN, CA (2002) Cross-shelf variation in calanoid copepod production during summer 1996 off the Oregon coast, USA. *Marine Biology* 141: 353-365.
- PHLEGER, CF, NELSON, MM, MOONEY, BD, and NICHOLS, PD (2002) Interannual and between species comparison of the lipids, fatty acids and sterols of Antarctic krill from the US AMLR Elephant Island survey area. *Comparative Biochemistry and Physiology Part B* 131: 733-747.
- PICKOVA, J, DUTTA, PC, LARSSON, P-O, and KIESSLING, A (1997) Early embryonic cleavage pattern, hatching success, and egg-lipid fatty acid composition: comparison between two cod (*Gadus morhua*) stocks. *Canadian Journal of Fisheries and Aquatic Science* 54: 2410-2416.
- POHNERT, G, LUMINEAU, O, CUEFF, A, ADOLPH, S, CORDEVANT, C, LANGE, M, and POULET, S (2002) Are volatile unsaturated aldehydes from diatoms the main line of chemical defence against copepods? *Marine Ecology Progress Series* 245: 33-45.

- POND, D, HARRIS, R, HEAD, R, and HARBOUR, D (1996) Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plymouth, UK. *Marine Ecology Progress Series* 143: 45-63.
- POULET, SA, IANORA, A, MIRALTO, A, and MEIJER, L (1994) Do diatoms arrest embryonic development in copepods? *Marine Ecology Progress Series* 111: 79-86.
- POULET, SA, LAABIR, M, IANORA, A, and MIRALTO, A (1995) Reproductive response of *Calanus helgolandicus*. I. Abnormal embryonic and naupliar development. *Marine Ecology Progress Series* 129: 85-95.
- POULET, SA, WICHARD, T, LEDOUX, JB, LEBRETON, B, MARCHETTI, J, DANCIE, C, BONNET, D, CUEFF, A, MORLIN, P, and POHNERT, G (2006) Influence of diatoms on copepod reproduction. I. Field and laboratory observations related to *Calanus helgolandicus* egg production. *Marine Ecology Progress Series* 308: 129-142.
- PUTT, D, and STOECKER, DK (1989) An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnology and Oceanography* 34: 1097-1103.
- REID, PC, HOLLIDAY, NP, and SMYTH, TJ (2001) Pulses in the eastern margin current and warmer water off the north west European shelf linked to North Sea ecosystem changes. *Marine Ecology Progress Series* 215: 283-287.
- RENZ, J (2006) Life-cycle and population dynamics of the calanoid copepod *Pseudocalanus* spp. in the Baltic Sea and North Sea, PhD thesis, Bremen University. Bremen, pp. 150.
- RENZ, J, and HIRCHE, H-J (2006) Life cycle of *Pseudocalanus acuspes* Giesbrecht (Copepoda, Calanoida) in the Central Baltic Sea: I. Seasonal and spatial distribution. *Marine Biology* 148: 567-580.
- REUSS, N, and POULSEN, LK (2002) Evaluation of fatty acids as biomarkers for a natural plankton community. A field study of a spring bloom and a post-bloom period off West Greenland. *Marine Biology* 141: 423-434.
- ROWLEY, AF, VOGAN, CL, TAYLOR, GW, and CLARE, AS (2005) Prostaglandins in non-insectan invertebrates: recent insights and unsolved problems. *The Journal of Experimental Biology* 208: 3-14.
- RUNGE, JA, and INGRAM, RG (1991) Under-ice feeding and diel migration by the planktonic copepods *Calanus glacialis* and *Pseudocalanus minutus* in relation to the ice algal production cycle in southeastern Hudson Bay, Canada. *Marine Biology* 108: 217-225.
- SARGENT, JR, and FALK-PETERSEN, S (1981) Ecological investigations on the zooplankton community in Balsfjorden, Northern Norway: lipids and fatty acids in *Meganyctiphanes norvegica*, *Thysanoessa raschi* and *T. inermis* during mid-winter. *Marine Biology* 62: 131-137.
- SARGENT, JR, and FALK-PETERSEN, S (1988) The lipid biochemistry of calanoid copepods. *Hydrobiologia* 167/168: 101-114.
- SARGENT, JR, and HENDERSON, RJ (1986) Lipids, 59-108. In E. D. S. Corner and S. C. M. O'Hara (eds), *The biological chemistry of marine copepods*. Clarendon Press. Oxford.
- SARGENT, JR, and WHITTLE, KJ (1981) Lipids and hydrocarbons in the marine food web, 491-533. In A. R. Longhurst (ed), *Analysis of marine ecosystems*. Academic Press. San Diego.

- SARGENT, JR, PARKES, RJ, MUELLER-HARVEY, I, and HENDERSON, RJ (1987) Lipid biomarkers in marine ecology, 119-138. *In* M. A. Sleigh (ed), *Microbes in the sea*. Ellis Horwood. Chichester.
- SARGENT, J, MCEVOY, L, and BELL, JG (1997) Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture* 155: 117-127.
- SARGENT, J, BELL, G, MCEVOY, L, TOCHER, D, and ESTEVEZ, A (1999a) Recent developments in the essential fatty acid nutrition of fish. *Aquaculture* 177: 191-199.
- SARGENT, J, MCEVOY, L, ESTEVEZ, A, BELL, G, BELL, M, HENDERSON, J, and TOCHER, D (1999b) Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* 179: 217-229.
- SCHLITZER, R (2002) Ocean Data View. <http://www.awi-bremerhaven.de/GEO/ODV>.
- SCHMIDT, J, MÖLLMANN, C, TEMMING, A, HERRMANN, J-P, FLOETER, J, SELL, A, and ST. JOHN, MA (2003) Unusual water mass advection affected Central Baltic key species 2: *Pseudocalanus* and the winter inflow. *International GLOBEC Newsletter* 9.2: 28-29.
- SCHMIDT, K, and JÓNASDÓTTIR, SH (1997) Nutritional quality of two cyanobacteria: How rich is 'poor' food? *Marine Ecology Progress Series* 151: 1-10.
- SCHNACK, S (1975) On the feeding biology of copepods (Crustacea) in Kiel Bay (in German), PhD thesis, Christian-Albrechts University. Kiel, pp. 141.
- SHAPORENKO, SI, KORENEVA, GA, PANTYULIN, AN, and PERTSOVA, NM (2005) Characteristics of the ecosystems of water bodies separating from Kandalaksha Bay of the White Sea. *Water Resources* 32: 469-483.
- SHIN, K, JANG, M-C, JANG, P-K, JU, S-J, LEE, T-K, and CHANG, M (2003) Influence of food quality on egg production and viability of the marine planktonic copepod *Acartia omorii*. *Progress in Oceanography* 57: 265-277.
- SIFERD, TD, and CONOVER, RJ (1992) Natural history of ctenophores in the Resolute Passage area of the Canadian High Arctic with special reference to *Mertensia ovum*. *Marine Ecology Progress Series* 86: 133-144.
- ST JOHN, MA, and LUND, T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. *Marine Ecology Progress Series* 131: 75-85.
- STARR, M, RUNGE, JA, and THERRIAULT, J-C (1999) Effects of diatom diets on the reproduction of the planktonic copepod *Calanus finmarchicus*. *Sarsia* 84: 379-389.
- STERNER, RW, and SCHULZ, K (1998) Zooplankton nutrition: recent progress and reality check. *Aquatic Ecology* 32: 261-279.
- STIGEBRANDT, A, and GUSTAFSSON, BG (2003) Response of the Baltic Sea to climate change – theory and observations. *Journal of Sea Research* 49: 243-256.
- STØTTRUP, JG, and JENSEN, J (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. *Journal of Experimental Marine Biology and Ecology* 141: 87-105.
- STØTTRUP, JG, BELL, JG, and SARGENT, JR (1999) The fate of lipids during development and cold-storage of eggs in the laboratory-reared calanoid copepod, *Acartia tonsa* Dana, and in response to different algal diets. *Aquaculture* 176: 257-269.
- STÜBING, D, and HAGEN, W (2003) Fatty acid biomarker ratios-suitable trophic indicators in Antarctic euphausiids? *Polar Biology* 26: 774-782.

- TANG, KW, and TAAL, M (2005) Trophic modification of food quality by heterotrophic protists: species-specific effects on copepod egg production and egg hatching. *Journal of Experimental Marine Biology and Ecology* 318: 85-98.
- TESHIMA, S, KANAZAWA, A, HORINOCHI, K, YAMASAKI, S, and HIRATA, H (1987) Phospholipids of the rotifer, prawn and larval fish. *Nippon Suisan Gakkaishi* 53: 609-615.
- TISELIUS, P, and JONSSON, PR (1990) Foraging behaviour of six calanoid copepods: observations and hydrodynamic analysis. *Marine Ecology Progress Series* 66: 23-33.
- UYE, S-I (1980) Development of neritic copepods *Acartia clausi* and *A. steueri*. I. Some environmental factors affecting egg development and the nature of resting eggs. *Bulletin of Plankton Society of Japan* 27: 1-9.
- UYE, S-I (1982) Population dynamics and production of *Acartia clausi* Giesbrecht (Copepoda: Calanoida) in inlet waters. *Journal of Experimental Marine Biology and Ecology* 57: 55-83.
- UYE, S-I (1996) Induction of reproductive failure in the planktonic copepod *Calanus pacificus* by diatoms. *Marine Ecology Progress Series* 133: 89-97.
- VARGAS, MA, RODRÍGUEZ, H, MORENO, J, OLIVARES, H, DEL CAMPO, JA, RIVAS, J, and GUERRERO, MG (1998) Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. *Journal of Phycology* 34: 812-817.
- VELOZA, AJ, CHU, F-LE, and TANG, KW (2005) Trophic modification of essential fatty acids by heterotrophic protists and its effects on the fatty acid composition of the copepod *Acartia tonsa*. *Marine Biology* 148: 779-788.
- VIITASALO, M (1992) Calanoid resting eggs in the Baltic Sea: implications for the population dynamics of *Acartia bifilosa* (Copepoda). *Marine Biology* 114: 397-405.
- VINCENT, D, and HARTMANN, HJ (2001) Contribution of ciliated microprotozoans and dinoflagellates to the diet of three copepod species in the Bay of Biscay. *Hydrobiologia* 443: 193-204.
- VOSS, R, KÖSTER, FW, and DICKMANN, M (2003) Comparing the feeding habits of co-occurring sprat (*Sprattus sprattus*) and cod (*Gadus morhua*) larvae in the Bornholm Basin, Baltic Sea. *Fisheries Research* 63: 97-111.
- VOSS, R, CLEMMESSEN, C, BAUMANN, H, and HINRICHSEN, HH (2006) Baltic sprat larvae: coupling food availability, larval condition and survival. *Marine Ecology Progress Series* 308: 243-254.
- WASMUND, N, and UHLIG, S (2003) Phytoplankton trends in the Baltic Sea. *ICES Journal of Marine Science* 60: 177-186.
- WASMUND, N, NAUSCH, G, and MATTHÄUS, W (1998) Phytoplankton spring blooms in the southern Baltic Sea - spatio-temporal development and long-term trends. *Journal of Plankton Research* 20: 1099-1117.
- WERNER, I, and AUDEL, H (2004) Environmental conditions and overwintering strategies of planktonic metazoans in and below coastal fast ice in the Gulf of Finland (Baltic Sea). *Sarsia* 89: 102-116.
- YEAGLE, PL (1989) Lipid regulation of cell membrane structure and function. *The FASEB Journal* 3: 1833-1842.



## CHAPTER I

Trophodynamics and seasonal cycle of the copepod *Pseudocalanus acuspes* in the Central Baltic Sea (Bornholm Basin):  
evidence from lipid composition.

Peters J, Renz J, van Beusekom J, Boersma M, Hagen W

published in *Marine Biology* (2006)

DOI 10.1007/s00227-006-0290-8.



## **TROPHODYNAMICS AND SEASONAL CYCLE OF THE COPEPOD *PSEUDOCALANUS ACUSPES* IN THE CENTRAL BALTIC SEA (BORNHOLM BASIN) – EVIDENCE FROM LIPID COMPOSITION**

### **Abstract**

Seasonal lipid dynamics of the copepod *Pseudocalanus acuspes* were studied in the Bornholm Basin (Central Baltic Sea) on a monthly basis from March 2002 until March 2003 and were interpreted in light of life cycle strategies and diet selection. The individual total lipid content of females ranged from 0.9 to 1.8 µg, with relative wax ester content reaching a significant maximum in May (44% of total lipids) and minimum (17% of total lipids) in April and November. Significant changes in size, lipid content, lipid classes and fatty acid composition of structural as well as storage lipids suggested five characteristic seasonal phases that were induced by different feeding histories and environmental conditions. Storage lipids were characterized by 18:1(n-9) as major component, which ranged between 44% of total fatty acids in June and 23% in February. The strong coherence between 18:1(n-9) in the seston lipids and the occurrence of ciliates emphasized the importance of ciliates in the diet of *P. acuspes*. As indicated by changes in the amounts of fatty acid markers, other food sources varied over the year, suggesting an opportunistic feeding behavior. The spring period was characterized by an increase in typical diatom and dinoflagellate markers, whereas other sources, potentially cyanobacteria, became more important during summer. The life cycle strategy is discussed with respect to extant adaptations to high latitudinal habitats.

### **Introduction**

*Pseudocalanus acuspes* is a key species in the Central Baltic Sea, as it serves as a major food organism for larval as well as for adult planktivorous fish (Hinrichsen et al. 2002, 2003, Möllmann and Köster 1999, 2002, Möllmann et al. 2003). Knowledge about the processes regulating population dynamics of *P. acuspes* in the Baltic Sea is essential to understand the principal mechanisms accounting for the high variability of copepod production and reproductive success of fish, which is a main focus of the German GLOBEC project.

*P. acuspes* mainly inhabits high latitudes (Frost 1989, Runge and Ingram 1991, Siferd and Conover 1992, Norrbin 1996) and due to its absence in the adjacent North Sea (Bucklin et al. 2003) and wide distribution in the Arctic, it is most likely a member of the Baltic glacial relict fauna. Different life cycles were described for *Pseudocalanus* spp. in high Arctic regions: from biennial (Cairns 1967) and annual cycles (Davis 1976, Conover and Siferd 1993, Lischka and Hagen 2005) up to cycles with two or more generations per year (Pertsova

1981, McLaren et al. 1989, Norrbin 1992). In temperate regions several generations per year are commonly observed (Marshall 1949, Digby 1950).

As a characteristic of the Baltic Sea, adult females of *P. acuspes* are more abundant in water layers below the thermocline and often concentrate near the halocline, presumably induced by the strong vertical stratification of the water column (Hernroth and Ackefors 1979, Hernroth 1985, Renz and Hirche 2006). Hence, sinking algae, detritus or microzooplankton are most likely the only available food sources. Feeding and growth conditions might therefore be suboptimal for this originally marine species (Renz and Hirche 2006) in the temperate brackish environment, with seasonal cycle and diet differing from those of other habitats.

Valuable information on the life cycle and overwintering strategy of *P. acuspes* in the Baltic Sea can be derived from seasonal dynamics in storage lipid content and fatty acid composition of polar, i.e. structural lipids as well as from size variations, as these attributes reflect environmental conditions and food supply during growth of different cohorts.

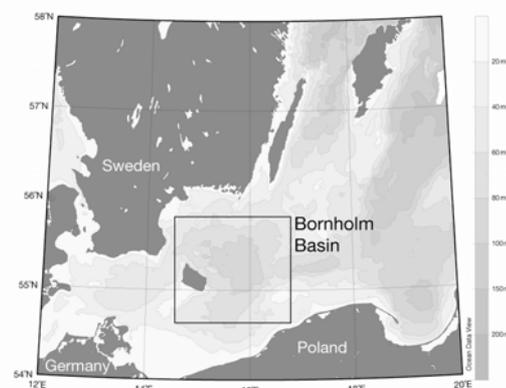
Studies on in situ grazing rates and food selection of *Pseudocalanus* spp. are scarce. It has been described that *Pseudocalanus* spp. exhibits a primarily herbivorous feeding behavior (e.g. Schnack 1975, Corkett and McLaren 1978, Fraser et al. 1989, Cotonnec et al. 2001), whereas other studies suggested a more omnivorous feeding mode (Båmstedt et al. 1990, Norrbin et al. 1990, Peters et al. 2004). To elucidate seasonal dynamics in diet we applied signature fatty acids to identify trophic relationships. We specifically focused on the fatty acid composition of storage, i.e. neutral lipids, in order to obtain unambiguous signals.

The use of specific fatty acids to characterize feeding on different taxonomic groups is well established, e.g. the assignment of 16:1(n-7) and 20:5(n-3) to diatoms (Nichols et al. 1993, Dunstan et al. 1994, Skerrat et al. 1995) and 18:4(n-3) and 22:6(n-3) to dinoflagellates (Sargent et al. 1987, Graeve et al. 1994). However, it is essential to validate those results in the studied ecosystem by comparing fatty acid profiles of the seston with its taxonomic composition. Beside the fatty acid dynamics in the neutral lipids of the copepods, we therefore provide data on the seasonal variation of the seston composition to reveal seasonal changes in the diet of *P. acuspes*.

## Materials and methods

### *Sampling and experiments*

Zooplankton and seston samples were collected in approximately monthly intervals from March 2002 until March 2003 (except for October and December) on eleven cruises in the Bornholm Basin (Fig. 1). To provide representative data for the whole basin, both stations in central and in marginal areas were sampled on



**Figure 1.**

Map of investigation area, generated with ODV software (Schlitzer 2005)

each cruise and combined in average values for each month, except for January and February 2003, where only samples from the central basin were available.

Zooplankton was sampled using a WP-2 net with a 10 l bucket end (vertically towed with 0.2 m/s, mesh size 200  $\mu\text{m}$ , 0.26 m<sup>2</sup> opening). Sampling depths were adjusted to hydrography covering the water column from the lower halocline up to the surface. Copepods were sorted on board under ambient temperature conditions into -80 °C pre-cooled glass vials. Depending on availability each sample consisted of 20 to 150 adult females of *Pseudocalanus acuspes* or copepodite stages V (CV), respectively. On three stations of each cruise prosoma lengths of 30 females were measured using formalin preserved samples (4% in seawater).

Seston samples from five depths were taken with 10 l water sampler bottles. Vertical resolution was adapted to the hydrographic structure of the water column, with samples taken from the upper water layer (5 m), from above the thermocline (10 m), from the midwater layer, from above the halocline and in the halocline. Depending on seston concentrations two to six liters of water were filtered with low pressure (-200 mbar) on precombusted (12 h at 400 °C) GF/C filters. All zooplankton organisms were carefully removed under the stereomicroscope immediately after filtration and prior to freezing, so that they did not bias the seston data. Zooplankton samples and filters were permanently stored at -80°C until further analysis.

For analyses of taxonomic seston composition aliquots of 100 ml were taken from water sampler bottles, preserved with 2% acid Lugol's solution and stored cool and dark until further investigation. Samples were analyzed using Uthermöhl microscopy and phytoplankton as well as protozooplankton cell size was converted to biomass according to Edler (1979) and Putt and Stoecker (1989), respectively.

### *Analytics*

After lyophilization dry mass of copepods was determined using a Sartorius micro-balance (+/- 2 $\mu\text{g}$ ). During weighing procedure, samples were temporarily stored in a vacuum desiccator to prevent unequal condensation on the tissue. Lipid extraction was performed with minor modifications as described in Folch et al. (1957) using ultrasonic disruption in dichloromethane:methanol (2:1/v:v) and a washing procedure with aqueous KCl solution (0.88%). For quantification of fatty acids, tricosanoic acid was added as an internal standard prior to extraction.

Lipid classes were separated by solid phase extraction, using 1 ml SiOH glass columns (CHROMABOND®, Macherey-Nagel) on a vacuum manifold. To remove residues the columns were washed with a solvent sequence of acetone, diethylether, and hexane:diethylether-mixtures, prior to sample load. After column conditioning with 4 ml of hexane, 4  $\mu\text{l}$  of lipid extract (lipid concentration approx. 5  $\mu\text{g}/\mu\text{l}$ ) were added. The neutral lipid fraction was washed out with 2.5 ml hexane:diethylether (95:5/v:v) and 2.5 ml hexane:diethylether (1:1/v:v). Polar lipids were eluted with 2.5 ml methanol and subsequently

5 ml of dichloromethane were added. The polar fraction was then washed with 2 ml aqueous KCl solution (0.88%).

For fatty acid analyses a subsample of total lipids as well as the total neutral and polar lipid fraction were hydrolyzed and fatty acids were converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulfuric acid at 80 °C for 4 h (Kattner and Fricke 1986). After cooling, 2 ml of Aqua bidest. were added, and FAMEs were extracted three times with 1 ml hexane. Samples were analyzed using a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) operated with a temperature program and helium as carrier gas. Samples were injected using a hot split/splitless inlet (250 °C, split mode 1:20) or a programmable temperature vaporizer injector (solvent vent mode). The FAMEs and fatty alcohols were detected by flame ionization and identified by comparing retention times with those derived from standards of known composition. The accurate identification of the substances was checked for selected peaks using GC MS.

#### *Calculations and statistical analyses*

The proportions of wax esters (WE), triacylglycerols (TAG) and polar lipids (PL) were estimated based on comparisons of the relative fatty acid and alcohol composition of the neutral lipid fraction and the total lipid extract, whereas the composition of the polar lipid fraction was used to verify the results. Taking the non-fatty acid components into account, the usually dominating phosphatidylcholine was assumed to be the only polar lipid component and the corresponding mass ratio was used. However, this method does not account for sterol esters and cholesterol. Furthermore, the WE content was calculated based on the alcohol content in the total lipid extract.

All statistical analyses were performed using the software SPSS. For all statistical operations that require normal distribution, percentage data (e.g. relative fatty acid composition) were transformed using an arc sine square root transformation. Normal distribution and homogeneity of variances were checked using the Shapiro-Wilk- and the Levene-test, respectively, according to sample size. For identification of coherences between fatty acid markers and seston taxa, as well as within the fatty acids of seston and storage lipids of *P. acuspes* females, principal component analyses (PCAs) were performed on the correlation matrix, extracting non-rotated components with eigenvalues >1. Relevant variables (i.e. length, biomass, total and storage lipid content) were analyzed using one-way ANOVA followed by a Tukey's HSD test for post-hoc comparisons with time as independent variable.

To detect seasonal changes between fatty acid compositions in the neutral and the polar lipid fraction of females, the relative amount of each fatty acid was tested between two adjacent months using a Student's T-test. If the difference between two months was only due to one fatty acid on a significance level of  $p < 0.01$  or two fatty acids on a significance level of  $p < 0.05$ , the months were fused to one group. Afterwards, these groups were tested against

each other. For months with less than three replicates, i.e. January and February, the months were assigned to the group with the highest similarity in a cluster analysis using the PRIMER software (based on Bray-Curtis similarity and complete linkage cluster mode, data not presented). Selectivity, here understood as ratio between availability of individual fatty acids in the seston and the incorporation into the storage lipids of *P. acuspes*, was calculated as ratio between their relative content in the seston and in the neutral lipids of the copepods using a logarithmic scale.

## Results

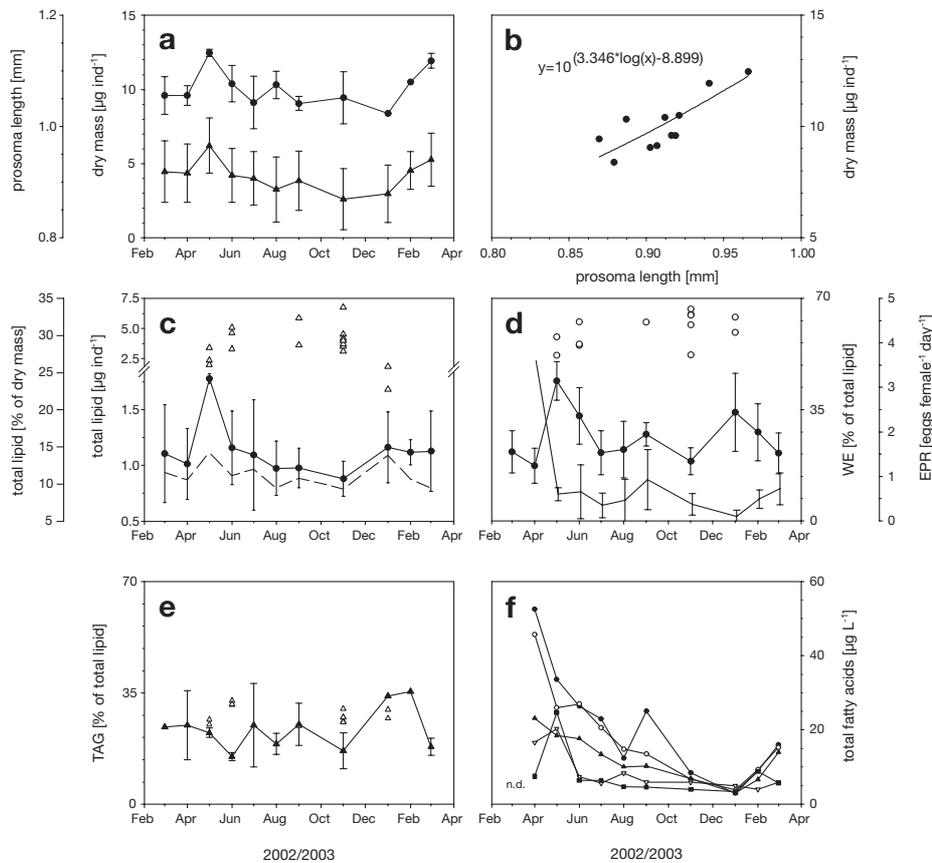
### *Pseudocalanus acuspes*

Females of *P. acuspes* differed substantially in size between succeeding months (Fig. 2a), with a highly significant increase of prosoma length as well as biomass in May up to an average of 966  $\mu\text{m}$  (significant difference to April and June  $p < 0.001$ ) and 12.5  $\mu\text{g}$  per individual, respectively. Over the summer their size decreased, reaching a minimum of 870 to 880  $\mu\text{m}$  in length in November (significant difference to September  $p < 0.01$ ) and January (significant difference to February  $p < 0.001$ ). From February on, females increased in size again. Dry mass-length ratios, based on monthly averages of variables, basically followed the relationship established by Hay et al. (1988) (Fig. 2b).

The lipid content in terms of total fatty acids and alcohols of the females ranged from 0.9 to 1.8  $\mu\text{g}$  per individual and from 9 to 14% of dry mass, respectively (Fig. 2c), with a maximum in May (significant difference to April  $p < 0.05$ ). There was no significant difference in May between the total individual lipid content of the females and the copepodite stage V (mean 2.6  $\mu\text{g}$  per individual). In all other months examined, the lipid amount of CV was clearly higher than that of the females, with an average individual lipid content between 4.7  $\mu\text{g}$  in September and 1.6  $\mu\text{g}$  in January.

In both stages, females and CV, wax esters (WE) as well as triacylglycerols (TAG) served as storage lipids throughout the year. Neutral lipids of females (WE and TAG) comprised about two thirds of total lipids in May and January, respectively and one third of total lipids in November (Fig. 2d, e).

The relative amount of TAG ranged between 15 and 35% of total lipids, but due to a high variability no seasonal trends could be identified, neither for females nor CV. In contrast, the relative WE content of the females changed over the year, reaching a maximum of 44% of total lipids in May (significant difference to April  $p < 0.001$ ) and lowest values of 17% in April and November. From November the wax ester content increased until January, afterwards it declined again until March. The WE content of CV was significantly higher during all months, except for May, when values were in the same range as for the females.



**Figure 2**

**a.** Prosoma length and dry mass of adult females of *Pseudocalanus acuspes*: triangles = monthly average of length, circles = monthly average of dry mass, error bars = standard deviation, **b.** Length dry mass relationship of adult females of *P. acuspes*: circles = monthly average of dry mass versus monthly average of prosoma length, line = length dry mass relationship determined by Hay et al. (1988); **c.** Total lipid content of *P. acuspes*: circles = monthly average of total fatty acids and alcohols (TL) per female, error bars = standard deviation, open triangles = TL per copepodite stage V (data points = stations, please note axis break), dashed line = monthly average of TL in percent of dry mass in adult females; **d.** Wax ester (WE) content and egg production rates (EPR) of *P. acuspes*: filled circles = monthly average of WE [% TL] in adult females, open circles = WE [% TL] in copepodite stage V (data points = stations), line without symbols = monthly average of daily individual egg production, EPR modified from Renz et al. (subm.), error bars = standard deviation; **e.** Triacylglycerol (TAG) content of *P. acuspes*: filled triangles = monthly average of TAG [% TL] in adult females, open triangles = TAG [% TL] in copepodite stage V (data points = stations), error bars = standard deviation; **f.** Monthly average of lipid concentration in the seston: filled circles = upper water layer (5 m), open circles = above thermocline (10 m), filled triangles = midwater layer (20-30 m), open triangles = above halocline (30-40 m), filled squares = in halocline (40-60 m)

Egg production data were adopted from Renz et al. (subm.). Daily egg production rates (EPR) showed an overall high variability in the Bornholm Basin (Fig. 2d), reaching a minimum of 0.1 eggs per female per day in January and increasing again in February. The highest EPR was measured in April, but was based only on data from one station, i.e. on the

average EPR of 30 females. Whereas in summer mean EPR and WE content paralleled, EPR increased with decreasing WE content in spring 2003.

**Table 1.**

Relative composition of fatty acids [% of total fatty acids] and fatty alcohols [% of total fatty alcohols] in total lipids of adult females and copepodite stage V (CV) of *Pseudocalanus acuspes*, values are calculated on basis of monthly averages, values below 1% not shown, min = minimum, max = maximum, sd = standard deviation, - = sd not calculated

|                       | females |      |      |     | CV   |      |      |     |
|-----------------------|---------|------|------|-----|------|------|------|-----|
|                       | min     | max  | mean | sd  | min  | max  | mean | sd  |
| <b>fatty acids</b>    |         |      |      |     |      |      |      |     |
| 14:0                  | <1      | 1.6  | 1.0  | 0.3 | <1   | <1   | <1   | -   |
| 15:0                  | <1      | 1.2  | <1   | -   | <1   | <1   | <1   | -   |
| 16:0                  | 10.3    | 20.7 | 15.0 | 2.9 | 6.0  | 8.3  | 7.1  | 1.0 |
| 17:0                  | <1      | 1.0  | <1   | -   | <1   | <1   | <1   | -   |
| 18:0                  | <1      | 3.7  | 2.5  | 0.6 | <1   | 1.3  | <1   | -   |
| 16:1(n-7)             | 1.1     | 6.5  | 2.4  | 1.5 | 3.3  | 7.6  | 4.9  | 1.6 |
| 16:1(n-9)             | <1      | 2.6  | <1   | -   | <1   | <1   | <1   | -   |
| 18:1(n-5)             | 1.6     | 4.1  | 2.8  | 0.9 | <1   | 2.1  | 1.3  | 0.6 |
| 18:1(n-7)             | 1.4     | 2.9  | 2.0  | 0.4 | 1.3  | 1.6  | 1.5  | 0.1 |
| 18:1(n-9)             | 10.1    | 26.1 | 18.9 | 5.2 | 28.9 | 39.6 | 34.8 | 4.4 |
| 24:1                  | 1.3     | 3.3  | 2.2  | 0.7 | <1   | <1   | <1   | -   |
| 16:2(n-4)             | <1      | 1.2  | <1   | -   | <1   | <1   | <1   | -   |
| 18:2(n-6)             | 2.9     | 8.5  | 5.6  | 1.9 | 4.2  | 10.7 | 7.1  | 2.8 |
| 16:3(n-4)             | <1      | 1.0  | <1   | -   | <1   | <1   | <1   | -   |
| 18:3(n-3)             | 1.7     | 4.8  | 3.3  | 1.1 | 2.2  | 6.9  | 5.1  | 1.9 |
| 18:3(n-6)             | <1      | 1.0  | <1   | -   | <1   | <1   | <1   | -   |
| 18:4(n-3)             | 1.3     | 5.8  | 3.6  | 1.4 | 4.1  | 7.2  | 6.1  | 1.2 |
| 20:4(n-3)             | <1      | 1.6  | 1.1  | 0.4 | 1.0  | 2.9  | 2.1  | 0.7 |
| 20:5(n-3)             | 12.4    | 19.5 | 15.2 | 2.4 | 9.8  | 14.3 | 12.0 | 2.1 |
| 22:6(n-3)             | 16.6    | 26.2 | 22.0 | 3.1 | 10.6 | 20.5 | 15.2 | 4.0 |
| <b>fatty alcohols</b> |         |      |      |     |      |      |      |     |
| 14:0                  | 21.4    | 33.2 | 26.7 | 3.7 | 22.9 | 30.0 | 27.2 | 2.7 |
| 16:0                  | 55.2    | 70.2 | 62.7 | 5.1 | 59.5 | 69.6 | 65.6 | 3.9 |
| 18:0                  | <1      | 11.5 | 5.1  | 3.7 | 1.5  | 2.6  | 2.0  | 0.4 |
| 18:1                  | 2.8     | 8.2  | 5.2  | 1.6 | 3.5  | 9.6  | 5.2  | 2.5 |
| 20:1                  | <1      | 1.6  | <1   | -   | <1   | <1   | <1   | -   |

The total fatty acid composition of females was characterized by high amounts of the typical membrane components 16:0, 20:5(n-3), 22:6(n-3), as well as by elevated levels of 18:1(n-9). Alcohols were dominated by 14:0 and 16:0, while 18:0 and 18:1 were found in much lower quantities (Table 1). Within the neutral lipid fraction ten important fatty acids (i.e. maximum values  $\geq 2\%$  of total fatty acids) were identified (Table 2). The fatty acid 18:1(n-9) dominated during all seasons, ranging between 44% of total fatty acids in June and 23% in February. Based on their relative fatty acid composition, females were merged into five seasonal groups, which exhibited highly significant differences (Table 2). The first group included females from March and April 2002 as well as from February and March 2003. This spring season was characterized by elevated amounts of the dinoflagellate marker 18:4(n-3), whereas the diatom marker 16:1(n-7) peaked in May. Both groups showed high percentages of 20:5(n-3) and 22:6(n-3), also indicating diatom- and dinoflagellate-based diets, respectively. In May, 18:1(n-9) strongly increased, reaching maximum values in June. From June to September fatty acids were characterized by rising levels of 18:2(n-6) and 18:3(n-3),

reaching up to 12% and 8%, respectively. In winter higher amounts of the unspecific fatty acids 16:0, 16:1(n-9) and 18:0 prevailed.

**Table 2.** Relative fatty acid composition of neutral lipids of females of *Pseudocalanus acuspes*, \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ , I-V seasonal groups, sd = standard deviation

|           | I                 |     | II           |     | III           |     | IV                  |     | V                |     | level of significance (Student's t-Test) |        |        |      |     |                |      |       |      |       |
|-----------|-------------------|-----|--------------|-----|---------------|-----|---------------------|-----|------------------|-----|--|--------|--------|------|-----|----------------|------|-------|------|-------|
|           | Feb-Apr<br>n = 11 |     | May<br>n = 3 |     | June<br>n = 4 |     | July-Sept<br>n = 14 |     | Nov-Jan<br>n = 4 |     | adjacent groups                          |        |        |      |     | distant groups |      |       |      |       |
|           | mean              | sd  | mean         | sd  | mean          | sd  | mean                | sd  | mean             | sd  | I:II                                     | II:III | III:IV | IV:V | I:V | I:III          | I:IV | II:IV | II:V | III:V |
| 16:0      | 13.3              | 2.5 | 8.2          | 1.3 | 9.4           | 3.1 | 14.8                | 2.8 | 17.7             | 4.4 | **                                       |        | **     |      | *   | *              |      | ***   | **   | *     |
| 18:0      | 3.0               | 1.1 | 1.8          | 0.6 | 2.6           | 1.1 | 3.0                 | 0.8 | 6.5              | 1.9 |  |        |        | ***  | *** |                |      | *     | **   | **    |
| 16:1(n-7) | 5.0               | 1.3 | 9.2          | 1.0 | 5.0           | 0.4 | 2.5                 | 0.5 | 3.8              | 1.1 | ***                                      | ***    | ***    |      |     |                | ***  | ***   | **   |       |
| 16:1(n-9) | 1.6               | 1.1 | 1.3          | 1.4 | 1.3           | 0.5 | 1.6                 | 1.2 | 7.3              | 2.8 |  |        |        | ***  | *** |                |      |       | **   | **    |
| 18:1(n-9) | 26.7              | 5.0 | 39.1         | 1.4 | 43.8          | 0.5 | 35.9                | 4.5 | 35.7             | 5.0 | ***                                      | **     | **     |      | **  | ***            | ***  |       |      | *     |
| 18:2(n-6) | 8.2               | 1.3 | 8.2          | 0.6 | 11.6          | 2.0 | 12.4                | 2.2 | 5.7              | 1.6 |  | *      |        | ***  | **  | **             | ***  | **    |      | **    |
| 18:3(n-3) | 5.7               | 0.9 | 3.0          | 0.3 | 5.2           | 1.5 | 7.9                 | 2.2 | 2.7              | 1.0 | ***                                      | *      | *      | ***  | *** |                | **   | ***   |      | *     |
| 18:4(n-3) | 8.3               | 1.9 | 3.8          | 0.3 | 3.7           | 1.8 | 4.3                 | 1.7 | 1.6              | 0.6 | ***                                      |        |        | **   | *** | ***            | ***  | ***   | **   | *     |
| 20:5(n-3) | 9.4               | 2.2 | 8.5          | 0.5 | 6.6           | 0.4 | 5.6                 | 0.8 | 4.7              | 1.4 |  | **     | *      |      | **  | ***            | ***  | ***   | *    |       |
| 22:6(n-3) | 6.3               | 3.3 | 8.6          | 0.9 | 5.5           | 0.6 | 3.6                 | 1.3 | 3.7              | 0.9 |  | **     | *      |      |     | **             | ***  | **    | *    |       |

The neutral lipids of CV showed a very similar fatty acid composition to those of the females (Fig. 3). Especially in May and June there was no significant difference between the fatty acids of both stages, whereas in autumn and winter the fatty acids 18:1(n-9), 18:2(n-6), 18:4(n-3) and 20:5(n-3) of CV showed higher percentages.

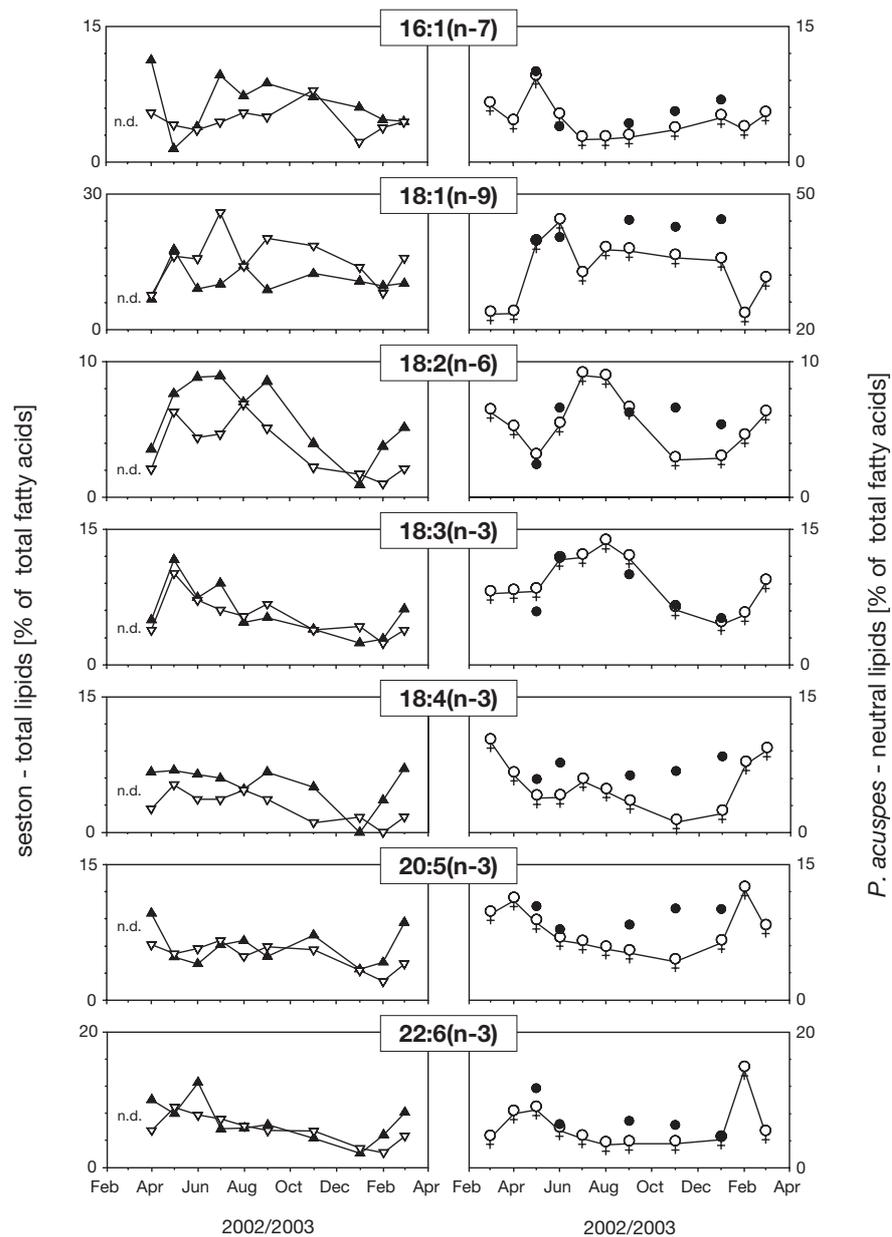
Principal component analysis (PCA) on the storage lipid composition of females extracted three components with eigenvalues  $>1$ . Only the major two, together explaining 68% of the variance, are presented (Fig. 4). The PCA revealed a strong coupling between the fatty acids 18:2(n-6) and 18:3(n-3), as well as between 22:6(n-3), 20:5(n-3) and 16:1(n-7). The fatty acids 16:1(n-9), 18:0 and 18:4(n-3) were important moieties to distinguish samples along component one, whereas 18:1(n-9), 22:6(n-3) and 16:1(n-7) mostly affected samples influenced by component two. Other fatty acids, like 18:3(n-3) and 18:2(n-6) had a high impact on both components and could not be assigned clearly. Samples from different storage months were separated, demonstrating seasonal changes in the fatty acid compositions of storage lipids.

Although polar lipids remained rather uniform throughout the year, with 16:0, 20:5(n-3) and 22:6(n-3) contributing between 50% and 73% of total fatty acids (Table 3), their fatty acid profiles divided into the same seasonal groups as the storage lipids. Small but significant differences were mainly due to changes of the 18:1 isomers, as well as of 18:3(n-3) and 18:4(n-3), with largest changes between early (March-April) and late spring (May).

### Seston

Maximum lipid concentrations in the seston were always found above the thermocline during spring and summer, whereas from autumn until spring, mixing caused more equally dis-

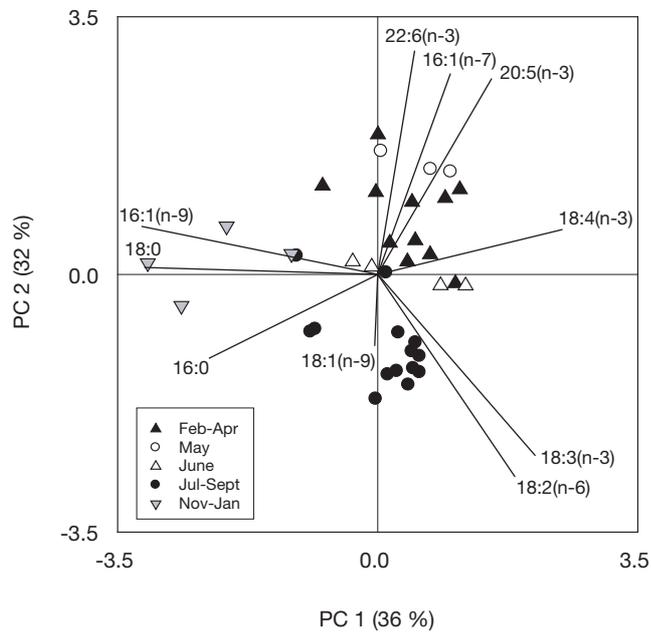
tributed lipid contents over the whole water column (Fig. 2f). In terms of total fatty acids and alcohols, maximal lipid contents with up to 52  $\mu\text{g}$  per liter were found in upper water layers in April. This lipid-rich seston reached lower water layers with a time delay of one month, resulting in a lipid peak near the halocline of 20 to 24  $\mu\text{g}$  per liter in May.



**Figure 3.** Seasonal development of mean fatty acid composition of seston (total lipids) and *Pseudocalanus acuspes* (neutral lipids): n.d. = no data, filled triangles = seston in upper water layer (5 m), open triangles = seston in halocline (40-60 m), filled circles = copepodite stage V, open circles with cross = adult females

**Figure 4.**

Principal component analysis on the relative fatty acid composition of neutral lipids of adult females of *Pseudocalanus acuspes*, scales were adjusted to combine plots: scales of principal components (PC) refer to sample plot, scale of variables reaches from -1 to +1 for both PCs



The PCA revealed a strong coherence within the relative seston composition in terms of biomass of different taxonomic groups and typical signature fatty acids (Fig. 5). There was a distinct correlation between 18:1(n-9) and ciliates and to a lesser degree flagellates, between 16:1(n-7), 20:5(n-3) and diatoms as well as a coherence between 22:6(n-3), 18:4(n-3) and dinoflagellates. The strong connection between 18:2(n-6) and 18:3(n-3) could not be assigned to a specific algal group, but they both had a very similar impact on component one as cyanobacteria and chlorophytes, whereas component two differentiated them. Due to their relative position on component three 18:2(n-6) grouped with chlorophytes, whilst 18:3(n-3) correlated with cyanobacteria.

Ciliates contributed significantly to seston biomass at all seasons (Table 4), maximum proportions of diatoms were found in spring, of ciliates and dinoflagellates in May. In contrast, other flagellates and cyanobacteria increased during the summer. No data were available for the winter season.

#### *Trophic interactions*

When compared with seston lipids, some fatty acids of *P. acuspes* females developed with a time lag of one to two months at the beginning of 2002, whereas in autumn and winter seston and copepods showed relatively parallel progressions (Fig. 3). Specifically the increase of 18:3(n-3) and 18:2(n-6) in May and June was reflected with some delay in the storage lipids of females. In May, the increase of 18:1(n-9) in females co-occurred with a rise in the seston

from lower water layers, whereas the peak in the upper water column in July was not found in the copepods.

As indicated by a selection index (Fig. 6), 16:0 and 18:0 were usually negatively selected during all seasons, whereas 18:1(n-9), 18:2(n-6) and 20:5(n-3) were elevated in the neutral lipids most of the time. Selectivity for all other fatty acids changed with time or depth. In early spring 18:3(n-3), 18:4(n-3) and 22:6(n-3) were accumulated in storage lipids as compared to the seston, as well as 16:1(n-7) in May and June.

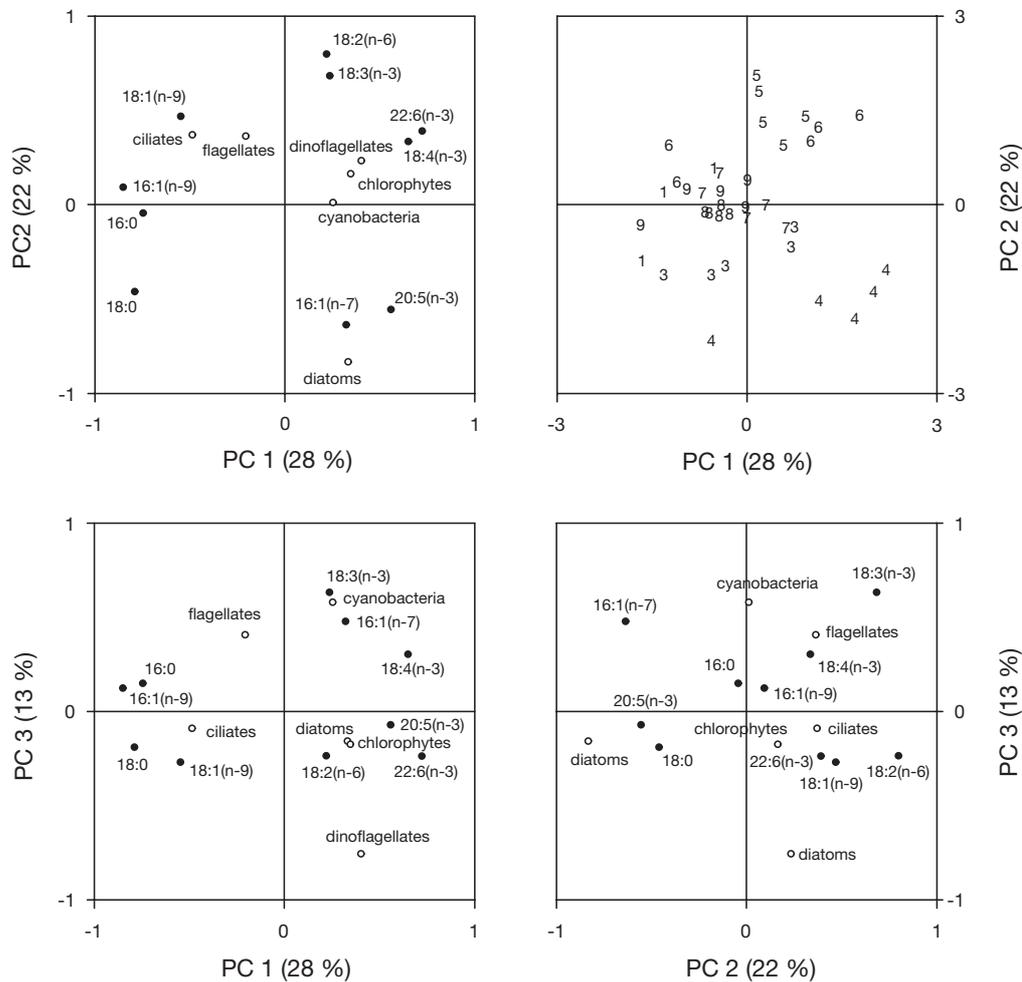
**Table 4.**

|                   | depth [m] | din | dia | chl | cya | div fl | cil |
|-------------------|-----------|-----|-----|-----|-----|--------|-----|
| <b>Feb-Apr</b>    |           |     |     |     |     |        |     |
| upper water layer | 5         | 24  | 18  | 0   | 3   | 9      | 45  |
| above thermocline | 10        | 20  | 23  | 1   | 1   | 14     | 41  |
| midwater layer    | 20-30     | 7   | 38  | 1   | 2   | 11     | 42  |
| above halocline   | 30-40     | 15  | 45  | 1   | 1   | 5      | 33  |
| in halocline      | 40-60     | 20  | 45  | 1   | 1   | 3      | 30  |
| <b>May</b>        |           |     |     |     |     |        |     |
| upper water layer | 5         | 24  | 1   | 0   | 2   | 7      | 66  |
| above thermocline | 10        | 20  | 0   | 0   | 1   | 8      | 71  |
| midwater layer    | 20-30     | 50  | 4   | 2   | 11  | 6      | 27  |
| above halocline   | 30-40     | 23  | 1   | 1   | 0   | 2      | 73  |
| in halocline      | 40-60     | 29  | 0   | 8   | 0   | 7      | 55  |
| <b>June</b>       |           |     |     |     |     |        |     |
| upper water layer | 5         | 43  | 1   | 1   | 19  | 17     | 19  |
| above thermocline | 10        | 23  | 1   | 17  | 17  | 15     | 27  |
| midwater layer    | 20-30     | 25  | 3   | 1   | 11  | 29     | 30  |
| above halocline   | 30-40     | 17  | 2   | 0   | 1   | 10     | 70  |
| in halocline      | 40-60     | 13  | 2   | 0   | 7   | 9      | 69  |
| <b>July-Sept</b>  |           |     |     |     |     |        |     |
| upper water layer | 5         | 5   | 4   | 0   | 19  | 17     | 56  |
| above thermocline | 10        | 3   | 11  | 2   | 9   | 17     | 57  |
| midwater layer    | 20-30     | 3   | 5   | 1   | 55  | 14     | 22  |
| above halocline   | 30-40     | 6   | 0   | 2   | 18  | 30     | 42  |
| in halocline      | 40-60     | 14  | 11  | 1   | 0   | 15     | 59  |

## Discussion

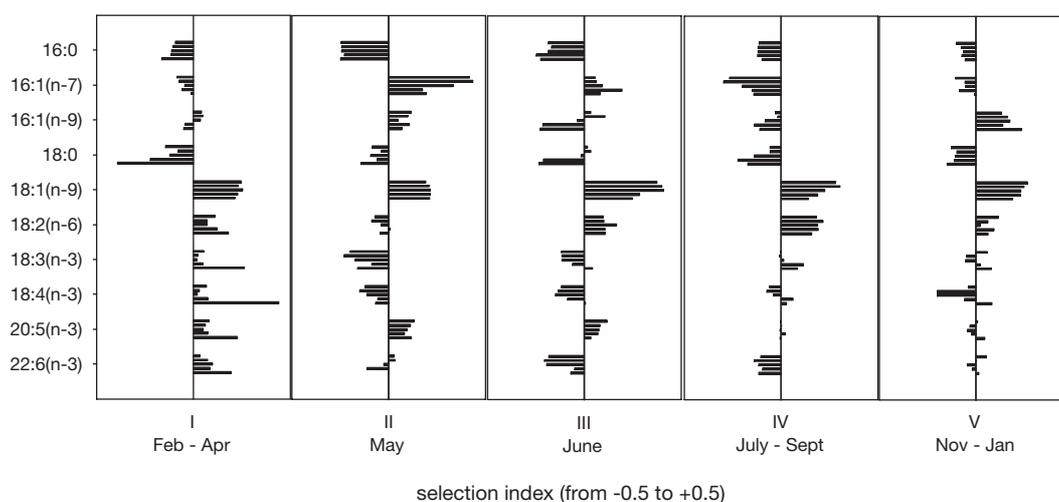
### *Seasonal cycle*

The seasonal cycle and condition of *Pseudocalanus acuspes* in the Bornholm Basin were described on the basis of lipid content and composition as well as prosoma length, to relate lipid dynamics and size variations to the life cycle of this originally Arctic copepod in the Baltic Sea. Pronounced changes in body size and fatty acid composition of structural lipids of females revealed five “environmental cohorts”, which obviously experienced similar biotic and abiotic conditions during development, thus leading to constant attributes of females: early spring (February-April), late spring (May), early summer (June), late summer (July-September) and winter (November-January). Recent studies on stage composition and growth measurements (Renz and Hirche 2006, Renz et al. subm.) indicate that *P. acuspes* basically follows an annual cycle in the Bornholm Basin, although the development of a second cohort in summer was also considered possible.



**Figure 5.** Principal component analysis on the relative taxonomic (in terms of biomass) and fatty acid composition of the seston: loading plots for the extracted principal components (PC) 1-3 (open circles = seston taxa, filled circles = fatty acids) and sample plot for the extracted PCs 1 and 2 (numbers = months of the year)

Aligning the “environmental cohorts” in this context, the early spring cohort consisted of females, which successively matured from older overwintering copepodite stages. Their growth was at least partly fueled by storage lipids, as indicated by the decrease in wax ester content. This cohort was followed by females in May, which were probably larger due to better feeding conditions and lower temperatures (Vidal 1980, Klein Breteler and Gonzalez 1988). Strong changes in size co-occurred with variations in the composition of structural lipids and in storage lipid content, indicating different feeding histories during growth. The May cohort probably derived from younger overwintering copepodids of the previous year, which encountered a high food supply in the upper water column in April. In May lipid-rich seston reached lower water layers, thus providing better feeding conditions for older copepo-



**Figure 6.** Selection index for fatty acids with vertical resolution (sorted top-down: upper water layer (5 m), above thermocline (10 m), midwater layer (20-30 m), above halocline (30-40 m), in halocline (40-60 m))

dite stages and females. It remains however a matter of conjecture, whether the drastic changes in May were due to successively maturing cohorts or rather to the appearance of a new generation.

To better understand the further progression of the seasonal cycle, valuable information can be derived from comparisons of storage lipid content of CV and females. In May the amounts of storage lipids of females and CV hardly differed, whereas in summer the copepodids were always richer in wax esters. A similar decrease in storage lipids, measured as oil sac volume, was observed by McLaren and co-authors (1989) in summer females of *P. acuspes* in the Bedford Basin, Nova Scotia. Two, not mutually exclusive mechanisms, causing the pronounced differences between females and copepodids, can be assumed:

(1) Food supply - The accumulated storage lipids were used up very quickly by the females for metabolic costs of last molt, gonad maturation and egg production. Due to reduced food availability, the depletion of reserves proceeded more quickly during summer than in May, explaining a high wax ester retention of females in late spring. Lipid retention is a direct expression for surplus of food. Apparently, the food supply alone was not sufficient to sustain egg production at ambient temperatures in summer. The pronounced utilization of storage lipids signifies that in summer food limitation might have been an important factor, whereas in May egg production was primarily determined by abiotic factors. Hence, sub-optimum growth conditions might reduce the number of generations per year.

(2) Onset of overwintering - Only the lipid-poor copepodids accomplished maturation during summer and autumn to produce potentially more successful offspring, whereas the lipid-rich copepodids passed into an “active diapause”, with ongoing feeding, suspended development and resting gonads (McLaren et al. 1989). According to this hypothesis, the females found in

the Bornholm Basin from summer to winter would represent a still maturing but minor part of the population. This is consistent with the drastic decline of female and nauplii abundance in the water column in July and August (Renz and Hirche 2006) and the slow developmental rates in late spring and summer (Renz et al. subm.). Such a continued development of only a minor part of the generation of *Pseudocalanus* sp. was also observed in the White Sea (Pertsova 1981). Norrbin (Norrbin et al. 1990, Norrbin 1996) suggested that it is less a continuous process, triggered by the physiological state as proposed by McLaren and co-authors (1989), but rather a specific switching date, at which *P. acuspes* copepodids stop maturation but proceed to accumulate lipids. Klein Breteler and Gonzalez (1988) suspected that changes to poor food conditions are necessary to induce hormonal cessation of development in favor of lipid production. Still, it remains unclear, how an external trigger, which would be effective early in the year, should function in the Bornholm Basin, with higher temperatures and longer periods of high food abundance as compared to high latitudinal habitats.

In conclusion, we found evidence that the life cycle of *P. acuspes* in the Baltic Sea resembles that of *Pseudocalanus* spp. in Arctic regions (e.g. Pertsova 1981, McLaren et al. 1989, Norrbin et al. 1990, Norrbin 1991, Conover and Siferd 1993), with highest reproductive activities in spring, a successive accumulation of resting copepodite stages starting in early summer and a potential interposition of minor summer generations. This is supported by the corresponding lipid-storing strategies of *P. acuspes*. Wax ester levels in the Baltic were similar to those found for CIV and CV of *P. acuspes* in Arctic regions (Båmstedt et al. 1990, Norrbin et al. 1990), with values reaching 72% of total lipids in autumn and around 55% in summer. To our knowledge, there are no data available on the wax ester content of *P. acuspes* females.

Based on these fundamental analogies, we hypothesize that life cycle and lipid-storing strategies of *P. acuspes* in the central Baltic Sea originate from extant adaptations to high latitudinal habitats.

### *Trophodynamics*

The five different phases of the seasonal cycle were also reflected in the fatty acid dynamics of neutral lipids, although they are less conservative than structural lipid composition and body size. We applied signature fatty acids (Lee et al. 1971, Sargent and Whittle 1981, Sargent et al. 1987, Graeve et al. 1994, Daalsgard et al. 2003) to identify feeding preferences and food selection of *Pseudocalanus acuspes*. Due to parallel analyses of the seston, we were able to assign the fatty acid markers to specific food sources.

Similar to all other studies dealing with the fatty acid composition of *Pseudocalanus* spp. (e.g. Kattner et al. 1981, Kattner and Krause 1989, Fraser et al. 1989, Norrbin et al. 1990, Cotonnec et al. 2001), we found 18:1(n-9) to be one of the most abundant fatty acids throughout the year. Apparently, this does not inevitably indicate similar feeding habits in different habitats, but rather a species-specific attribute, probably affected by metabolic

processes. This fatty acid is not only known to be characteristic for carnivorous or detritivorous feeding (Sargent and Falk-Petersen 1981, Falk-Petersen et al. 1990), it is also synthesized de novo by copepods (Pascal and Ackman 1976, Sargent and Henderson 1986, Kattner et al. 1994, Kattner and Hagen 1998). Thus, a trophic assignment of 18:1(n-9) remains problematic. Nevertheless, as revealed by principal component analysis we found a strong coherence between 18:1(n-9) levels in the seston lipids and the occurrence of ciliates. Lipid profiles of ciliates have been reported to reflect, at least within species-specific ranges, the fatty acid composition of their diet (Ederington et al. 1995, Harvey et al. 1997, Broglio et al. 2003). Therefore, a comparison of field data with fatty acid profiles derived in laboratory studies is rather difficult. However, our data emphasize a high relevance of ciliates in the food spectrum of *P. acuspes*. The apparently intense use of heterotrophic organisms and/or detritus might be explained by the vertically stratified environment in the Baltic Sea. Due to the concentration of older copepodite stages of *P. acuspes* in deeper water layers (Hernroth and Ackefors 1979, Renz and Hirche 2006), their potential food mainly consisted of sinking material from the surface and organisms inhabiting the lower stratum of the water column. At least in May and June, those were mainly ciliates, representing approximately 75% of living biomass, co-occurring with very high 18:1(n-9) levels in the females. Feeding of *Pseudocalanus* spp. on ciliates (Klein Breteler et al. 2004) and heterogeneous particulate matter was documented in laboratory studies (Poulet 1974, 1976, Pavlovskaya and Pechen'Finenko 1975 as cited by Corkett and McLaren 1978). We did not quantify detritus, although an accumulation of degraded material on the halocline is to be expected.

A comparison between the fatty acid and taxonomic composition of seston revealed a relationship between 18:4(n-3), 22:6(n-3) and the biomass of dinoflagellates, as well as coherence between 18:4(n-3) and other flagellates. Those fatty acids are known to reach high levels in dinoflagellates and cryptophytes (Sargent et al. 1987, Graeve et al. 1994, 2001 and references therein, Daalsgard et al. 2003). In our study, the biomarker 18:4(n-3) was found in significantly higher amounts in early spring, and 22:6(n-3) was also more abundant from February until May. This indicates a preferential ingestion of flagellates or dinoflagellates in spring time, although dinoflagellates showed a rather constant portion of total biomass during all seasons examined, whereas other flagellates increased later in the year. *Pseudocalanus* spp. selectively feeds on flagellates such as cryptophytes and dinoflagellates (e.g. Geen and Hargrave 1966, Zagorodnyaya 1974). This high quality food (Brown et al. 1997) enhances growth, egg production and lipid accumulation and also decreases mortality (Klein Breteler et al. 1990, Koski et al. 1998, Koski and Klein Breteler 2003).

Diatom blooms, which have reappeared in the Bornholm Basin since 1999, were mainly restricted to early spring (February-April) (Wasmund et al. 2003, present study). However, the diatom marker 16:1(n-7) reached its maximum in *P. acuspes* not until May, when diatoms were of only marginal importance in the water column and their fatty acid markers in the seston had already decreased significantly. This suggests that lipids observed in the new females in May probably derived from lipid reserves built up during earlier copepodite

stages. This time shift between fatty acid levels in seston and copepods related to the period of higher lipid accumulation or retention by females. Low lipid levels reflect changes much quicker, probably causing the more synchronous progression of fatty acid composition of seston and storage lipids later in the season. Alternatively, in spite of low standing stocks of diatoms, their production rates may have been high, as the production potential of diatoms was evident from a small diatom bloom during July.

Diatom marker levels were rather low in the Baltic. Especially in polar regions with more pronounced diatom and ice algal blooms 16:1(n-7) may reach values of up to 20% of total fatty acids in CIV and CV of *P. acuspes* in the Arctic summer (Norrbín et al. 1990), thus exceeding twice the maximum value found for CV in the present study. Very similar results, indicating diatom-based feeding, were found for other *Pseudocalanus* species in polar regions with 16:1(n-7) levels reaching up to 40% of total fatty acids (Peters et al. 2004, Lischka and Hagen subm.).

The rather limited ingestion of diatoms seems to be characteristic for temperate regions, as all studies show similarly low marker amounts (Kattner et al. 1981, Kattner and Krause 1989, Fraser et al. 1989, Cotonnec et al. 2001). Still, the levels of 16:1(n-7) found in our study belong to the lowest ever measured for *Pseudocalanus* spp., indicating a more intense use of other food sources. Especially cyanobacteria have to be considered as potential diet in the Baltic Sea, as they usually bloom intensively during summer, except for 2002, when only a minor bloom was registered (Wasmund et al. 2003). However, in our study cyanobacteria values reached up to 55% of seston biomass.

Cyanobacteria are very variable in their fatty acid compositions, with marked differences occurring even in the same genus (Gugger et al. 2002). Due to the coexistence of different cyanobacteria species in the Baltic Sea, it is not easy to identify a clear trophic signal. Many studies reported that a characteristic fatty acid pattern of cyanobacteria consists of 16:0, 16:1, 18:2(n-6) and 18:3(n-3) (e.g. Murata and Nishida 1987 and references therein, Vargas et al. 1998, Gugger et al. 2002). The simultaneous occurrence of 18:3(n-3) and 18:2(n-6) with cyanobacteria and chlorophytes in the seston, indicated that elevated amounts of these fatty acids in *P. acuspes* were due to an augmented use of cyanobacteria in summer, since chlorophytes were only of minor importance. Hoppe (1981) showed that cyanobacteria and microzooplankton often build up agglomerates especially in the late bloom phase, which might improve food quality and attractiveness for copepods, leading to a more intensive use in the later phases (Meyer-Harms et al. 1999). This might explain why cyanobacteria were reflected in the storage lipids with a delay of some months.

We conclude that *P. acuspes* displays a basically opportunistic feeding behavior in the Baltic Sea. Five different seasonal fatty acid profiles were determined in the neutral lipids with high levels of 18:1(n-9) at all times, indicating a species-specific storage pattern as well as a ciliate-dominated diet. Other food sources varied over the year. In early spring dinoflagellates were increasingly utilized, whereas in late spring diatom markers were most strongly re-

flected in the fatty acid composition. During summer, cyanobacteria, and probably to a lesser degree chlorophytes, seemed to contribute substantially to the diet of *P. acuspes*.

### Acknowledgements

The study was funded by the German Federal Ministry for Education and Research within the GLOBEC GERMANY project (03F0320C). We wish to thank the crews and scientific parties of the RV Alkor, Heincke and A.v.Humboldt for the excellent support during the field phase. We also thank M. Graeve for GC-MS measurements. The critical revision and improvement of the manuscript by H. Auel, D. Stübing and three anonymous reviewers are gratefully acknowledged. The original publication is available at [www.springerlink.com](http://www.springerlink.com)

### Literature

- Båmstedt U, Håkanson JL, Brenner-Larsen J, Bjørnsen PK, Geertz-Hansen O, Tiselius P (1990) Copepod nutritional condition and pelagic production during autumn in Kosfjorden, western Sweden. *Mar Biol* 104: 197-208
- Broglio E, Jónasdóttir SH, Calbet A, Jakobsen HH, Saiz E (2003) Effect of heterotrophic versus autotrophic food on feeding and reproduction of the calanoid copepod *Acartia tonsa*: relationship with prey fatty acid composition. *Aquat Microb Ecol* 31: 267-278
- Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture. *Aquaculture* 151: 315-331
- Bucklin A, Frost BW, Bradford-Grieve J, Allen LD, Copley NJ (2003) Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Mar Biol* 142: 333-343
- Cairns AA (1967) The zooplankton of Tanquary Fjord, Ellesmere Island, with special reference to the calanoid copepods. *J Fish Res Board Can* 24: 555-568
- Conover RJ, Siferd TD (1993) Dark-season survival strategies of coastal zone zooplankton in the Canadian Arctic. *Arctic* 46: 303-311
- Corkett CJ, McLaren IA (1978) The biology of *Pseudocalanus*. *Adv Mar Biol* 15: 1-231
- Cotonnec G, Brunet C, Sautour N, Thoumelin G (2001) Nutritive value and selection of food particles by copepods during a spring bloom of *Phaeocystis* sp. in the English Channel, as determined by pigment and fatty acid analyses. *J Plankton Res* 23: 693-703
- Daalsgard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol* 46: 225-340
- Davis CC (1976) Overwintering strategies of common planktic copepods in some north Norway fjords and sounds. *Astarte* 9: 37-42
- Digby PSB (1950) The biology of the small planktonic copepods of Plymouth. *J Mar Biol Assoc UK* 29: 393-438

- Dunstan GA, Volkman JK, Barrett SM, LeRoi J-M, Jeffrey SW (1994) Essential polyunsaturated fatty acids from 14 species of diatoms (Bacillariophyceae). *Phytochemistry* 35: 155-161
- Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. *Limnol Oceanogr* 40: 860-867
- Edler L (1979). Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. The Baltic Marine Biologists, Publication 5: 1-38
- Falk-Petersen S, Hopkins CCE, Sargent JR (1990) Trophic relationships in the pelagic, Arctic food web. In: Barnes M, Gibson RN (eds) Trophic relationships in the marine environment - Proceedings of the 24th European Marine Biological Symposium. Aberdeen University Press, Aberdeen, pp 315-333
- Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497-509
- Fraser AJ, Sargent JR, Gamble JC (1989) Lipid class and fatty acid composition of *Calanus finmarchicus* (Gunnerus), *Pseudocalanus* sp. and *Temora longicornis* Muller from nutrient-enriched seawater enclosure. *J Exp Mar Biol Ecol* 130: 81-92
- Frost BW (1989) A taxonomy of the marine calanoid copepod genus *Pseudocalanus*. *Can J Zool* 67: 525-551
- Geen GH, Hargrave BT (1966) Primary and secondary production in Bras d'Or Lake, Nova Scotia, Canada. *Verh Int Ver Theor* 16: 333-340
- Graeve M, Kattner G, Hagen W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. *J Exp Mar Biol Ecol* 182: 97-110
- Graeve M, Dauby P, Scailteur Y (2001) Combined lipid, fatty acid and digestive tract content analyses: a penetrating approach to estimate feeding modes in Antarctic amphipods. *Polar Biol* 24: 853-862
- Gugger M, Lyra C, Suominen I, Tsitko I, Humbert J-F, Salkinoja-Salonen MS, Sivonen K (2002) Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria). *Int J Syst Evol Microbiol* 52: 1007-1015
- Harvey HR, Ederington MC, McManus G.B. (1997) Lipid composition of the marine ciliates *Pleuronema* sp. and *Fabrea salina* : shifts in response to changes in diet. *J Euk Microbiol* 44: 189-193
- Hay SJ, Evans GT, Gamble JC (1988) Birth, growth and death rates for enclosed populations of calanoid copepods. *J Plankton Res* 10: 431-454
- Henroth L (1985) Recommendations on methods for marine biological studies in the Baltic Sea: Mesozooplankton biomass assessment. The Baltic Marine Biologists Publication 10: 1-32

- Henroth L, Ackefors H (1979) The zooplankton of the Baltic proper: a long-term investigation of the fauna, its biology and ecology. Report Fish Bd Sweden, Institute for Marine Research 2: 1-160
- Hinrichsen H-H, Möllmann C, Voss R, Köster FW, Kornilovs G (2002) Biophysical modeling of larval Baltic cod (*Gadus morhua*) growth and survival. Can J Fish Aquat Sci 12: 1858-1873
- Hinrichsen H-H, Lehmann A, Möllmann C, Schmidt J (2003) Dependency of larval fish survival on retention/dispersion in food limited environments: the Baltic as a case study. Fish Oceanogr 12: 425-433
- Hoppe HG (1981) Blue-green algae agglomeration in surface water: a microhabitat of high bacterial activity. Kieler Meeresforschung Sonderhefte 5: 291-303
- Kattner G, Fricke HSG (1986) Simple gas-liquid chromatography method for simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. J Chromatogr 361: 263-268
- Kattner G, Hagen W (1998) Lipid metabolism of the Antarctic euphausiid *Euphausia crystallorophias* and its ecological implications. Mar Ecol Prog Ser 170: 203-212
- Kattner G, Krause M (1989) Seasonal variations of lipids (wax esters, fatty acids and alcohols) in calanoid copepods from the North Sea. Mar Chem 26: 261-275
- Kattner G, Krause M, Trahms J (1981) Lipid composition of some typical North Sea copepods. Mar Ecol Prog Ser 4: 69-74
- Kattner G, Graeve M, Hagen W (1994) Ontogenetic and seasonal changes in lipid and fatty acid/alcohol compositions of the dominant Antarctic copepod *Calanus propinquus*, *Calanoides acutus* and *Rhincalanus gigas*. Mar Biol 118: 637-644
- Klein Breteler WCM, Gonzalez SR (1988) Influence of temperature and food concentration on body size, weight and lipid content of two Calanoid copepod species. In: Boxshall GA, Schminke HK (eds) Biology of copepods. Kluwer Academic Publishers, Dordrecht, pp 201-210
- Klein Breteler WCM, Schogt N, Gonzalez SR (1990) On the role of food quality in grazing and development of life stages, and genetic change of body size during cultivation of pelagic copepods. J Exp Mar Biol Ecol 135: 177-189
- Klein Breteler WCM, Koski M, Rampen S (2004) Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate. Mar Ecol Prog Ser 274: 199-208
- Koski M, Klein Breteler WCM (2003) Influence of diet on copepod survival in the laboratory. Mar Ecol Prog Ser 264: 73-82
- Koski M, Klein Breteler W, Schogt N (1998) Effect of food quality on the rate of growth development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda: Calanoida). Mar Ecol Prog Ser 170: 169-187
- Lee RF, Nevenzel JC, Paffenhöfer G-A (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9: 99-108

- Lischka S, Hagen W (2005) Life histories of the copepods *Pseudocalanus minutus*, *P. acuspes* (Calanoida) and *Oithona similis* (Cyclopoida) in the Arctic Kongsfjorden (Svalbard). *Polar Biol* 28: 910-921
- Lischka S, Hagen W (subm) Seasonal lipid dynamics of the copepods *Pseudocalanus minutus* (Calanoida) and *Oithona similis* (Cyclopoida) in the Arctic Kongsfjorden (Svalbard)
- Marshall SM (1949) On the biology of the small copepods in Loch Striven. *J Mar Biol Assoc UK* 28: 45-122
- McLaren IA, Laberge E, Corkett CJ, Sévigny J-M (1989) Life cycles of four species of *Pseudocalanus* in Nova Scotia. *Can J Zool* 67: 552-558
- Meyer-Harms B, Reckermann M, Voß M, Siegmund H, Bodungen Bv (1999) Food selection by calanoid copepods in the eutrophic layer of the Gotland Sea (Baltic Proper) during mass occurrence of N<sub>2</sub>-fixing cyanobacteria. *Mar Ecol Prog Ser* 191: 243-250
- Möllmann C, Köster FW (1999) Food consumption by clupeids in the Central Baltic: evidence for top-down control? *ICES J Mar Sci* 56: 100-113
- Möllmann C, Köster FW (2002) Population dynamics of calanoid copepods and the implications of their predation by clupeid fish in the Central Baltic Sea. *J Plankton Res* 24: 959-978
- Möllmann C, Kornilovs G, Fetter M, Köster FW, Hinrichsen H-H (2003) The marine copepod *Pseudocalanus elongatus* as a mediator between climate variability and fisheries in the Central Baltic Sea. *Fish Oceanogr* 12: 360-368
- Murata N, Nishida I (1987) Lipids of blue-green algae (cyanobacteria). In: Stumpf PK (ed) *The biochemistry of plants*. Academic Press, New York, pp 315-347
- Nichols DS, Nichols PD, Sullivan CW (1993) Fatty acid, sterol and hydrocarbon composition of Antarctic sea ice diatom communities during the spring bloom in McMurdo Sound. *Antarct Sci* 5: 271-278
- Norrbin MF (1991) Gonad maturation as an indication of seasonal cycles for several species of small copepods in the Barents Sea. In: Sakshaug E, Hopkins CCE, Øritsland NA (eds) *Proceedings of the Pro Mare Symposium on Polar Marine Ecology*, pp 421-432
- Norrbin F (1992) Overwintering strategies of small copepods in high latitude marine environments. Dissertation. Faculty of Natural Sciences, Göteborg
- Norrbin MF (1996) Timing of diapause in relation to the onset of winter in the high-latitude copepods *Pseudocalanus acuspes* and *Acartia longiremis*. *Mar Ecol Prog Ser* 142: 99-109
- Norrbin MF, Olsen R-E, Tande KS (1990) Seasonal variation in lipid class and fatty acid composition of two small copepods in Balsfjorden, northern Norway. *Mar Biol* 105: 205-211
- Pascal JC, Ackman RG (1976) Long chain monoethylenic alcohol and acid isomers in lipids of copepods and capelin. *Chem Phys Lipids* 16: 219-223

- Pavlovskaya TV, Pechen'-Finenko GA (1975) Comparison of the relative role of living and non-living organic matter in the nutrition of *Pseudocalanus elongatus* (Boeck) (in Russian). *Biologiya Morya*, Kiev 34: 65-70
- Pertsova NM (1981) Number of generations and their span in *Pseudocalanus elongatus* (Copepoda, Calanoida) in the White Sea (in Russian). *J Zool* 60: 673-684
- Peters J, Tuschling K, Brandt A (2004) Zooplankton in the arctic Laptev Sea - feeding ecology as indicated by fatty acid composition. *J Plankton Res* 26: 227-234
- Poulet SA (1974) Seasonal grazing of *Pseudocalanus minutus* on particles. *Mar Biol* 25: 109-123
- Poulet SA (1976) Feeding of *Pseudocalanus minutus* on living and non-living particles. *Mar Biol* 34: 117-125
- Putt D, Stoecker DK (1989) An experimentally determined carbon:volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. *Limnol Oceanogr* 34: 1097-1103
- Renz J, Hirche H-J (2006) Life cycle of *Pseudocalanus acuspis* Giesbrecht (Copepoda, Calanoida) in the Central Baltic Sea: I. Seasonal and spatial distribution. *Mar Biol* 148: 567-580
- Renz J, Peters J, Hirche H-J (subm) Life cycle of *Pseudocalanus acuspis* Giesbrecht (Copepoda, Calanoida) in the Central Baltic Sea: II. Reproduction, growth and secondary production
- Runge JA, Ingram RG (1991) Under-ice feeding and diel migration by the planktonic copepods *Calanus glacialis* and *Pseudocalanus minutus* in relation to the ice algal production cycle in southeastern Hudson Bay, Canada. *Mar Biol* 108: 217-225
- Sargent JR, Falk-Petersen S (1981) Ecological investigations on the zooplankton community in Balsfjorden, Northern Norway: lipids and fatty acids in *Meganctiphanes norvegica*, *Thysanoessa raschi* and *T. inermis* during mid-winter. *Mar Biol* 62: 131-137
- Sargent JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SCM (eds) *The biological chemistry of marine copepods*. Clarendon Press, Oxford, pp 59-108
- Sargent JR, Whittle KJ (1981) Lipids and hydrocarbons in the marine food web. In: Longhurst AR (ed) *Analysis of marine ecosystems*. Academic Press, San Diego, pp 491-533
- Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. In: Sleight MA (ed) *Microbes in the sea*. Ellis Horwood, Chichester, pp 119-138
- Schlitzer, R (2005) Ocean Data View. <http://www.awi-bremerhaven.de/GEO/ODV>
- Schnack S (1975) Studies on the feeding biology of copepods (Crustacea) in the Kiel Bight (in German). Doctoral Thesis, Kiel University
- Siferd TD, Conover RJ (1992) Natural history of ctenophores in the Resolute Passage area of the Canadian High Arctic with special reference to *Mertensia ovum*. *Mar Ecol Prog Ser* 86: 133-144

- Skerratt JH, Nichols PD, McMeekin TA, Burton HR (1997) Identification of dominant taxa in coastal Antarctic water and ice core samples using lipid signatures. In: Battaglia B, Valencia J, Walton DWH (eds) Antarctic communities. Species, structure and survival. Cambridge University Press, Cambridge, pp 79-85
- Vargas MA, Rodríguez H, Moreno J, Olivares H, Del Campo JA, Rivas J, Guerrero MG (1998) Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. J Phycol 34: 812-817
- Vidal J (1980) Physioecology of zooplankton. I. Effects of phytoplankton concentration, temperature, and body size on the growth rate of *Calanus pacificus* and *Pseudocalanus* sp. Mar Biol 56: 111-134
- Wasmund N, Pollehne F, Postel L, Siegel H, Zettler ML (2003) Assessment of the biological state of the Baltic Sea in 2002 (in German). Meereswiss Ber 56: 1-78
- Zagorodnyaya YA (1974) Nutrition and migration of Black Sea *Pseudocalanus elongatus* (Boeck) in the winter period (in Russian). Gidrobiologicheskii zhurnal 10: 49-56

## CHAPTER II

Life-cycle of *Pseudocalanus acuspes* Giesbrecht (Copepoda, Calanoida)  
in the Central Baltic Sea: II. Reproduction, growth and secondary  
production

Renz J, Peters J, Hirche HJ

submitted to *Marine Biology*



---

## LIFE CYCLE OF *PSEUDOCALANUS ACUSPES* GIESBRECHT (COPEPODA, CALANOIDA) IN THE CENTRAL BALTIC SEA: II. REPRODUCTION, GROWTH AND SECONDARY PRODUCTION

### Abstract

The population dynamics of *Pseudocalanus acuspes* in the Central Baltic Sea were studied from March 2002 to May 2003 on a monthly basis. All stages were present year round with a stage shift from nauplii to older copepodite stages over the course of the year. Biomass, estimated from prosome length, peaked between May and September with maximum recorded values of 594 and 855 mg C m<sup>-2</sup> in May 2002 and 2003, respectively. Differences in biomass between stations up to a factor of 20 were observed especially in April/ May and October. Mean egg production rate (EPR) showed a seasonal course and was highest in April 2002 and 2003 with 3.6 and 2.1 eggs f<sup>-1</sup> d<sup>-1</sup>, respectively, corresponding to a mean weight-specific egg production rate (SEPR) of 0.13 and 0.04. Egg production seems to be limited by food from May on. Stage durations determined from moulting experiments turned out to be extremely long. Maximum growth rates based on stage durations of 15-25 days at 4°C in May and July 2003 amounted for 0.03-0.05 d<sup>-1</sup> in CI-CIV. Comparing these rates with rates derived from temperature-development relationships for *P. acuspes* from the literature resulted in 5 times higher growth rates for the latter case. Secondary production reached values up to 9.1 mg C m<sup>-2</sup> d<sup>-1</sup> (method for continuously reproducing populations) and 10.5 mg C m<sup>-2</sup> d<sup>-1</sup> (increment summation).

### Introduction

Life history traits of copepods, including egg production and growth provide information essential to understand the energy transfer in marine pelagic food webs (Lee et al. 2003), as calanoid copepods are the most important grazers on phytoplankton and prey for higher trophic levels. Physical processes, food and predation are the principle mechanisms accounting for variability in abundance and growth of natural populations (Ohman 1985). While the effect of temperature and food on growth rates of copepods is well studied in the laboratory, annual coverage of population dynamics in the field is scarce for many important species.

In the Central Baltic Sea, *Pseudocalanus acuspes* is a key species, serving as a major food item for commercially important zooplanktivorous fish. Recent studies emphasise the key role of this copepod in the stock dynamics of herring and cod (Möllmann et al. 2003, Hinrichsen et al. 2003). Originating from the Arctic and the Norwegian Sea, *P. acuspes* is considered to be a relict species and lives at marginal physiological conditions in this brackish water system characterised by a permanent halocline and a summer thermocline. Its on-

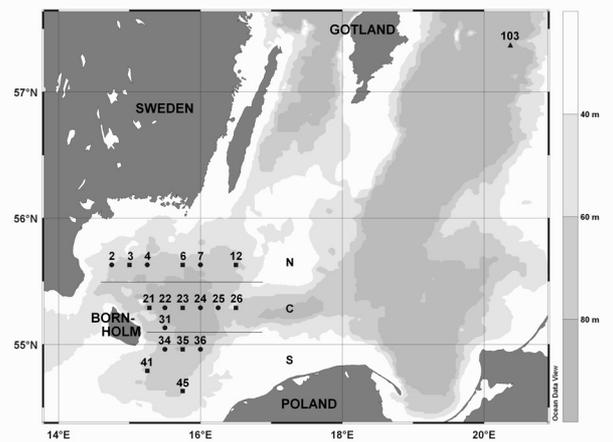
togenetic vertical distribution with youngest stages in the upper water column and older stages concentrated within the halocline is probably a result of stage specific physiological requirements (Renz and Hirche 2006). A study by Möllmann and Köster (2002) based on four collection dates per year found an annual shift in stage composition from younger to older copepodids. A similar stage composition resulted also from our previously published results (Renz and Hirche 2006), suggesting the production of only one generation per year. In the Canadian Arctic Conover and Siferd (1993) described one generation for this species, while in Bedford Basin it produced 3 generations with most of the population in a resting phase as CIII and CIV for several months. Only a small number of individuals completed the life cycle at rates determined by the prevailing temperature (McLaren et al. 1989a). A similar situation was reported for *Pseudocalanus* sp. from the White Sea (Pertsova 1981). In contrast, in Dabob Bay under a temperature regime comparable to the Baltic Sea the congener species *Pseudocalanus* sp. produced several generations per year (Ohman 1985).

This study aims to describe the population dynamics and production of *Pseudocalanus acuspes* in the Bornholm Basin in relation to its physical environment, using data from a high resolution sampling program during German GLOBEC, and to discuss it in light of life cycle strategies and feeding conditions. The measurement of egg production and moulting rates together with data on abundance and stage composition were used to estimate growth and production.

## Materials and methods

### Sampling

Sampling of zooplankton was conducted on 16 cruises between March 2002 and May 2003 on a station grid in the Bornholm Basin (BB), Central Baltic Sea (Fig. 1). On every station double oblique bongo net hauls (mouth opening 0.2 m<sup>2</sup>, mesh size 150 µm, towing speed 3 kn) were taken vertically from above the bottom to the surface; additionally on 9 focus stations sampling was performed using a multinet (Hydrobios, 0.25 m<sup>2</sup> mouth opening, 50 µm mesh size, towing speed 0.2 m s<sup>-1</sup>) towed vertically with a 10 m resolution from the bottom to the surface. Furthermore at the focus stations samples were taken with a WP-2 net (UNESCO 1968, mouth opening 0.57 cm, mesh size



**Figure 1.**

Study area Gotland Basin and Bornholm Basin (Baltic Sea); *square* focus stations, *circle* stations for length measurements, *stations 23 and 103* measurement of moulting rates

200  $\mu\text{m}$ , towing speed  $0.2 \text{ m s}^{-1}$ ) towed vertically from above the bottom to the surface. Bongo and multinet samples were immediately preserved in a 4% borax-buffered formaldehyde-seawater solution.

In the laboratory, subsamples of the multinet hauls were analysed for developmental stages until at least 150 individuals of *Pseudocalanus acuspes* were counted. Individuals were identified to nauplii, 5 copepodite stages and adult males (AM) and females (AF). While in earlier publications the species was often called *P. elongatus* (e.g. Möllmann and Köster 1999, 2002), *P. minutus* (e.g. Dahmen 1995) or *P. minutus elongatus* (e.g. Hernroth 1985) a recent genetic publications found this species to be mainly *P. acuspes* (Bucklin et al. 2003). Therefore we consider it to be *P. acuspes*.

Concurrent to the zooplankton sampling, vertical profiles of temperature and salinity were recorded using a CTD-probe (SBE 911+, ME).

#### Length measurement

For each cruise, prosome length of 30 preserved adult females (AF) from either bongo net or WP-2 net hauls from one northern (N), one central (C) and one southern station (S)(Fig. 1), was measured using a stereo microscope (Leica MZ 16) with a resolution of 80x. The prosome length of 30-60 preserved copepodite stages I-V (CI-CV) and adult males (AM) was measured for 11 cruises (Fig. 1) from 50  $\mu\text{m}$  multinet hauls from station 23. During time of highest egg production rate (Fig. 5b) the total length of nauplii stages I-VI (NI-NVI) was measured in April and May 2002 and March, April and May 2003 with a resolution of 200x.

Total biomass for every cruise and focus station was calculated using the length-weight relationship for *Pseudocalanus elongatus* by Hay et al. (1988).

$$\text{NI-NVI: } \text{LogDM} = 0.989 \log \text{PL} - 2.712$$

$$\text{CI-CIV: } \text{LogDM} = 3.346 \log \text{PL} - 8.899$$

where DM is the dry mass ( $\mu\text{g}$ ), TL the total length and PL the prosome length ( $\mu\text{m}$ ) of an individual. This relationship revealed the best length-weight regression for *P. acuspes* females from the Bornholm Basin (Peters et al. 2006). As length measurements of nauplii and copepodids were missing in some months, a mean between the previous and following month was used to calculate biomass. No correction was made for shrinkage of individuals by fixation as changes in prosome length of preserved copepods are contradictorily discussed. While some authors assumed only small changes in length after preservation with formalin (Williams and Robins 1982, Böttger and Schnack 1986), Kaipris et al. (1997) reported significant reduction of length after preservation of 4.4-15.1%, depending on sex and temperature. Halliday (2001) considered a general reduction of 8%. Assuming a reduction of 8% for all stages would lead to up to 25% higher biomass values during our study.

Differences in prosome length of copepodids between sampling dates as well as differences in prosome length of AF between stations were tested with one-way ANOVA using Tukey's honestly significant difference as the post-hoc test.

#### Egg production

For measurement of in-situ egg production at the focus stations 30 females from the WP-2 net hauls were randomly sorted out immediately after capture under ambient temperature conditions. Niehoff et al. (1999) and Harris et al. (2000) have demonstrated that incubation in small volumes does not affect egg production and egg cannibalism of an egg carrying species can nearly be excluded, females were incubated individually in 15 ml cell wells for 48h under in-situ temperature using 50 µm prefiltered water from the upper halocline. Clutch size and number of reproducing females were recorded and females were preserved in a 4% borax-buffered formaldehyd-seawater solution for later length measurement.

At low temperatures, egg production rates (EPR) might be underestimated when females are incubated for only 24h. We therefore extended our incubation period to 48h. No differences were observed between 24h and 48h incubation periods. As in some cases only 24h incubations are available, we displayed EPR of the first 24h.

#### Moulting rate

Moulting rates of copepodids were measured at the same station as EPR in the BB at station (sta.) 23 in May and July 2003 in short-term incubations (Klein Breteler et al. 1998). As no young copepodids were found in the BB in July 2003, an experiment from sta. 103 in the Gotland Basin (GB) was chosen to get the information on moulting rates of these stages in the Baltic Sea. In May, experiments were incubated at ambient temperature conditions in 50 µm prefiltered water from above the halocline. In July different temperature and salinity conditions (Table 2) were chosen to simulate habitat conditions (Renz and Hirche 2005). For each experiment, 4 sub-samples containing 100-150 copepodids CI-CV each were taken from a WP2 haul. Old exuviae of *Pseudocalanus acuspes* were removed and sub-samples were incubated for 4 days in 1 l Kautex bottles. Every 24h one sub-sample was preserved in a 4% borax-buffered formaldehyd-seawater solution for later enumeration of moults by counting of exuviae. The moulting rate (*MR*) of each stage was calculated after Peterson et al. (1991) as

$$MR_i = \frac{Ex_{i+1}}{[N_i + Ex_{i+1}]} * \frac{24}{T}$$

where  $N_i$  is the number of individuals in stage  $i$  at the beginning of the experiment,  $Ex_{i+1}$  is the number of exuviae in stage  $i+1$  at the end of the experiment and  $T$  is the incubation time

(in hours). The minimum, maximum and mean moulting rate of each stage per experiment was calculated from the 4 sub-samples (Table 2).

#### Weight-specific growth rate

The growth rate ( $g_i$ ) of nauplii and copepodite stages CI-CV was calculated from the expression

$$g_i = \ln\left(\frac{W_{i+1}}{W_i}\right) * \frac{1}{D}$$

where  $W_{i+1}$  is the mass calculated from length measurements of the stage moulted to and moulted from, respectively. No correction for shrinkage of individuals by fixation was made.  $D$  is the stage duration of stage  $i$ . An isochronal development was assumed and  $D$ , calculated for copepodite stages, was adopted for nauplii.

The carbon-specific growth rate of AF ( $g_f$ ) was calculated by first converting the number of eggs to carbon of eggs using  $0.14 * 10^{-6} \mu\text{g C } \mu\text{m}^{-3}$  (Kjørboe et al. 1985, Huntley and Lopez 1992). Egg size for Baltic Sea *P. acuspes* was determined from unpreserved eggs in March 2004 from 10 egg production experiments (mean diameter eggs  $130 \mu\text{m}$ ). Length of AF was determined from preserved samples and we allowed for shrinkage of 8% by fixation (Halliday 2001) when calculating female dry mass and converting to carbon assuming  $0.4 \mu\text{g C } \mu\text{g}^{-1}$  dry mass (Parsons et al. 1984). Female growth rate is then

$$g_f = \frac{W_{eggs}}{W_{female}} * \frac{24}{T}$$

where  $T$  is the incubation time (hours),  $W_{eggs}$  is the carbon mass of eggs ( $\mu\text{g}$ ) produced per day and  $W_{AF}$  the carbon mass of a female.

#### Production and productivity

Production and productivity (P/B) were calculated by 2 different methods: 1. a standard method for continuously reproducing populations, 2. the increment summation method.

Method 1: Instantaneous growth method for continuously reproducing populations (Rigler and Downing 1984)

Production of nauplii, CI-CV and AF was calculated as

$$P_i = (g_i B_i)$$

where  $P_i$  is the production of stage  $i$  ( $\mu\text{g}$  dry mass produced  $\text{m}^{-2}$ ),  $g_i$  is the growth rate of single stage  $i$  and  $B_i$  the biomass of stage  $i$ . Production of the whole population was calculated by summing up the production of every single stage.

Method 2: Increment summation according to Rigler and Downing 1984 (modified in Hirche et al. 2001) for populations where cohorts can be followed

This method computes production from stage to stage by daily increment in biomass as

$$P_{i,i+1} = (M_{i+1} - M_i) * (X_i / D_i + X_{i+1} / D_{i+1}) / 2$$

where  $P_{i,i+1}$  is the daily production,  $X_{i,i+1}$  is the abundance,  $M_{i,i+1}$  are the mean masses and  $D_{i,i+1}$  are the stage durations of stages  $i$  and  $i+1$ .

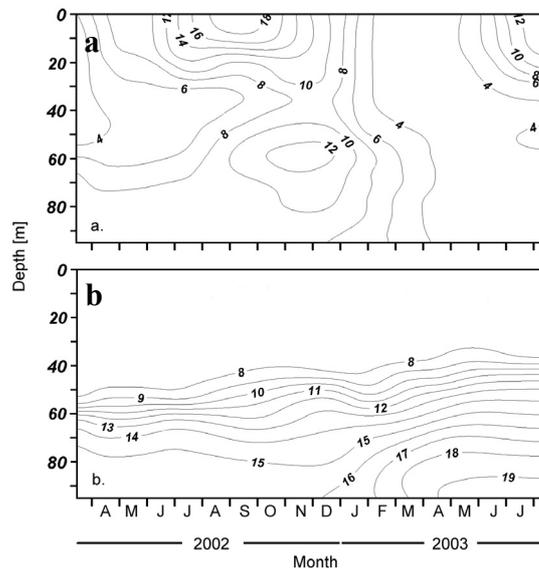
## Results

### Hydrography

Hydrography in the Bornholm Basin was characterised by a permanent halocline (Fig. 2b), which was located at around 40-50 m depth at station 23 between March 2002 and July 2003. From March to December 2002, salinity averaged 7 above the halocline and increased towards the bottom up to 16. Temperature (Fig. 2a) in March 2002 averaged 4°C above and 8°C below the halocline. A summer thermocline was developing from May on, leading to temperatures up to 20°C in the upper 20 m and between 4°C and 10°C in the intermediate layer. After a Major Baltic Inflow event (MBI, Feistel et al. 2004) in January 2003, salinity below the halocline reached values up to 20.

#### Figure 2.

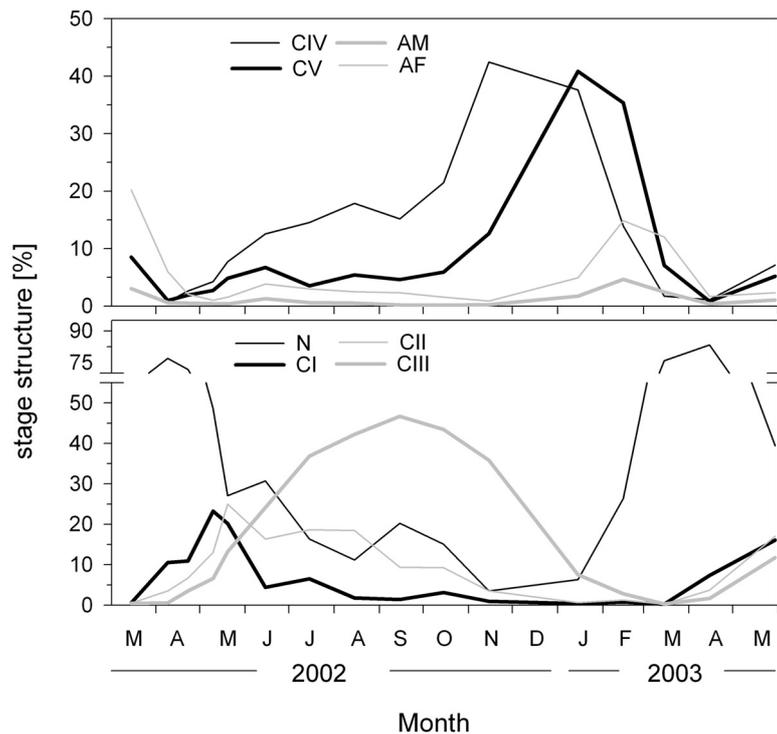
Hydrography in the Bornholm Basin at station 23 from March 2002 to May 2003; **a.** temperature [°C], **b.** salinity



### Stage structure

The mean relative stage composition in the study area showed a seasonal stage shift from nauplii in spring and early summer to older copepodids in August (Fig. 3). Nauplii made up >63% of the total population in March and April 2002. Their proportion declined over the

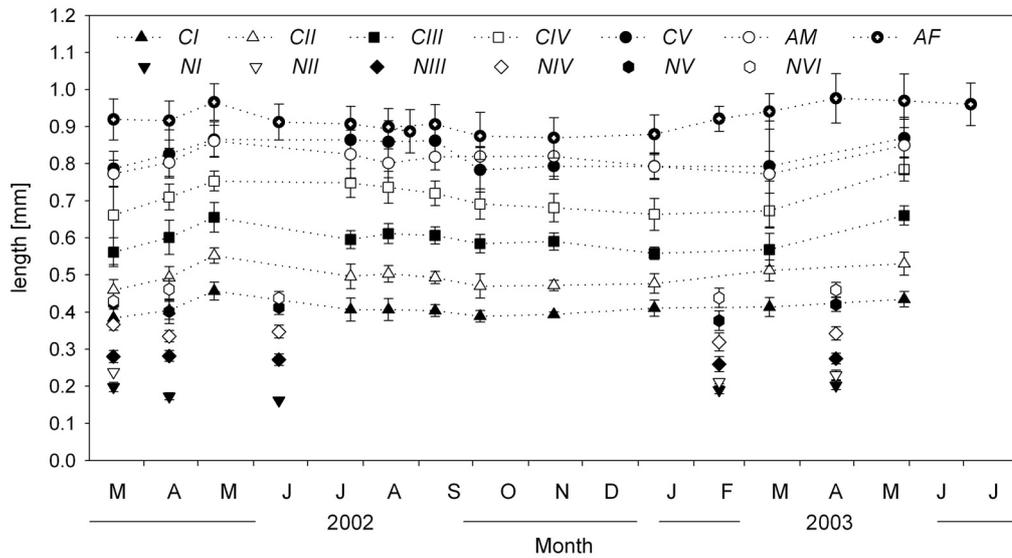
following months and was lowest during November 2002 (3.5%). The highest proportion of CI was found in May, followed by CII in May to July and CIII from August to October 2002. CIV and CV dominated the late autumn and early winter population, they made up >55% of the population from November to January.



**Figure 3.** Mean stage structure of *Pseudocalanus acuspes* in the Bornholm Basin from March 2002 to May 2003

#### Prosome length of developmental stages

The mean prosome length of the developmental stages of *P. acuspes* is shown in Fig. 4. The length of copepodite stages ranged from 0.384 mm (CI) to 0.976 mm (adult female, AF). Nauplii were only measured in spring; their total length ranged from 0.162 mm (NI) to 0.462 mm (NVI). Length of all copepodids and adults increased by 0.04-0.05 mm between March and May 2002, except for the naupliar stages, which became smaller during this period. Subsequently length decreased in all stages measured starting in May with CI-CIII, followed by CIV in July and CV in September. This period of smaller individuals was followed by an increase in length from February/March on especially in the older stages and adults. Statistical analysis of mean prosome length of AF using one-way ANOVA and the associated post-hoc test showed significant horizontal differences ( $p < 0.05$ ) in length only between the N and S stations in April and June 2002 and between the N and S as well as S and C stations in July 2003 (data not shown).



**Figure 4.** Total length of *Pseudocalanus acuspis* nauplii stages and prosome length of copepodite stages and adults from March 2002 to July 2003 in the Bornholm Basin; *symbols* mean values, *error bars* standard deviation

Statistical analysis of mean prosome length of copepodite stages and adults using one-way ANOVA showed significant differences ( $p < 0.05$ ) of mean length between sampling months (data not shown). The post hoc test, comparing pairs of means, showed that especially CI-III and AF during May in both years were significantly larger than those found from August to November ( $p < 0.05$ ), while size of CIV and CV in May differed significantly in size from those found from October 2002 to March 2003 ( $p < 0.05$ ). Length variation in males was small and differences were only significant ( $p < 0.05$ ) between May 2002 and August 2002 as well as January and March 2003.

### Biomass

The biomass of *Pseudocalanus acuspis* (Table 1) showed a pronounced seasonal trend with highest values between May and September 2002 and in May 2003, when nauplii and younger stages dominated the population. Maximum biomass was observed in May 2002 ( $594 \text{ mg C m}^{-2}$ ) and in May 2003 ( $855 \text{ mg C m}^{-2}$ ). Concentrations below  $20 \text{ mg C m}^{-2}$  were recorded in autumn and winter. After the inflow of cold, saline North Sea water the maximum biomass was 1.4 times higher compared to the previous year. Differences in biomass between stations up to a factor of 20 were observed in May and September 2002.

## Reproduction

Egg production rate (EPR)(Fig. 5b) showed high variability and ranged from 0 at several stations to a maximum of 3.6 eggs  $f^{-1} d^{-1}$  at station 23 in April 2002 and 2.1 eggs  $f^{-1} d^{-1}$  at station 35 in April 2003. Mean EPR per cruise ranged from 0.1 eggs  $f^{-1} d^{-1}$  in winter to 1.2 eggs  $f^{-1} d^{-1}$  in spring and showed a peak in April and May 2003. A second small peak could be identified in August 2002. In 2002 highest EPR were recorded in the marginal areas of the BB, while in 2003 the central station 35 showed also high values.

**Table 1.** Cruisedates, minimum, maximum and mean biomass [B][mg C  $m^{-2}$ ] of *Pseudocalanus acuspes*

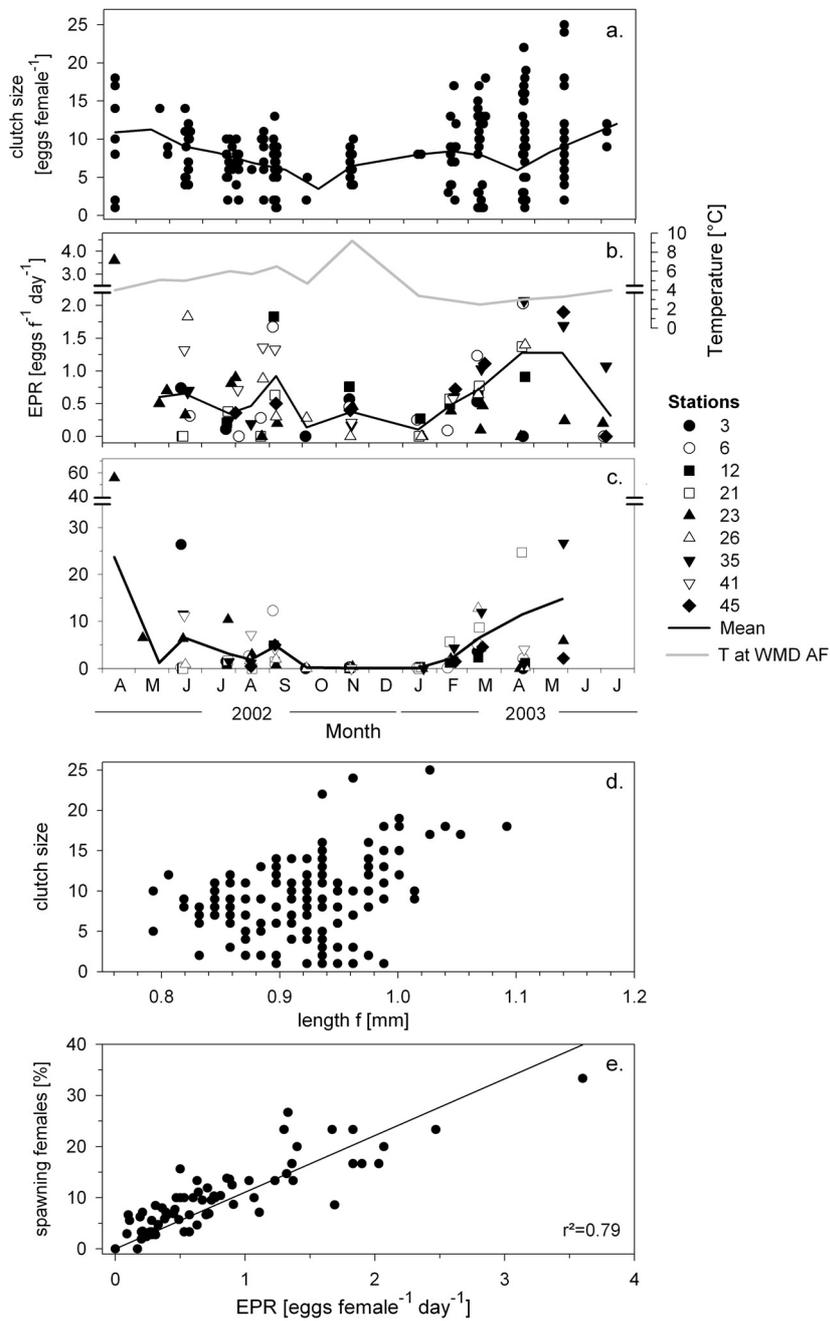
| Date                | B <sub>min</sub> | B <sub>max</sub> | B <sub>mean</sub> |
|---------------------|------------------|------------------|-------------------|
| 12.03. - 22.03.2002 | 50.74            | 57.79            | 53.62             |
| 02.04. - 16.04.2002 | 47.03            | 178.64           | 102.64            |
| 16.04. - 30.04.2002 | 22.86            | 344.24           | 166.8             |
| 05.05. - 24.05.2002 | 32.55            | 271.76           | 103.2             |
| 15.05. - 30.05.2002 | 30.29            | 595.29           | 305.94            |
| 11.06. - 23.06.2002 | 121.8            | 494.26           | 296.46            |
| 22.07. - 07.08.2002 | 82.98            | 501.5            | 304.8             |
| 12.08. - 21.08.2002 | 85.78            | 309.45           | 201.96            |
| 03.09. - 13.09.2002 | 120.48           | 383.73           | 240.96            |
| 30.09. - 10.10.2002 | 19.36            | 196.5            | 96.47             |
| 11.11. - 29.11.2002 | 18.56            | 70.31            | 50.14             |
| 13.01. - 24.01.2003 | 17.36            | 92.71            | 46.8              |
| 10.02. - 21.02.2003 | 30.21            | 129.54           | 58.6              |
| 03.03. - 22.03.2003 | 30.11            | 202.41           | 74.3              |
| 17.04. - 28.04.2003 | 30.01            | 510.49           | 154.34            |
| 15.05. - 03.06.2003 | 81.39            | 855.03           | 339.49            |
| 01.07. - 19.07.2003 | No data          | No data          | No data           |

Clutch size (Fig. 5a) ranged between 2 and 25 eggs  $f^{-1}$  with minimum and maximum mean values of 3.5 eggs  $f^{-1}$  in October 2002 and 12 in July 2003. The correlation between the proportion of spawning females and the EPR (Fig. 5e) revealed a significant positive relationship ( $r^2=0.79$ ,  $p<0.001$ ). In contrast to other studies there was no significant correlation between clutch size and female prosome length ( $r^2=0.14$ )(Fig. 5d), though a trend of larger clutches could be observed for larger females.

Population egg production rate (eggs  $m^{-2} d^{-1}$ ) was calculated for each station where egg production was measured (Fig. 5c). Mean population egg production is shown using mean female abundance on the grid and mean egg production for the cruise. April to June was clearly the phase of highest egg production of the population, followed by a period of intermediate production in summer and a period of hardly any egg production, which lasted until February 2003. The proportion of nauplii (Fig. 3) closely reflects the seasonal population egg production.

## Moulting rates and stage duration

Moulting rates varied between different stages, sampling dates and regions (Table 2). In the GB CI and CII showed lower moulting rates than CIII. CIV and CV did not moult at all in this area during the 4 days of incubation. In May moulting rates of all stages examined were higher in the BB than in the GB and decreased in July. In the BB CIV/CV moulted during the experiments both in May and in July.



**Figure 5.** Reproduction of *Pseudocalanus acuspes* in the Bornholm Basin April 2002 to July 2003; **a.** clutch size, *black line* mean clutch size, **b.** egg production rate (EPR), *black line* mean EPR, *grey line* temperature [°C] at weighted mean depth (WMD) of females, **c.** population egg production, **d.** correlation between number of eggs and length of females, **e.** correlation between proportion of spawning females and EPR

**Table 2:** Moulting rates (M) and stage duration (D) of *Pseudocalanus acuspes* at station (sta.) 103 in the Gotland Basin (GB) and sta. 23 in the Bornholm Basin (BB) at *in situ* temperature (T) and salinity

| Area          | Date   | Stage | T [°C] | Salinity | M [%]        |      | D [days]    |      |
|---------------|--------|-------|--------|----------|--------------|------|-------------|------|
|               |        |       |        |          | range        | mean | range       | mean |
| GB (sta. 103) | Jul-03 | CI    | 3      | 7        | 1.62 - 5.15  | 3.81 | 19.4 - 61.7 | 26.2 |
|               |        | CII   | 3      | 7        | 2.17 - 7.14  | 3.74 | 14.0 - 46.1 | 26.7 |
|               |        | CIII  | 3      | 10.5     | 1.66 - 4.17  | 2.76 | 24.0 - 60.2 | 36.2 |
|               |        | CIV-V | 3      | 10.5     | 0            | -    | -           | -    |
| BB (sta. 23)  | May-03 | CII   | 3.7    | 14.8     | 4.63 - 14.29 | 6.25 | 7.0 - 21.6  | 16   |
|               |        | CIII  | 3.7    | 14.8     | 3.33 - 13.33 | 7.01 | 7.5 - 30.0  | 14.3 |
|               |        | CIV-V | 3.7    | 14.8     | 1.30 - 7.32  | 4.53 | 13.7 - 77.0 | 22   |
|               | Jul-03 | CIII  | 4      | 16.2     | 2.35 - 4.44  | 3.39 | 22.5 - 42.6 | 29.5 |
|               |        | CIV-V | 4      | 16.2     | 2.38 - 4.26  | 3.4  | 23.5 - 42.0 | 29.4 |

The temperatures and salinities chosen for the experiments reflected the environmental conditions at which the animals were collected. The increase of moulting rates with increasing salinity in Table 2 suggests a strong relationship. However, experiments on the salinity influence on stage duration revealed no clear relation (Renz unpublished data).

#### Weight-specific growth rates

Stage durations in our experiment lasted 2-3 times longer than those calculated from temperature-development relationships for *Pseudocalanus* sp. from the literature (in Eiane and Ohman 2004). Therefore we used fixed stage durations of 15 and 25 days for each stage for further calculation of growth rates and production; no temperature effect was considered. (Fig. 6b, c). Continuous growth throughout the year was assumed. The resulting growth rates at 15 d stage durations were highest in CI to CIV (0.03 to 0.05 d<sup>-1</sup>). CV developing to small AM and larger AF, showed smallest growth (0 to 0.02 d<sup>-1</sup>). A 25 d stage duration reduced growth rates for CI-CIII down to 0.02 to 0.03 d<sup>-1</sup> for young copepodite stages and to below 0.1 d<sup>-1</sup> for CV. For comparison we estimated stage durations at different temperatures from generation times reported for *P. acuspes* from Nova Scotia by McLaren et al. (1989b), assuming an isochronal development. Application of these durations produced a pronounced peak in October and November and growth rates up to 5-9 times higher than for stage durations of 15 and 25 d (Fig. 6d).

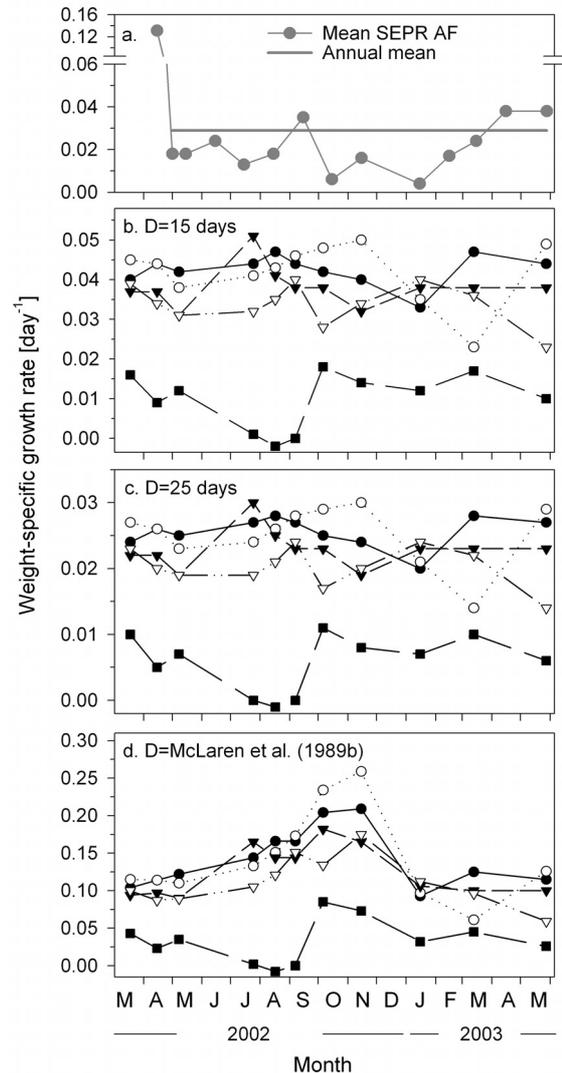
Except for the maximum in April 2002 (0.13 d<sup>-1</sup>), weight-specific growth rates of females varied between 0.01 and 0.04 d<sup>-1</sup> with a mean of 0.03 d<sup>-1</sup>, when the high value in April 2002 is excluded (Fig. 6a). During times of highest reproduction in September 2002 as well as April and May 2003 they were comparable to growth rates of young copepodids for a stage duration of 15 d. Over the rest of the year, growth rates of females were comparable to those of older copepodids at a stage duration of 25 days.

## Secondary production

Cumulative secondary production (method 1) of all stages using the stage durations described before is shown in Fig. 7b-d; for calculation of female production, female weight-specific egg production was used. The main productive period of *Pseudocalanus acuspes* copepodids in the BB lasted from May to September, female production started one month earlier. The seasonal course was basically unimodal and consisted of a long increase originating from production of CI-II while the peak in July and the slow decrease until September were mainly caused by production of CIII and CIV. Applying a stage duration of 15 d, highest production of  $4.8 \text{ mg C m}^{-2} \text{ d}^{-1}$  was recorded for CIII in July 2002, followed by values of  $3.8 \text{ mg C m}^{-2} \text{ d}^{-1}$  of females in April 2002. The cumulative mean production of all stages (eggs to CV) was highest in July ( $9.1 \text{ mg C m}^{-2} \text{ d}^{-1}$ ). A stage duration of 25 d reduced production of copepodite stages by 25% for CI-CII and 35-40% for CIII-CV. This led to a decrease in overall production of all stages of 35%.

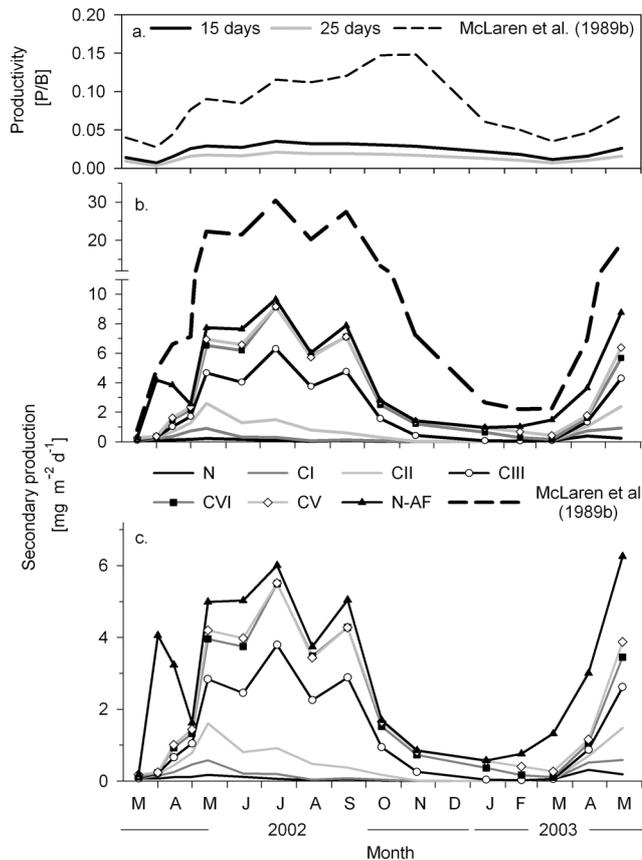
Using the temperature dependent stage durations from generation times for *P. acuspes* reported by McLaren et al. (1989b), the seasonal course in production confirmed our data well (Fig. 7b). However, production exceeded our values up to 3.3 times and led to a cumulative mean secondary production up to  $30 \text{ mg C m}^{-2}$  adding up production of single stages.

A comparison of two methods for the estimation of secondary production and productivity is presented in Table 3 with both methods using a stage duration of 15 d. The increment summation (method 2) estimated daily mean production of the population to be up to  $10.5 \text{ mg C m}^{-2} \text{ d}^{-1}$  in July 2002, 14% higher than estimated by the method for continuously reproducing populations (method 1). The corresponding productivities were 0.035 and 0.031, respectively. Lowest production ( $<0.6 \text{ mg C m}^{-2} \text{ d}^{-1}$ ) was measured in March and early April 2002. In general, comparing method 1 and 2 resulted in similar production and productivity values.



**Figure 6.**

**a.** Weight-specific growth rate (=specific egg production rate, SEPR) of AF and **b.-d.** Weight-specific growth rates of nauplii and copepodite stages of *Pseudocalanus acuspes* from March 2002 to May 2003; *filled circle* CI, *open circle* CII, *filled triangle down* CIII, *open triangle down* CIV, *square* CV



**Figure 7.**

**a.** Mean productivity (P/B) of *Pseudocalanus acuspes* between March 2002 and May 2003 in the Bornholm Basin,  
**b.-d.** Cumulative mean secondary production of nauplii and copepodite stages for stage durations (D) of 15 days (b.), 25 days (c.) and a temperature dependent stage duration derived by a generation time from McLaren et al. (1989b) (grey line) (b.) together with production of adult females (AF) (b. and c.)

## Discussion

### Biomass

In temperate ecosystems biomass of copepods undergoes seasonal changes with a typical unimodal distribution and a peak usually during late spring and summer (Colebrook 1979). During our study biomass of *Pseudocalanus acuspes* showed a seasonal cycle with a minimum in March and an increase during a phytoplankton bloom in April (van Beusekom et al. submitted) It peaked between May and July with maxima in May 2002 ( $594 \text{ mg C m}^{-2}$ ) and May 2003 ( $858 \text{ mg C m}^{-2}$ ). Möllmann and Köster (1999), reporting historical biomass data collected in the Gdansk Deep during July found concentrations similar to our 2002 values.

Time series analysis showed significantly higher biomass of *Pseudocalanus* in years with higher salinities (Dippner et al. 2000; Möllmann et al. 2000; 2003). It was substituted by *Acartia* spp. in the long period of lower salinity in the Central Baltic Sea before 1993 (Kononen et al. 1996). The increased biomass at one deeper station in May 2003 as compared to 2002 could therefore be related to the inflow of cold, saline North Sea water with the MBI in the beginning of 2003. Renz and Hirche (2006) discussed several mechanisms responsible for an increased biomass such as advection of *P. acuspes* populations from the western Baltic Sea or the increase of the habitat in the deep basins. However, an effect of improved living conditions was to be expected only with some delay after our sampling period.

Differences in biomass between stations up to a factor of 20 were observed especially in April/ May and October. The spatial variability in biomass clearly emphasizes the spatial and temporal resolution required for representative time series studies.

### Reproduction

*Pseudocalanus acuspes* produced eggs throughout the study period, though there was a seasonal trend with highest rates in April 2002 and 2003 and lowest values in October 2002 and January 2003. As egg production of this species has not been measured before comparisons are only possible with congeners. Our maximum (3.6 eggs  $f^{-1} d^{-1}$ ) coincides with the 2.5 eggs  $f^{-1} d^{-1}$  in the North Sea (Kiørboe and Johansen 1986)

and 3.3 eggs  $f^{-1} d^{-1}$  at 12-14°C in *Pseudocalanus* sp. from Dabob Bay (Ohman 1985). In laboratory experiments with *P. elongatus* from the North Sea Koski et al. (1998) reported 2-5 eggs  $f^{-1} d^{-1}$  at 15°C and good food quality while Corkett and Zillioux (1975) found 1.1 eggs  $f^{-1} d^{-1}$  at 4°C, 2.3 at 8°C and 3.4 eggs  $f^{-1} d^{-1}$  at 16°C; similarly Paffenhöfer and Harris (1976) observed between 3.1 and 4.7 nauplii  $f^{-1} d^{-1}$  at 12.5°C. Highest egg production was reported by Halsband and Hirche (2001) for the German Bight, where *P. elongatus* produced up to 8 eggs  $f^{-1} d^{-1}$  at 3°C; however, these females were more than 150  $\mu m$  larger.

The relatively low egg production in this genus seems to be related to the large size of their eggs (diameter 130  $\mu m$ ). Our maximum egg production in April 2002 corresponds to a specific egg production rate (SEPR) of 0.13. This is very high when compared to the SEPR of 0.04 in April/ May 2003 and might result from a combination of warmer temperature and more suitable food conditions. However, only one experiment for the measurement of EPR was determined in April 2002. Our annual mean SEPR (0.03) is similar to the 0.055 (mean 0.014) of Kiørboe and Johansen (1986) in September at 9-12°C in the northern North Sea. For the same species, a weight-specific fecundity of 0.078 (Corkett and Zillioux 1975), 0.09 (Paffenhöfer and Harris 1976) and 0.03-0.11 at good food quality (Koski et al. 1998) is reported at 15°C.

The seasonal cycle of egg production is usually controlled by female size, food and temperature (Corkett and McLaren 1978 and references therein; Jónasdóttir 1989, Halsband and

**Table 3.**

Mean secondary production (P) and productivity (P/B) of *Pseudocalanus acuspes* in the Bornholm Basin; method [M] 1=method for continuously reproducing populations, M 2=increment summation method

| Month       | P [mg C m <sup>-2</sup> d <sup>-1</sup> ] |       | P/B   |       |
|-------------|---|-------|-------|-------|
|             | M 1                                       | M 2   | M 1   | M 2   |
| <b>2002</b> |   |       |       |       |
| March       | 0.27                                      | 0.53  | 0.005 | 0.011 |
| April_1     | 0.23                                      | 0.45  | 0.005 | 0.008 |
| April_2     | 1.54                                      | 1.89  | 0.013 | 0.015 |
| May_1       | 2.35                                      | 2.6   | 0.024 | 0.026 |
| May_2       | 6.9                                       | 8.24  | 0.027 | 0.030 |
| June        | 6.54                                      | 7.62  | 0.024 | 0.027 |
| Jul         | 9.14                                      | 10.45 | 0.031 | 0.035 |
| Aug         | 5.71                                      | 6.7   | 0.029 | 0.034 |
| Sep         | 7.09                                      | 8.14  | 0.029 | 0.034 |
| Oct         | 2.75                                      | 3.14  | 0.029 | 0.033 |
| Nov         | 1.39                                      | 1.58  | 0.028 | 0.032 |
| <b>2003</b> |   |       |       |       |
| Jan         | 0.95                                      | 1.24  | 0.019 | 0.011 |
| Feb         | 0.66                                      | 1.09  | 0.011 | 0.008 |
| March       | 0.41                                      | 0.81  | 0.004 | 0.015 |
| April       | 1.75                                      | 1.96  | 0.013 | 0.026 |
| May         | 6.36                                      | 7.58  | 0.023 | 0.030 |

Hirche 2001). In the BB female size showed relatively small variations (Renz and Hirche 2006), and the correlation between egg production and prosome length was not significant. Only little effect could be expected from temperature, which slightly increased at WMD of females from 3°C to 6.5°C, except a short peak in November (9°C). Primary production in the BB is basically restricted to the waters above the thermocline (van Beusekom et al. submitted) which forms the upper boundary of the habitat of *P. acuspes* (Renz and Hirche 2006). Only in late April 2002 a sedimenting phytoplankton bloom reached down to the bottom (van Beusekom et al. submitted) and probably was responsible for the peak in egg production. This is also documented in an increase in diatom and dinoflagellate fatty acid markers (Peters et al. 2006). Surprisingly, during this period the females performed a descend and concentrated near the halocline (Renz and Hirche 2006). As indicated by signature fatty acids, the diet of *P. acuspes* mainly consists of microzooplankton and sinking detritus. Due to reduced food sedimentation in summer and winter, diet might limit egg production throughout most of the year. This is consistent with very low storage lipid amounts of females during summer and winter (Peters et al. 2006).

While in some *Pseudocalanus* species females are hardly present during winter in the Greenland Sea (Richter 1994), *P. elongatus* in the Kattegat (Kjørboe and Nielsen 1994) and in the North Sea (Halsband and Hirche 2001) and *P. acuspes* in the Central Baltic Sea are breeding throughout the year, though the proportion of spawning females is low during this time. Based on lipid content of CV and females in winter (total lipid 1.8 and 1.2 µg per individual, respectively; Peters et al. 2006) it is unlikely that females are able to fuel egg production from lipid reserves alone. Therefore food uptake was necessary to maintain the egg production observed during winter. McLaren et al. (1989a) further suggested that only copepodids with insufficient lipid content accomplish maturation during summer and autumn to potentially produce more successful offspring.

#### Moulting, development and growth

Stage durations of the younger copepodids in May and July derived from moulting rates were low and not isochronous, but increased with age and depth inhabited (Renz and Hirche 2006). The older stages CIV/CV in the GB in July hardly moulted at all, indicating arrested development, while they moulted in the BB. According to temperature-growth relationships established for *Pseudocalanus* spp. by Eiane and Ohman (2004) from various sources, stage durations are around 8 d (CI-CIII), 6 d (CIV) and 11 d (CV) at 3-4°C. In the field, Ohman (1994) found generation times of approx. 40 days at 8°C and 26-27 days at 13.4 °C in Dabob Bay, which agree well with laboratory measurements by Klein Breteler et al. (1982) and Paffenhöfer and Harris (1976). At 5°C, Klein Breteler et al. (1995) reported stage durations of ~5 days for most developmental stages and a generation time of ~60 days. Our stage durations are extremely long when compared to field populations of the congener *P. elongatus* from the North Sea (Renz unpublished data) and at least twice those predicted from temperature-growth relationships. This results in considerably lower growth rates than reported for

*Pseudocalanus* species from the literature (Paffenhöfer and Harris 1976, Klein Breteler et al. 1982, McLaren et al. 1989b, Peterson et al. 1991, Koski et al. 1998), but our growth rates for stage durations of 15 days are in good agreement with those reported by Ciszewski and Witek (1977) for *Pseudocalanus "elongatus"* from the Gdansk Bay at 5°C (0.026-0.058).

There are some uncertainties in our approach to calculate growth rate. The adoption of copepodid stage durations on nauplii might miscalculate growth rates as the first nauplii stages are known not to feed and have short developmental times. Furthermore, different copepodite stages experience different biotic and abiotic conditions due to their ontogenetic vertical distribution (Renz and Hirche 2006) and may therefore not grow at the same rate.

The large variability in the moulting experiments and the long incubation times necessary here without doubt suggest that our results have to be taken with caution, as previously discussed by Hirst et al. (2005) for such conditions. On the other hand, our observations show interesting similarities to observations by McLaren et al. (1989a) in Bedford Basin. They estimated generation times longer by approximately 30% than expected and proposed the population was in a resting phase as CIII and CIV for most of the year with only a small number of individuals maturing and producing further generations in summer and autumn. In the BB the portion of older copepodite stages was also high during more than six months of the year. The lipid content of CV, which from May on contains a large portion of wax esters until January when compared to the very low values of females over summer (Peters et al. 2006) further supports the possibility of an arrested development in older copepodite stages. The low salinity in the BB may also contribute to the slow development. It puts strong constraints on the habitat of *P. acuspes* and forces older copepodids and adult stages to stick to the halocline where food is limited, as this habitat is for most of the time separated from the euphotic layer.

### Life cycle

The fact that *Pseudocalanus acuspes* was breeding continuously and most stages were present all the time (Fig. 3) together with obvious inhomogeneous horizontal distribution complicates understanding of the life cycle. Furthermore, the role of advection has to be taken into account, as several inflow events were observed during the study period. For these reasons our interpretation has to be tentative until better data are available. The appearance of a relatively large portion of males in the BB in February/March clearly indicates the beginning of the spawning period. According to Corkett and McLaren (1978) appearance of adult males is a good indicator of recruitment of a new generation in *Pseudocalanus* as they are short-lived. The highest proportion of females in 2002 was recorded during March and April. However, an female peak in February/March 2003 suggests that we may have missed the early peak in 2002. The main reproductive period started in March/April, as indicated by the high population egg production rate and the steep increase in nauplii abundance, and lasted until June. A mean stage duration of 15 days as found for CII-CIV in May 2003 in the Born-

holm Basin would lead to a generation time of >150 days and the first adults of this generation would be expected at the end of August. Indeed this was the time when a second small peak in reproduction was recorded, though it was followed only by a minor peak in nauplii. Beginning in July CIII-CV were accumulating and formed the overwintering stock, which persisted until February. From stage composition, changes in prosome length and estimates of developmental times a further cohort may have developed during summer.

#### Secondary production and productivity (P/B)

The measurement of copepod secondary production in the field requires detailed knowledge on the life cycle with abundance, mass determination and stage duration to be the regulating factors. Our mean estimates of secondary production in the BB using a stage duration of 15 days, is in the lower range of the 8-24 mg C m<sup>-2</sup> day<sup>-1</sup> found by Peterson et al. (1991) for *Pseudocalanus* sp. in August in the Skagerrak. Kiørboe and Nielsen (1994) found highest cumulative production for several copepod species in the Kattegat in March-April and August-September, associated with phytoplankton blooms. They related variation in copepod biomass primarily to mortality, rather than to temperature. Production of youngest stages, living in the upper part of the water column (Renz and Hirche 2006), started to increase with the phytoplankton peak in April. Highest production was recorded in July when the characteristic vertical distribution pattern already restricted *P. acuspes* from direct utilisation of food in the euphotic zone. From May on food might therefore be a limiting factor. Additionally, this distribution exposed *P. acuspes* to his main predators, herring and cod (Möllmann et al. 2003, Hinrichsen et al. 2003). Mortality might therefore be an important factor regulating production of this species in the BB. A potential arrest of development of older stages further would limit secondary production of *P. acuspes* in the BB. Productivity in the study area was well in the range of values reported by Ciszewski and Witek (1977) for *Pseudocalanus "elongatus"* in the Gdansk Bay at 5 °C with highest values observed between May and November.

Two different methods were applied to estimate production. While the increment summation describes production of populations, where cohorts can be followed, the method for continuously reproducing populations is applied for populations in a steady state (Rigler and Downing 1984). Both approaches do not exactly fit our observations and can only result in an approximation, as on the one hand no clear cohorts could be followed due to difficulties with interpreting the life cycle. On the other hand reproduction was not continuously high but decreased after April/ May. The resulting estimates of highest production in July however were very similar, 10.5 and 9.1 mg C m<sup>-2</sup>, corresponding to a productivity of 0.029 and 0.034, respectively. Stage duration is the factor influencing both methods and the exemplified year-round adoption of long stage durations, measured in May and July 2003, must result in comparably low production. Clearly, no concrete predictions can be made for stage durations beyond the investigation period. Especially an adequate food situation in spring might shorten developmental times. If this is the case, our estimates for winter and early

spring would fairly underestimate secondary production of *P. acuspes*. This emphasizes the importance of measuring stage durations in field populations. The application of temperature-development relationships from the literature (McLaren et al 1989b) might therefore firstly give best information. The comparably long stage durations in May and July however suggest that the generally used temperature dependent stage durations are insufficient to describe year round production of *P. acuspes* in the Central Baltic Sea.

### Acknowledgements

We like to thank the crews and scientific parties of the RV Alkor, Heincke and A.v.Humboldt for the provided support during the field phase, K. Barz for help with the egg production experiments and U. Holtz for help with length measurements. The critical revision and improvement of the manuscript by three anonymous reviewers is gratefully acknowledged. The study was funded by the German Federal Ministry for Education and Research within the GLOBEC GERMANY project (03F0320C).

### Literature

- Böttger R, Schnack D (1986) On the effects of formaldehyde fixation on the dry weight of copepods. *Meeresforschung* 31: 141-152
- Bucklin A, Frost BW, Bradford-Grieve J, Allen LD, Copley NJ (2003) Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. *Mar Biol* 142: 333-343
- Ciszewski P, Witek Z (1977) Production of older stages of copepods *Acartia bifilosa* Giesb. and *Pseudocalanus elongatus* Boeck in Gdansk Bay. *Pol Arch Hydrobiol* 24: 449-459
- Colebrook JM (1979) Continuous plankton records: seasonal cycles of phytoplankton and copepods in the north Atlantic Ocean and the North Sea. *Mar Biol* 51: 23-32
- Conover RJ, Siferd TD (1993) Dark-season survival strategies of coastal zone zooplankton in the Canadian Arctic. *Arctic* 46: 303-311
- Corkett CJ, McLaren IA (1978) The biology of *Pseudocalanus*. *Adv Mar Biol* 15: 1-231
- Corkett CJ, Zillioux EJ (1975) Studies on the effect of temperature on the egg laying of three species of calanoid copepods in the laboratory (*Acartia tonsa*, *Temora longicornis* and *Pseudocalanus elongatus*). *Bull Plankton Soc Jap* 21: 77-85
- Dahmen K (1995) Vertikalverteilung und produktionsbiologische Bedeutung des Mesozooplanktons im Bornholm-Becken (Südliche Ostsee). *Berichte aus dem Institut für Meereskunde an der Universität Kiel Nr. 273*: pp 175
- Dippner JW, Kornilovs G, Sidrevics L (2000) Long-term variability of mesozooplankton in the Central Baltic Sea. *J Mar Syst* 25: 23-32
- Eiane K, Ohman MD (2004) Stage-specific mortality of *Calanus finmarchicus*, *Pseudocalanus elongatus* and *Oithona similis* on Fladen Ground, North Sea, during a spring bloom. *Mar Ecol Prog Ser* 268: 183-193
- Feistel R, Nausch G, Matthäus W, Lysiak-Pastuszek E, Seifert T, Hansen IS, Mohrholz V, Krüger S, Buch E, Hagen E (2004) Background data to the exceptionally warm inflow into the Baltic Sea in late summer of 2002. *Mar Sci Rep* 58: 1-59

- Halliday NC (2001) A comparison of morphometric and geometric methods for the estimation of individual zooplankton volumes. *Sarsia* 86: 101-105
- Halsband C, Hirche HJ (2001) Reproductive cycles of dominant calanoid copepods in the North Sea. *Mar Ecol Prog Ser* 209: 219-229
- Harris RP, Wiebe PH, Lenz J, Skjoldal HR, Huntley M (eds)(2000) *Zooplankton Methodology Manual*. Academic Press, pp 410-412
- Hay SJ, Evans GT, Gamble JC (1988) Birth, growth and death rates for enclosed populations of calanoid copepods. *J Plankton Res* 10: 431-454
- Hernroth L (1985) Recommendations on methods for marine biological studies in the Baltic Sea: Mesozooplankton Biomass Assessment. *The Baltic Marine Biologists* 10: 1-32
- Hinrichsen H, Lehmann A, Möllmann C, Schmidt J (2003) Dependency of larval fish survival on retention/dispersion in food limited environments: the Baltic Sea as a case study. *Fish Oceanogr* 12: 425-433
- Hirche HJ, Brey T, Niehoff B (2001) A high-frequency time series at Ocean Weather Ship Station M (Norwegian Sea): Population dynamics of *Calanus finmarchicus*. *Mar Ecol Prog Ser* 219: 205-219
- Hirst AG, Peterson WT, Rothery P (2005) Errors in juvenile copepod growth rate estimates are widespread: Problems with the Moulting Rate method. *Mar Ecol Prog Ser* 296: 263-279
- Huntley ME, Lopez MDG (1992) Temperature-dependent production of marine copepods: A global synthesis. *Am Nat* 140: 201-242
- Jónasdóttir SH (1989) Effects of food concentration on egg-production rates of two species of *Pseudocalanus*: Laboratory observations. *J Exp Mar Biol Ecol* 130:33-43
- Kaipris K, Miliou H, Moraitou-Apostolopoulou M (1997) Effects of formaldehyde preservation on biometrical characters, biomass and biochemical composition of *Acartia clausi* (Copepoda, Calanoida). *Helgoländer Meeresunters* 51: 95-106
- Kjørboe T, Møhlenberg F, Hamburger K (1985) Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar Ecol Prog Ser* 26: 85-97
- Kjørboe T, Johansen K (1986) Studies of a larval herring (*Clupea harengus* L.) patch in the Buchan area. 4. Zooplankton distribution and productivity in relation to hydrographic features. *Dana* 6: 37-51
- Kjørboe T, Nielsen TG (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 1. Copepods. *Limnol Oceanogr* 39: 493-507
- Klein Breteler WCM, Franz HG, Gonzalez SR (1982) Growth and development of four calanoid copepod species under experimental and natural conditions. *Neth J Sea Res* 16: 195-207
- Klein Breteler WCM, Gonzalez SR, Schogt N (1995) Development of *Pseudocalanus elongatus* (Copepoda, Calanoida) cultured at different temperature and food conditions. *Mar Ecol Prog Ser* 119: 99-110
- Klein Breteler WCM, Gonzalez SR, Schogt N, Franz HG (1998) Feasibility of incubation experiments to estimate moulting rates in marine copepods. *J Plankton Res* 20: 257-269
- Kononen K, Kuosa H, Leppänen J, Olsonen R, Kuparinen J, Postel L, Behrends G (1996) Overall assessment. Pelagic biology. HELCOM 1996: *Baltic Sea Envir Proc* 64 B: 215-222
- Koski M, Klein Breteler WCM, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). *Mar Ecol Prog Ser* 170: 169-187

- Lee HW, Ban S, Ikeda T, Matsuishi T (2003) Effect of temperature on development, growth and reproduction in the marine copepod *Pseudocalanus newmani* at satiating food condition. *J Plankton Res* 25: 261-271
- McLaren IA, Laberge E, Corkett CJ, Sevigny JM (1989a) Life cycles of four species of *Pseudocalanus* in Nova Scotia. *Can J Zool* 67: 552-558
- McLaren IA, Sevigny JM, Corkett CJ (1989b) Temperature-dependent development in *Pseudocalanus* species. *Can J Zool* 67: 559-564
- Möllmann C, Köster FW (1999) Food consumption by clupeids in the Central Baltic: evidence for top-down control?. *ICES J Mar Sci* 56: pp 100-113
- Möllmann C, Kornilovs G, Sidrevics L (2000) Long-term dynamics of main mesozooplankton species in the Central Baltic Sea. *J Plankton Res* 22: 2015-2038
- Möllmann C, Köster FW (2002) Population dynamics of calanoid copepods and the implications of their predation by clupeid fish in the Central Baltic Sea, *J Plankton Res* 24: 959-978
- Möllmann C, Kornilovs G, Fetter M, Köster F, Hinrichsen H (2003) The marine copepod, *Pseudocalanus elongatus*, as a mediator between climate variability and fisheries in the Central Baltic Sea. *Fish Oceanogr* 12: 360-368
- Niehoff B, Klenke U, Hirche HJ, Irigoien X, Head R, Harris R (1999) A high frequency time-series at Weathership M, Norwegian Sea, during the 1997 spring bloom: the reproductive biology of *Calanus finmarchicus*. *Mar Ecol Prog Ser* 176: 81-92
- Ohman MD (1985) Resource-satiated population growth of the copepod *Pseudocalanus* sp. *Arch Hydrobiol Beih Ergebn Limnol* 21: 15-32
- 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 and Oceanogr* 39: 21-36
- Paffenhöfer GA, Harris RP (1976) Feeding, growth and reproduction of the marine planktonic copepod *Pseudocalanus elongatus* Boeck. *J Mar Biol Assoc UK* 56: 327-344
- Parsons TR, Takahashi M, Hargrave (1984) Chemical composition/ Zooplankton. In: *Biological Oceanographic Processes*. Pergamon Press, pp 323
- Peters J, Renz J, van Beusekom J, Boersma M, Hagen W (2006) Trophodynamics and seasonal cycle of the copepod *Pseudocalanus acuspes* in the Central Baltic Sea (Bornholm Basin): evidence from lipid composition. *Mar Biol* DOI 10.1007/s00227-006-0290-8
- Peterson WT, Tiselius P, Kiørboe T (1991) Copepod egg production, moulting and growth rates, and secondary production, in the Skagerrak in August 1988. *J Plankton Res* 13: 131-154
- Pertsova NM (1981) Number of generations and their life span in *Pseudocalanus elongatus* (Copepoda, Calanoida) in the White Sea (in Russian). *Zool Zh LX*: 673-684
- Renz J, Hirche HJ (2006) Life cycle of *Pseudocalanus acuspes* Giesbrecht (Copepoda, Calanoida) in the Central Baltic Sea: I: Seasonal and spatial distribution. *Mar Biol* 148: 567-580
- Richter C (1994) Regional and seasonal variability in the vertical distribution of mesozooplankton in the Greenland Sea. *Rep Pol Res* 154: pp 87
- Rigler FH, Downing JA (1984) The calculation of secondary productivity. In: Downing JA, Rigler FH (eds) *A manual on methods for the assessment of secondary productivity in fresh waters*. Blackwell Science, Oxford, pp19-58
- Williams R, Robins DB (1982) Effects of preservation on net weight, dry weight, nitrogen and carbon contents of *Calanus helgolandicus*. *Mar Biol* 71: 271-281

## CHAPTER III

Trophodynamics and condition of the copepods *Temora longicornis* and  
*Acartia longiremis* in the Bornholm Basin (Baltic Sea)  
– indications from lipid composition

Peters J, Dutz J, Hagen W

planned for submission to *Journal of Plankton Research*.



---

## TROPHODYNAMICS AND CONDITION OF THE COPEPODS *TEMORA LONGICORNIS* AND *ACARTIA LONGIREMIS* IN THE BORNHOLM BASIN (BALTIC SEA) – INDICATIONS FROM LIPID COMPOSITION

### Abstract

Seasonal lipid dynamics of the copepods *Temora longicornis* and *Acartia longiremis* were studied in the Bornholm Basin (Central Baltic Sea) on a monthly basis from March 2002 to March 2003 and were interpreted in light of life cycle strategies and diet selection. Lipid reserves (triacylglycerols) in *T. longicornis* females ranged from 6% in August to 42% of total lipids in November. Lipid accumulation for overwintering started in September, while reproduction still proceeded. More constant lipid levels in *A. longiremis* females and strongly reduced wax ester (WE) amounts suggested insufficient food supply or the absence of external triggers for the induction of WE anabolism. Significant interspecific and interseasonal changes were observed in fatty acid (FA) markers in storage lipids of copepods, reflecting different trophic niches and a seasonal succession in diet composition. *A. longiremis* showed constantly higher levels of C18-polyunsaturated FAs, while 16:1(n-7), 20:5(n-3) and 22:6(n-3) were elevated in *T. longicornis*. The observed strong rejection of diatoms by *A. longiremis* probably derived from the performance of ambush feeding induced by prevailing ratios of diatoms and large heterotrophic prey during the study period. In contrast, diatoms were of occasional importance for *T. longicornis* as indicated by elevated 16:1(n-7) levels in times of intense reproduction or lipid accumulation. Both species showed indications for feeding on cyanobacteria. Markers in seston and in neutral lipids of copepods paralleled, with significant correlations for 18:2(n-6) and 18:3(n-3). Elevated levels of 18:1(n-9) characterised FA profiles of seston (max. 16% of total FAs) and copepods, suggesting ciliates as an important dietary component. No significant differences in the FA profiles of *A. longiremis* and *A. bifilosa* were observed in May 2002.

### Introduction

Over the last decades, the pelagic system of the Baltic Sea has experienced severe changes, spanning almost all trophic levels, thus affecting biomass and recruitment of fish stocks as well as abundance and composition of phyto- and zooplankton (Flinkman et al. 1998, Möllmann et al. 2000, Wasmund and Uhlig 2005). Most likely induced by climatic forcing, a decrease in the standing stock of *Pseudocalanus acuspes* was observed, contrasting with a general increase in *Acartia* spp. and *Temora longicornis* biomass (Möllmann et al. 2000). Diatom blooms failed to appear in the Baltic Sea since the late 1980s, probably due to decreases in convective mixing, while dinoflagellates, which prefer stable water columns, gained in importance (Wasmund et al. 1998). However, since 1999 increasing diatom

abundances were reported for the Bornholm Basin (Wasmund et al. 2003). These fluctuations most likely have a strong impact on the food web structure of the pelagic system. A detailed knowledge on trophic interactions is fundamental to understand and assess involved mechanisms, such as hydrographic and top-down-, bottom-up-processes. So far little is known about the seasonal changes in diet composition of *A. longiremis* and *T. longicornis* in the Bornholm Basin, which provide - next to *P. acuspes* - the major food source for sprat and herring (Möllmann and Köster 1999, Möllmann et al. 2004). Hence, our study aims at elucidating their trophodynamics and assessing dietary implications for the life cycle strategies of both species. Since their vertical distribution largely overlaps with highest abundances below the thermocline in summer (Hernroth and Ackefors 1979, unpublished data), the provided food spectrum will be similar, yet its exploitation might strongly differ between the two species.

While numerous studies are dealing with food selection of *T. longicornis* (e.g. De Mott 1988, Vincent and Hartmann 2001, Guisande et al. 2002, Koski et al. 2005) and *Acartia* spp. (e.g. Turner and Tester 1989, Wiadnyana and Rassoulzadegan 1989, Pagano et al. 2003, Katechakis et al. 2004), knowledge on the diet of *A. longiremis* is extremely scarce and most information is deduced from comparisons of chl *a* with egg production (Peterson et al. 1991, Gómez-Guitérrez and Peterson 1999, Peterson et al. 2002). *In situ* grazing experiments showed high proportions of heterotrophic protists in the diet (Levinsen et al. 2000), whereas during strong diatom blooms no clearance on ciliates was detectable (Fessenden and Cowels 1994). Since diatom blooms occur only moderately in the Bornholm Basin, the question as to the role of diatoms in the diet of *A. longiremis* arises.

We applied fatty acids (FA) as trophic markers, which allow an integrated measurement at the population level with a high seasonal resolution and comparatively moderate effort. Distinct changes in fatty acid composition were found in mono-algal feeding experiments with *A. tonsa* and *T. longicornis* after very few days (Veloza et al. 2005, Kreibich et al. in prep.), thus markers most likely reflect *in situ* ingestion for both species on a recent time scale. To prevent a masking of incorporated markers by conservative FA profiles of structural lipids, that temporarily dominate in these lipid-poor copepods, we specifically focused on seasonal changes within the storage lipids. Little is known so far of their composition in *T. longicornis* and *A. longiremis* (Fraser et al. 1989, Norrbin et al. 1990).

The use of signature FAs to describe feeding on specific taxa is well established (Daalsgard et al. 2003). We will therefore only briefly outline, how specific markers were applied in the present study. Identification of some food taxa by signature FAs is rather unambiguous, e.g. 16:1(n-7) and 20:5(n-3) originating from diatoms (Nichols et al. 1993, Dunstan et al. 1994) or 18:4(n-3) and 22:6(n-3) from flagellates and dinoflagellates (Sargent et al. 1987, Graeve et al. 1994), and was validated for the Bornholm Basin by multivariate analyses of seston composition (Peters et al. 2006). Other FAs are more difficult to assign to specific food taxa. A characteristic of the Baltic Sea is the appearance of cyanobacteria blooms over summer and an important objective of this study was to assess their dietary importance for *A.*

*longiremis* and *T. longicornis*. FA compositions of cyanobacteria are very variable (Gugger et al. 2002) and due to the coexistence of different cyanobacteria species in the Baltic Sea, it is not easy to identify clear trophic signals. Many studies reported 16:1, 18:2(n-6) and 18:3(n-3) as a characteristic fatty acid pattern of cyanobacteria (e.g. Murata and Nishida 1987 and references therein, Vargas et al. 1998, Gugger et al. 2002) and due to coherences found between those FAs and cyanobacteria abundance in the Bornholm Basin (Peters et al. 2006) their use as marker for cyanobacteria appears to be justified.

The present study provides a comprehensive dataset on fatty acid markers in copepods and seston to describe seasonal and interspecific changes in the trophodynamics of *T. longicornis* and *A. longiremis*. To deduce information on food limitation and overwintering strategies of both species, we furthermore determined the lipid content and for *T. longicornis* also the lipid class composition.

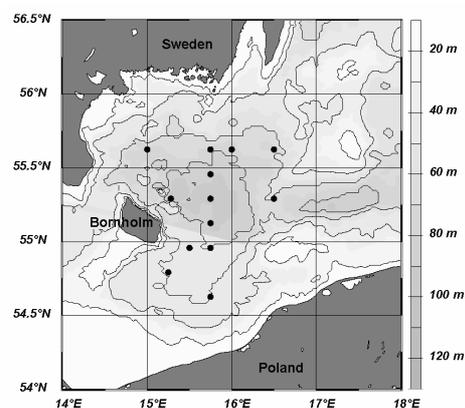
## Materials and Methods

### Sampling

Zooplankton and seston samples were collected in approximately monthly intervals from March 2002 until March 2003 (except for October and December) on eleven cruises in the Bornholm Basin (fig.1). To provide representative data for the whole basin, stations in central and in marginal areas were sampled on each cruise and combined in average values for each month.

Zooplankton was sampled using a WP-2 net with a 10 l bucket end (vertically towed with  $0.2 \text{ m s}^{-1}$ , mesh size  $200 \mu\text{m}$ ,  $0.26 \text{ m}^2$  opening). Sampling depths were adjusted to hydrography covering the water column from the lower halocline to the surface. Copepods were sorted on board under ambient temperature conditions into  $-80^\circ\text{C}$  precooled glass vials. Depending on availability each sample consisted of 20 to 200 adult females of *Acartia longiremis*, *A. bifilosa* (only in May) and *Temora longicornis*, respectively.

Daily egg production of 30 females per station was measured in spawning chambers with an inner compartment equipped with a false bottom made of net gauze of  $100 \mu\text{m}$  mesh size, filled with  $48 \mu\text{m}$  pre-screened water as group (1 l beaker) or single (250 ml beaker) incubations.



**Figure 1.**

Study area, maps were created with ODV (Schlitzer 2002), Sampling grid Bornholm Basin (Baltic Sea).

Seston samples from four depths were taken with 10 l water sampler bottles. Vertical resolution was adapted to the hydrographical structure of the water column, with samples taken from the upper water layer (5 m), from above the thermocline (10 m), from the midwater layer and from above the halocline. Depending on seston concentrations two to six litres of water were filtered with low pressure (-200 mbar) on precombusted (12 h at 400°C) GF/C filters. All zooplankton organisms were carefully removed under the stereomicroscope immediately after filtration and prior to freezing, not to bias the seston data. Zooplankton samples and filters were permanently stored at -80°C until further analysis.

### *Analytics*

After lyophilisation, dry mass of copepods was determined using a Sartorius micro-balance (+/- 2 µg). During weighing procedure, samples were temporarily stored in a vacuum desiccator to prevent unequal condensation on the tissue. Lipid extraction was performed with minor modifications as described in Folch et al. (1957) using ultrasonic disruption in dichloromethane:methanol (2:1/v:v) and a washing procedure with aqueous KCl solution (0.88%). For quantification of FAs, tricosanoic acid was added as an internal standard prior to extraction.

Lipid classes were separated by solid phase extraction, using 1 ml SiOH glass columns (CHROMABOND®, Macherey-Nagel) on a vacuum manifold. To remove residues the columns were washed with a solvent sequence of acetone, diethylether, and hexane:diethylether mixtures, prior to sample load. After column conditioning with 4 ml of hexane, 4 µl of lipid extract (lipid concentration approx. 5 µg µl<sup>-1</sup>) were added. The neutral lipid fraction was washed out with 2.5 ml hexane:diethylether (95:5/v:v) and 2.5 ml hexane:diethylether (1:1/v:v).

For fatty acid analyses a subsample of total lipids as well as the total neutral lipid fraction were hydrolysed and FAs were converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulphuric acid at 80°C for 4 h (Kattner and Fricke 1986). After cooling, 2 ml of aqua bidest. were added, and FAMES were extracted three times with 1 ml hexane. Samples were analysed (modified from Kattner and Fricke 1986) using a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 µm film thickness) operated with helium as carrier gas (constant flow 0.8 ml min<sup>-1</sup>) using the following temperature program: 80°C (5 min), 30°C min<sup>-1</sup>, 165°C, 4°C min<sup>-1</sup>, 240°C (15 min). Samples were injected using a hot split/splitless inlet (250°C, split mode 1:20) or a programmable temperature vaporiser injector (Gerstel® CIS3) with a baffled, deactivated siltek glass liner: solvent vent mode, injection volume 10 µl, injection temperature 25°C (0.21 min, 12°C min<sup>-1</sup>, 280°C), injection speed 0.88 µl min<sup>-1</sup>, purge flow 125 ml min<sup>-1</sup>, purge time 0.2 min, split flow 30 ml min<sup>-1</sup>, splitless time 2.5 min. The FAMES and fatty alcohols were detected by flame ionisation and identified by comparing retention times with those derived from standards of known composition.

Lipid class composition of *T. longicornis* was analysed in triplicates by thin layer chromatography – flame ionization detection on an Iatroscan Mk V according to Fraser et al. (1985). Single compound standards were used for calibration, with dipalmitoyl-phosphatidylcholine, cholesterol, triolein and oleic acid palmityl ester. Due to the adding of an internal FA standard prior to extraction, free FAs were excluded from calculations.

### Statistics

Statistical analyses were performed using the software SPSS. For all statistical operations that require normal distribution, percentage data on fatty acid composition was transformed using an arc sine square root transformation. A principal component analysis (PCA), based on the monthly averaged fatty acid composition of neutral lipids of *T. longicornis* and *A. longiremis* was performed on the correlation matrix, extracting non-rotated components with eigenvalues >1. Fatty acid markers were analysed using one-way ANOVA followed by a Dunnett-T3 test for *post-hoc* comparisons with time as independent variable. Comparisons between FA markers in seston and copepods were conducted with averaged seston composition (weighted for respective lipid concentrations) from below the thermocline (midwater layer and above halocline) and if the thermocline was not established from the corresponding depths.

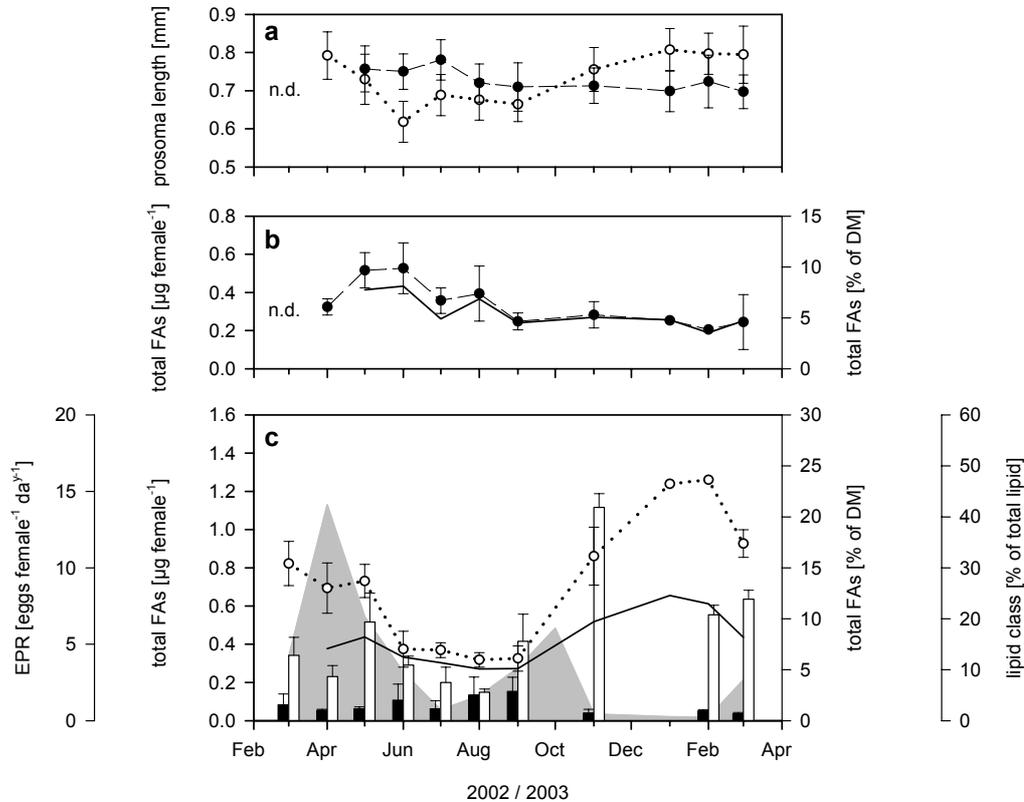
## Results

### *Total lipids, lipid classes and egg production of copepods*

The individual total fatty acid (total FA) content of *Temora longicornis* ranged between 0.3 to 1.2 µg per female and 5 to 12% of dry mass, respectively (fig. 2). Storage lipid amounts of triacylglycerol (TAG) were lowest in April, June and July (6-9% of total lipids (TL)) and increased up to 42% of TL in November. Sterols accounted for less than 5% of TL year round (data not shown). Wax esters showed low variability between 2 to 6% of TL. Individual egg production of *T. longicornis* reached 14 eggs per day in April and strongly declined over the summer to only 0.7 eggs per day, while a second smaller peak in reproduction occurred in October.

The individual total FA content of *Acartia longiremis* resembled that of *T. longicornis* with values from 4 to 8% of dry mass, with a trend of higher lipid amounts in April and May, while in contrast to *T. longicornis* no lipid accumulation was observed in autumn.

Total lipids of both species were dominated by the typical membrane FAs 16:0, 20:5(n-3) and 22:6(n-3), however, the respective levels showed interspecific differences year round (fig. 3). While *T. longicornis* was relatively enriched in 22:6(n-3) with up to 36% of total FAs and also to a lesser degree in 20:5(n-3), *A. longiremis* showed constantly higher amounts of the C18-FAs 18:1(n-9), 18:2(n-6), 18:3(n-3) and 18:4(n-3).



**Figure 2.** Seasonal changes in **a.** prosoma lengths of *Temora longicornis* females (open circles) and *Acartia longiremis* (filled circles) **b.** *A. longiremis*: lipid content as total fatty acids per female (filled circles) and in percentage of dry mass (DM) (line) **c.** *T. longicornis*: lipid content as total fatty acids per female (open circles) and in percentage of dry mass (DM) (line), individual daily egg production rate (EPR) (grey area) and percentage of wax ester (filled column) and triacylglycerols (open column); monthly means with standard deviation (error bars)

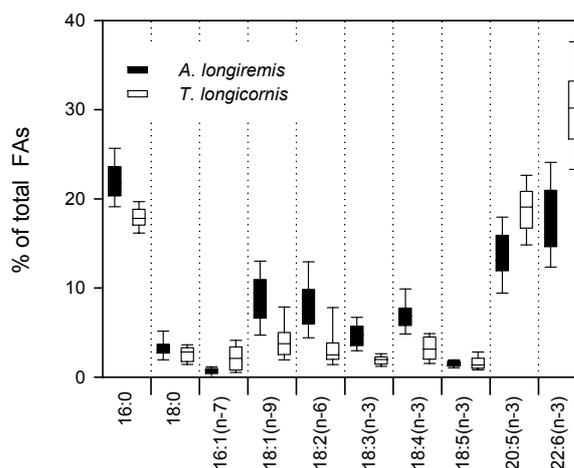
#### Fatty acid marker in copepods and seston

While both species showed stable levels of 16:0 in the storage lipids, oscillating around 20% of total FAs (fig. 4), signature FAs strongly differed between both species. The PCA separated both species on PC1 mainly due to higher values of 16:1(n-7), 20:5(n-3) and 22:6(n-3) in *T. longicornis* and C18-FAs in *A. longiremis*, while the PC2 divided the species seasonally (fig. 5). The seasonal development of markers in *T. longicornis* showed significant changes (ANOVA  $p = 0.000 - 0.005$ ). The diatom markers 16:1(n-7) and 20:5(n-3) were elevated in March and April 2002 with 6% and 16% of total FAs, respectively (fig. 4). A second peak occurred in July, especially for 16:1(n-7), and both markers strongly rose again in November. Levels of 22:6(n-3) were similar to 20:5(n-3), although the former showed a much lower variation, and 22:6(n-3) ascended in November together with 18:4(n-3) up to 18% and 5% of total FAs, respectively. The progression of the abundant 18:1(n-9) paralleled

between *T. longicornis* and *A. longiremis* and significantly peaked in May (ANOVA Dunnett-T3,  $p = 0.000 - 0.006$ , except for April and August) and, on a lower level, in August. C18-polyunsaturated FAs were always higher in *A. longiremis* compared to *T. longicornis*, with maximum values of 18:2(n-6), 18:4(n-3) and 18:3(n-3) with 20%, 11% and 9% of total FAs, respectively. We found no significant differences between the FA profiles of *A. bifilosa* and *A. longiremis* females in May (fig. 6).

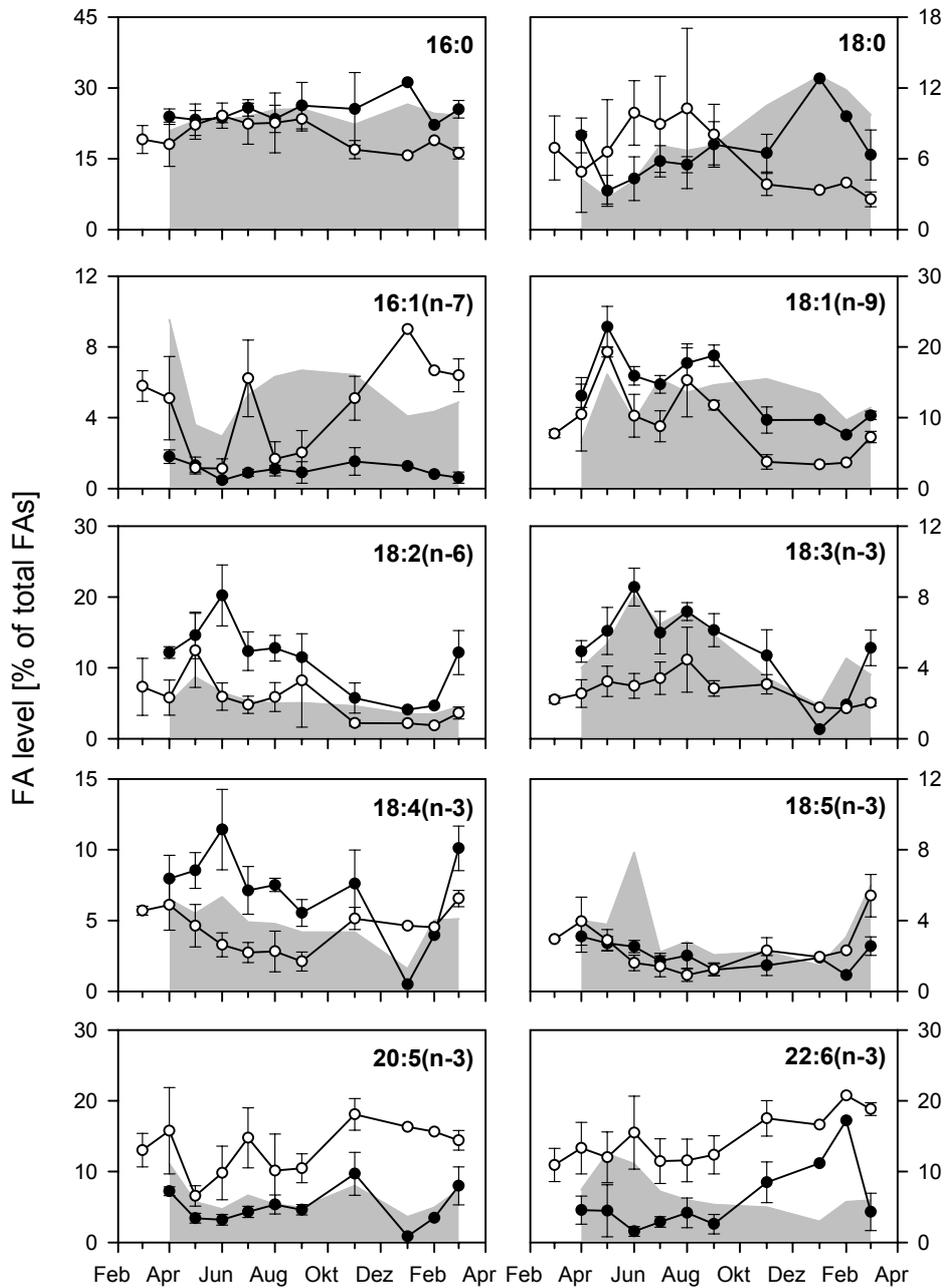
**Figure 3.**

Relative fatty acid composition of total lipids of *A. longiremis* (filled boxes,  $n = 54$  samples, 5250 individuals) and *T. longicornis* (open boxes,  $n = 67$  samples, 6190 individuals)



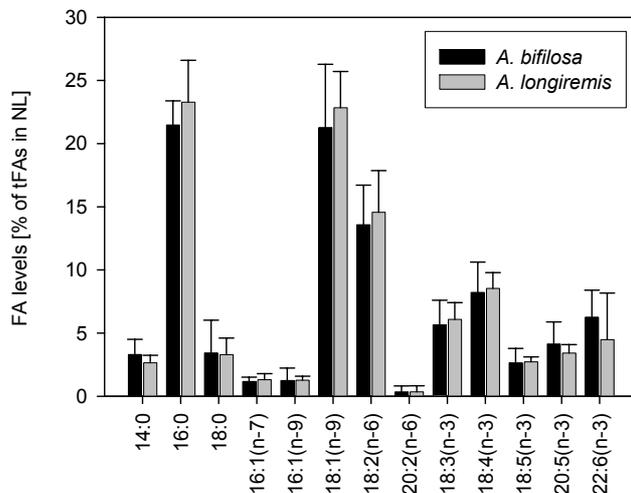
Significant correlations for several FA markers in copepods with relative FA levels in seston were observed (fig. 7). Especially 18:2(n-6) and 18:3(n-3) showed constant relations to the seston lipids for most of the time in both species.

Seston lipid concentrations in terms of total FAs and fatty alcohols in the midwater layer (below thermocline depth) reached maximum values in April and May with  $21 \mu\text{g l}^{-1}$  (data not shown). After a sharp decline in June to  $13 \mu\text{g l}^{-1}$ , they steadily decreased to  $4 \mu\text{g l}^{-1}$  in January. Levels in the upper water layer (above thermocline depths) were normally 1.5 to 2.5 times higher, while in November vertical mixing led to a homogeneous distribution. The FA 16:0 dominated seston lipids at all times (up to  $5 \mu\text{g l}^{-1}$ ) ranging between 22% and 25% of total FAs in the midwater layer (fig. 8 and fig. 4). A distinct increase of 18:1(n-9) was observed in May ( $3.5 \mu\text{g l}^{-1}$ ), corresponding to 16% of total FAs at that time. Maximum values of the highly polyunsaturated FAs 20:5(n-3) and 22:6(n-3) were recorded in April ( $2.2 \mu\text{g l}^{-1}$ ) and May ( $2.7 \mu\text{g l}^{-1}$ ), respectively (fig. 8). Parallel progressions were observed between the diatom markers 16:1(n-7) and 20:5(n-3) as well as between the dinoflagellate markers 18:4(n-3) and 22:6(n-3) (fig 4). 16:1(n-7) reached maximum proportions in April (9% of total FAs) and after a sharp decline in May, it increased again in July (7% of total FAs) and remained on these levels until November. In contrast, 18:4(n-3) showed lower variability and declined only slightly from maximum levels in April (6% of total FAs) to November (4% of total FAs), reaching minimum levels in January. Proportions of 18:2(n-6) showed relative constant amounts with a maximum in May (9% of total FAs) and June (7% of total FAs), while relative values of 18:3(n-3) were elevated from June to August (6-8% of total FAs).



**Figure 4.** Seasonal changes in the relative fatty acid levels in the neutral lipids (NL) of *Temora longicornis* (filled circles) and *Acartia longiremis* (open circles) and in seston (grey area) from below the thermocline; monthly means with standard deviation (error bars)



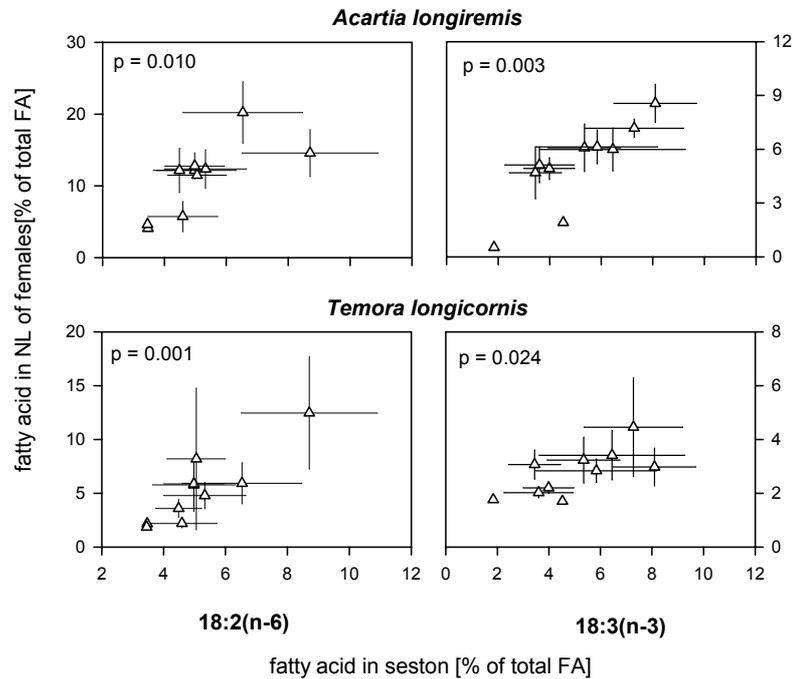


**Figure 6.**

Relative fatty acid composition of neutral lipids of *Acartia longiremis* and *A. bifilosa* in May 2002

Lipid accumulation as preparation for the overwintering of *T. longicornis* females started again in September, although reproduction continued. Seston lipid concentrations in the upper water layers were slightly elevated at that time and a deep-layer chlorophyll maximum near the thermocline occurred (van Beusekom et al. subm.). Unfortunately, data on the seston lipids in October are not available, which was probably an important month for the build-up of the overwintering stages. Female size significantly increased from September to November, suggesting that the lipid-rich and poorly reproducing females in November represented a new generation or cohort, that was produced by summer females. A comparable strong accumulation of lipids of *T. longicornis* for overwintering is not known in the North Sea (Kattner et al. 1981). However, those data were derived from bulk measurements of *T. longicornis* and did not specifically focus on females. In contrast to other habitats (Lindley 1990, Castellani and Lucas 2003), no production of resting eggs was recorded in the Baltic Sea so far (Madhupratap et al. 1996), thus leaving more energy for the investment in overwintering of females. TAG were strongly utilised over winter and beside general metabolic costs, they might have been invested in maturation processes. Such a transfer of lipid reserves into the development of primary oocytes in females was described for instance for *A. longiremis* (Norrbin 2001). While TAG served as major energy deposit in *T. longicornis* and varied strongly, WE were constantly present, although at low levels.

Fatty alcohol levels were even lower in *A. longiremis*, indicating a minor importance of WE. Although *Acartia* spp. is described to mainly store TAG, much higher WE levels than in our study were recorded for *A. longiremis* and *A. clausi* in polar regions with up to 25% of TL (Båmstedt 1990, Norrbin et al. 1990, Peters et al. 2004), and *A. bifilosa* even reached values up to 62% of TL in the northern Baltic Sea (Werner and Auel 2004). Wax esters are assumed to serve as long term storage, while TAG provide fast mobilised energy for short-term needs and their synthesis was suggested to be the first step in lipid accumulation (Lee et al. 2006). Eventually storage lipid amounts in *A. longiremis* never reached levels that



**Figure 7.** Correlations between fatty acid (FA) levels in neutral lipids (NL) of *Acartia longiremis* and *Temora longicornis* females,  $p$  = level of significance (Pearson correlation)

induced wax ester production, thus indicating that food amount was limited for most of the time. This is consistent with the generally low lipid levels, which were only slightly increased in spring, and the lack of lipid accumulation for overwintering. Genetic variability between populations or the absence of an external trigger, such as strong changes in daylight, food supply or food composition might explain the strongly reduced lipid storage of *A. longiremis*. Due to more constant feeding conditions in the Bornholm Basin compared to higher latitudes the need to build up large lipid reserves might be less pronounced.

### *Trophodynamics*

#### *Temora longicornis*

Significant changes in the signature FA compositions of *T. longicornis* were observed over the course of the year, indicating a seasonal progression in diet composition. The FA profiles in storage lipids were characterised by relatively high levels of 16:0, 18:1(n-9) and the highly unsaturated FAs 20:5(n-3) and 22:6(n-3). In March and April 2002 similar values of 18:4(n-3) and 16:1(n-7), as well as 20:5(n-3) and 22:6(n-3) occurred, indicating basically similar feeding rates on diatoms, which were dominated by *Skeletonema costatum* at that time, and dinoflagellates, e.g. *Peridiniella catenata* (van Beusekom et al. subm). While diatom markers showed a strong seasonal variability, typical dinoflagellate FAs declined only gently

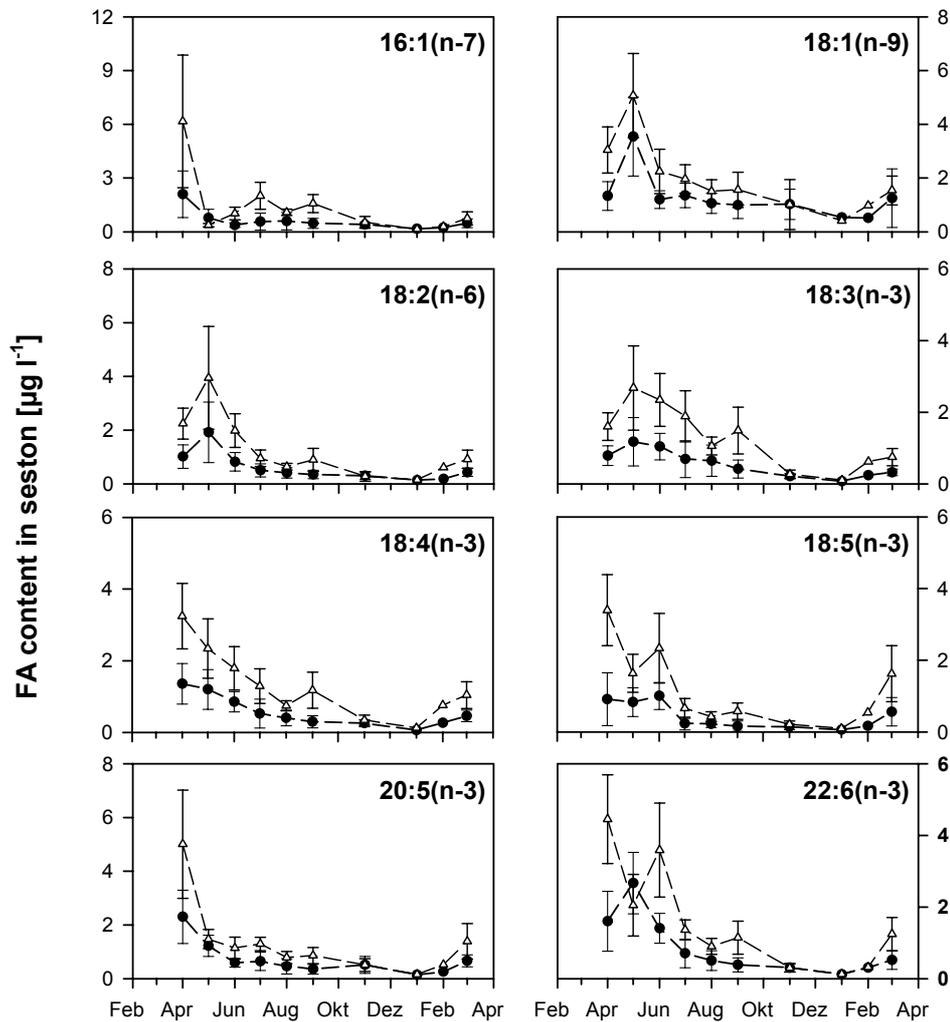
during the summer, indicating that dinoflagellates contributed to the diet most of the time. In May a drastic decrease in diatom markers and an increase of 18:1(n-9) coincided with a rise in storage lipid content. This FA also showed maximum values in the seston lipids at that time, probably reflecting high abundances of the ciliate *Myrionecta rubra* (van Beusekom et al. subm) or a spring bloom degradation. The difficulties in the assignment of 18:1(n-9) to specific food taxa were discussed earlier (Peters et al. 2006). However, due to the correlation of 18:1(n-9) with the abundance of ciliates in the seston, its increase in *T. longicornis* seems to indicate feeding on ciliates, thus providing a food source of high energy, as emphasised in the build-up of larger storage lipid reserves.

In July, a drastic increase of the diatom markers 16:1(n-7) and 20:5(n-3) in the storage lipids occurred as a reaction to a second, smaller diatom bloom mainly consisting of *Podosira stelliger* (van Beusekom et al. subm.). The levels of 16:1(n-7) in the seston remained relatively high over the summer months (August and September), while they dropped sharply in the copepods. It remains a matter of conjecture, whether abundant diatoms at that time were used only inefficiently or food limitation inhibited any anabolism of storage lipids at that time, so that ingested FAs were not transferred into TAG.

The large lipid reserves in November were built up by feeding again on diatoms and dinoflagellates, since both pairs of markers, i.e. 16:1(n-7) and 18:4(n-3), as well as 20:5(n-3) and 22:6(n-3), start to increase again to similar amounts. Hence, diatoms and dinoflagellates were important nutritional components for reproduction in early spring as well as lipid accumulation in late autumn and provided food of relatively high quality as indicated by the elevated levels of the highly polyunsaturated FAs 20:5(n-3) and 22:6(n-3) in *T. longicornis*. Even though the 16:1(n-7) signal was occasionally increased in the lipids of *T. longicornis*, the diatom marker levels were generally low when compared with other habitats, where 16:1(n-7) can reach up to three times higher levels (Peters et al. subm.) than the maximum found in the Bornholm Basin. This signifies a comparatively moderate role of diatoms in the nutrition of *T. longicornis* in the Baltic Sea.

#### *Acartia* spp.

In contrast to the at least occasionally enhanced importance of diatoms in the diet of *T. longicornis*, *A. longiremis* showed an extremely reduced diatom signal, with 16:1(n-7) values never exceeding 2% of total FAs in the storage lipids. According to the FA composition of *T. longicornis* the peak of 18:1(n-9) in May, as well as the second smaller increase in August and September might have originated from feeding on ciliates or degraded material. Apart from this, *A. longiremis* was characterised by higher levels of polyunsaturated C18-FAs with a strong increase in 18:3(n-3), 18:2(n-6) and 18:4(n-3) in June. While 18:3(n-3) and probably also 18:2(n-6) reflected feeding on cyanobacteria (e.g. *Microcystis aeruginosa* and *Aphanizomenon "baltica"*), which started to strongly develop in the upper water layers in June and July (Wasmund et al. 2003, van Beusekom et al. subm.), 18:4(n-3) indicated an



**Figure 8.** Seasonal changes in the fatty acid (FA) concentration of seston from mean depths: 5 and 10 m (open triangles) and two sample depth from below the thermocline (filled circles); monthly means with standard deviation (error bars)

ingestion of flagellates. In contrast to *T. longicornis*, the 22:6(n-3) marker signal remained relatively low, most likely indicating that different types of flagellates were used by *A. longiremis*. November was again characterised by elevated levels of 18:4(n-3), 20:5(n-3) and 22:6(n-3).

We found no significant differences in the FA profiles of *A. longiremis* and *A. bifilosa* in May, indicating similar feeding preferences of both species. However, the thermocline was not fully established in May and their diet composition might differ over the summer, when both species exhibit a vertical separation, with highest abundances of *A. bifilosa* above and *A. longiremis* below the thermocline (Hernroth and Ackefors 1979).

*Diet selection*

Although some FAs in the storage lipid profiles of *T. longicornis* and *A. longiremis* showed quite similar trends over the course of the year, their respective levels differed significantly. This might be a consequence of a species-specific lipid metabolism leading to different trophic signals, even after ingestion of similar food sources. However, the extent of differences strongly emphasise interspecific distinctions in their diet spectrum. While *T. longicornis* utilised diatoms more strongly, *A. longiremis* probably ingested relatively large amounts of heterotrophic prey, which is consistent with other field observations (Levinsen et al. 2000) or at least of non-diatom food components.

It was proposed that unsuitable cell size or chain formation could be a reason for low grazing on diatoms by *A. longiremis*, leading to a decoupling of chl *a* and egg production in several studies (Peterson et al. 1991, Gómez-Guitérrez and Peterson 1999, Peterson et al. 2002). *Acartia* spp. is generally capable of feeding on chain-forming diatoms, as is evident from direct observations (Hasset 2004, Lincoln 2001, Katechakis et al. 2004, St John and Lund 1996) and from the coupling of egg production rates with blooms of *Skeletonema costatum* in the Baltic Sea. (Schmidt et al. 1998). However, *A. longiremis* did not graze on diatoms to a larger extent in the Bornholm Basin, which may be explained by their ability to switch from a suspension to an ambush feeding mode in times of low or moderate food conditions or in the presence of large motile prey, like some dinoflagellates and especially ciliates (Tiselius and Jonsson 1990, Kiørboe et al. 1996, Jakobsen et al. 2005). It was shown that, when offered in similar amounts, ciliates were preferred over diatoms or dinoflagellates by *A. clausi* (Wiadnyana and Rassoulzadegan 1989). Hence, the prevailing relation between diatoms and mobile prey, like *Myrionecta rubra* and possibly also larger dinoflagellates in the Bornholm Basin, probably favoured ambush feeding of *A. longiremis* for most of the time, leading to the strong selection against diatoms. This feeding mode might change under high diatom abundance, since *A. longiremis* showed no detectable clearance of ciliates during upwelling bloom events (Fessenden and Cowels 1994) and much higher diatom marker levels than in our study have been recorded in the FA profiles of *A. longiremis* (Norrbin et al. 1990, Peters et al. 2004) and other *Acartia* species (Kattner et al. 1981, Cotonnec et al. 2001, Werner and Auel 2004).

Contrasting the ambush feeding of *A. longiremis*, leading to a predominant feeding on motile prey, *T. longicornis* is a typical suspension feeder (Tiselius and Jonsson 1990). While at the beginning of the year, the progression between marker levels in the seston and the neutral lipids of *T. longicornis* generally paralleled, differences were more pronounced later in the year, indicating a more selective feeding. Data on food selectivity of *T. longicornis* are controversial. This species is often described as a non- or weakly selective omnivore (e.g. De Mott 1988, Koski et al. 2005, Kozłowsky-Suzuki et al. 2006) with strong herbivorous tendencies (Sautour and Castel 1999). It was suggested that *T. longicornis* is basically a size-selective feeder (Koski et al. 2005, Dam and Petersen 1991, Peterson and Kimmerer 1994, Tang et al. 1998), whereas other studies found prey taxa-specific selectivity, e.g. on ciliates

(Vincent and Hartmann 2001, Anatjan 2004), cryptophytes (Breton et al. 1999, Cotonnec et al. 2001) as well as diatoms, dinoflagellates and chlorophytes (Antajan 2004, Guisande et al. 2002). Our data emphasise, that *T. longicornis* exhibits a stronger coupling to primary production in the upper water layers than *A. longiremis*, which might not only be a result of different feeding modes, but also of its slightly higher vertical distribution.

A specific characteristic of the Baltic Sea is the mass occurrence of cyanobacteria during the summer months. Field observations (Meyer-Harms et al. 1999, Koski et al. 2002) and feeding experiments (Heerkloss et al. 1984, Schmidt and Jónasdóttir 1997) on *T. longicornis* and *Acartia* spp. showed that both species utilise cyanobacteria at least to some degree, although their qualitative value is still subject to discussion (Schmidt and Jónasdóttir 1997, Schmidt et al. 1998, Koski et al. 2002). Our data suggest that *A. longiremis* grazed more efficiently on cyanobacteria than *T. longicornis*. Especially in the late bloom phase, agglomerates of cyanobacteria are often associated with ciliates (Hoppe 1981). Those patches might be highly attractive for *A. longiremis* and may explain the increase of cyanobacteria signals in the FA profiles and possibly also to the second peak of 18:1(n-9) in August.

In conclusion, we were able to show significant interseasonal and interspecific variations in the trophic signals of *T. longicornis* and *A. longiremis*. Generally, diet composition in the Bornholm Basin seems to differ from that in other habitats by the relatively moderate role of diatoms and the much larger importance of heterotrophic food components. The species-specific characteristics in the exploitation of available food sources were most likely related to species-specific feeding modes and potentially reflect the formation of trophic niches of these two species, which exhibit generally similar vertical distribution patterns in the Baltic Sea.

The observed differences in life cycles and in particular overwintering strategies of both copepods might be related with to respective diet spectra. While *T. longicornis* females apparently utilised the small bloom of diatoms and dinoflagellates in the upper water layer to accumulate lipids in autumn, *A. longiremis* females showed no build-up of energy storage for overwintering. Potentially, preferred food abundance for *A. longiremis*, e.g. heterotrophic prey, extends longer into winter but is less pulsed in autumn. As a possible consequence, ingested energy might be rather invested into the production of eggs, that will hatch from the sediment to build up the next-year population, as was reported for *A. bifilosa* in the Baltic Sea (Viitasalo 1992), than into lipid buffers against starvation, as it was proposed for *A. longiremis* females in high latitudinal regions (Norrbin et al. 1990).

### Acknowledgements

The study was funded by the German Federal Ministry for Education and Research within the GLOBEC GERMANY project (03F0320C). We wish to thank the crews and scientific parties of the RV *Alkor*, *Heincke* and *A.v.Humboldt* for the excellent support during the field phase. We also thank S. Borchardt for the excellent assistance with lipid analyses.

### References

- Antajan, E (2004) Responses of calanoid copepods to changes in phytoplankton dominance in the diatom - *Phaeocystis globosa* dominated Belgium coastal waters. PhD thesis. University of Brussel
- Båmstedt U, Håkanson JL, Brenner-Larsen J, Björnsen PK, Geertz-Hansen O, Tiselius P (1990) Copepod nutritional condition and pelagic production during autumn in Kosterfjorden, western Sweden. *Marine Biology* 104: 197-208
- Breton, E, Sautour, B, Brylinski, J-M (1999) No feeding on *Phaeocystis* sp. as solitary cells (post-bloom period) by the copepod *Temora longicornis* in the coastal waters of the English Channel. *Hydrobiologia* 414: 13-23.
- Calliari D, Andersen CM, Thor P, Gorokhova E, Tiselius P (2006) Salinity modulates the energy balance and reproductive success of co-occurring copepods *Acartia tonsa* and *A. clausi* in different ways. *Marine Ecology Progress Series* 312: 177-188
- Castellani, C, Lucas, IAN (2003) Seasonal variation in egg morphology and hatching success of the calanoid copepods *Temora longicornis*, *Acartia clausi* and *Centropages hamatus*. *Journal of Plankton Research* 25: 527-536.
- Cotonnec G, Brunet C, Sautour N, Thoumelin G (2001) Nutritive value and selection of food particles by copepods during a spring bloom of *Phaeocystis* sp. in the English Channel, as determined by pigment and fatty acid analyses. *Journal of Plankton Research* 23: 693-703
- Daalsgard J, St John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. *Advances in Marine Biology* 46: 225-340
- Dam HG, Peterson WT (1991) *In situ* feeding behaviour of the copepod *Temora longicornis*: effects of seasonal changes in chlorophyll size fractions and female size. *Marine Ecology Progress Series* 71: 113-123
- DeMott WR (1988) Discrimination between algae and artificial particles by freshwater and marine copepods. *Limnology and Oceanography* 33: 397-408
- Dunstan GA, Volkman JK, Barrett SM, LeRoi J-M, Jeffrey SW (1994) Essential polyunsaturated fatty acids from 14 species of diatoms (Bacillariophyceae). *Phytochemistry* 35: 155-161
- Fessenden L, Cowles TJ (1994) Copepod predation on phagotrophic ciliates in Oregon coastal waters. *Marine Ecology Progress Series* 107: 103-111

- Flinkman J, Aro E, Vuorinen I, Viitasalo M (1998) Changes in northern Baltic zooplankton and herring nutrition from 1980s to 1990s: top-down and bottom-up processes at work. *Marine Ecology Progress Series* 165: 127-136
- Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 226: 497-509
- Fraser AJ, Sargent JR, Gamble JC (1989) Lipid class and fatty acid composition of *Calanus finmarchicus* (Gunnerus), *Pseudocalanus* sp. and *Temora longicornis* Muller from nutrient-enriched seawater enclosure. *Journal of Experimental Marine Biology and Ecology* 130: 81-92
- Fraser AJ, Tocher DR, Sargent JR (1985) Thin-layer chromatography - flame ionization detection and the quantification of marine neutral lipids and phospholipids. *Journal of Experimental Marine Biology and Ecology* 88: 91-99
- Gómez-Guitérrez J, Peterson WT (1999) Egg production rates of eight calanoid copepod species during summer 1997 off Newport, Oregon, USA. *Journal of Plankton Research* 21: 637-657
- Graeve M, Kattner G, Hagen W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. *Journal of Experimental Marine Biology and Ecology* 182: 97-110
- Gugger M, Lyra C, Suominen I, Tsitko I, Humbert J-F, Salkinoja-Salonen MS, Sivonen K (2002) Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (Cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology* 52: 1007-1015
- Guisande C, Maneiro I, Riveiro I, Barreiro A, Pazos Y (2002) Estimation of copepod trophic niche in the field using amino acids and marker pigments. *Marine Ecology Progress Series* 239: 147-156
- Hasset RP (2004) Supplementation of a diatom diet with cholesterol can enhance copepod egg production rates. *Limnology and Oceanography* 49: 488-494
- Heerkloss R, Borner R, Krummhaar B, Arndt H, Schnese W (1986) Growth inhibition in estuarine zooplankters due to blue-green algae. *Ophelia supplement* 3: 45-49
- Hernroth L, Ackefors H (1979) The zooplankton of the Baltic proper: a long-term investigation of the fauna, its biology and ecology. *Report, Fishery Board of Sweden, Institute for Marine Research* 2: 1-160.
- Hoppe HG (1981) Blue-green algae agglomeration in surface water: a microbiotop of high bacterial activity. *Kieler Meeresforschung Sonderhefte* 5: 291-303
- Jakobsen HH, Halvorsen E, Hansen BW, Visser AW (2005) Effect of prey motility and concentration on feeding in *Acartia tonsa* and *Temora longicornis*: the importance of feeding modes. *Journal of Plankton Research* 27: 775-785
- Katechakis A, Stibor H, Sommer U, Hansen T (2004) Feeding selectivities and food niche separation of *Acartia clausi*, *Penilia avirostris* (Crustacea) and *Doliolum denticulatum* (Thaliacea) in Blanes Bay (Catalan Sea, NW Mediterranean). *Journal of Plankton Research* 26: 589-603

- Kattner G, Fricke HSG (1986) Simple gas-liquid chromatography method for simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *Journal of Chromatography* 361: 263-268
- Kattner G, Krause M, Trahms J (1981) Lipid composition of some typical North Sea copepods. *Marine Ecology Progress Series* 4: 69-74
- Kjørboe T, Saiz E, Viitasalo M (1996) Prey switching behaviour in the planktonic copepod *Acartia tonsa*. *Marine Ecology Progress Series* 143: 65-75
- Koski M, Dutz J, Klein Breteler WCM (2005) Selective grazing of *Temora longicornis* in different stages of a *Phaeocystis globosa* bloom - a mesocosm study. *Harmful Algae* 4: 915-927
- Koski M, Schmidt K, Engström-Ost J, Viitasalo M, Jónasdóttir S, Repka S, Sivonen K (2002) Calanoid copepods feed and produce eggs in the presence of toxic cyanobacteria *Nodularia spumigena*. *Limnology and Oceanography* 47: 878-885
- Kozłowsky-Suzuki, B, Carlsson, P, Rühl, A, Granéli, E (2006) Food selectivity and grazing impact on toxic *Dinophysis* spp. by copepods feeding on natural plankton assemblages. *Harmful Algae* 5: 57-88.
- Lee RF, Hagen W, Kattner G (2006) Lipid storage in marine zooplankton. *Marine Ecology Progress Series* 307: 273-306
- Levinsen H, Turner JT, Nielsen TG, Hansen BW (2000) On the trophic coupling between protists and copepods in Arctic marine ecosystems. *Marine Ecology Progress Series* 204: 65-77
- Lincoln JA, Turner JT, Bates SS, Léger C, Gauthier DA (2001) Feeding, egg production and egg hatching success of the copepods *Acartia tonsa* and *Temora longicornis* on diets of the toxic diatom *Pseudo-nitzschia multiseriata* and the non-toxic diatom *Pseudo-nitzschia pungens*. *Hydrobiologia* 453/454: 107-120
- Lindley, JA (1990) Distribution of overwintering calanoid copepod eggs in sea-bed sediments around southern Britain. *Marine Biology* 104: 209-217.
- Madhupratap, M, Nehring, S, Lenz, J (1996) Resting eggs of zooplankton (Copepoda and Cladocera) from the Kiel Bay and adjacent waters (southwestern Baltic). *Marine Biology* 125: 77-87.
- Meyer-Harms B, Reckermann M, Voß M, Siegmund H, Bodungen Bv (1999) Food selection by calanoid copepods in the eutrophic layer of the Gotland Sea (Baltic Proper) during mass occurrence of N<sub>2</sub>-fixing cyanobacteria. *Marine Ecology Progress Series* 191: 243-250
- Möllmann C, Kornilovs G, Fetter M, Köster FW (2004) Feeding ecology of central Baltic Sea herring and sprat. *Journal of Fish Biology* 65: 1563-1581
- Möllmann C, Kornilovs G, Sidrevics L (2000) Long-term dynamics of main mesozooplankton species in the central Baltic Sea. *Journal of Plankton Research* 22: 2015-2038
- Möllmann C, Köster FW (1999) Food consumption by clupeids in the Central Baltic: evidence for top-down control? *ICES Journal of Marine Science* 56: 100-113

- Murata N, Nishida I (1987) Lipids of blue-green algae (Cyanobacteria). *In*: Stumpf PK (ed) *The biochemistry of plants*. Academic Press, New York, pp 315-347
- Nichols DS, Nichols PD, Sullivan CW (1993) Fatty acid, sterol and hydrocarbon composition of Antarctic sea ice diatom communities during the spring bloom in McMurdo Sound. *Antarctic Science* 5: 271-278
- Norrbin F (2001) Ultra-structural changes in the reproductive system of overwintering females of *Acartia longiremis*. *Marine Biology* 139
- Norrbin MF, Olsen R-E, Tande KS (1990) Seasonal variation in lipid class and fatty acid composition of two small copepods in Balsfjorden, northern Norway. *Marine Biology* 105: 205-211
- Paganon M, Kouassi E, Saint-Jean L, Arfi R, Bouvy M (2003) Feeding of *Acartia clausi* and *Pseudodiaptomus hessei* (Copepoda: Calanoida) on natural particles in a tropical lagoon (Ebrié, Côte d'Ivoire). *Estuarine, Coastal and Shelf Science* 56: 433-445
- Peters J, Dutz J, Hagen W (subm) Impact of food quantity and quality on the reproductive success of the copepod *Temora longicornis* in the North Sea - the role of essential fatty acids
- Peters J, Renz J, van Beusekom J, Boersma M, Hagen W (2006) Trophodynamics and seasonal cycle of the copepod *Pseudocalanus acuspes* in the Central Baltic Sea (Bornholm Basin): evidence from lipid composition. *Marine Biology* DOI 10.1007/s00227-006-0290-8
- Peters J, Tuschling K, Brandt A (2004) Zooplankton in the arctic Laptev Sea—feeding ecology as indicated by fatty acid composition. *Journal of Plankton Research* 26: 227-234
- Peterson WT, Gómez-Guitérrez J, Morgan CA (2002) Cross-shelf variation in calanoid copepod production during summer 1996 off the Oregon coast, USA. *Marine Biology* 141: 353-365
- Peterson WT, Kimmerer WJ (1994) Processes controlling recruitment of the marine calanoid copepod *Temora longicornis* in Long Island Sound: egg production, egg mortality, and cohort survival rates. *Limnology and Oceanography* 39: 1594-1605
- Peterson WT, Tiselius P, Kiørboe T (1991) Copepod egg production, moulting and growth rates, and secondary production, in the Skagerrak in August 1988. *Journal of Plankton Research* 13: 131-154
- Sargent JR, Parkes RJ, Mueller-Harvey I, Henderson RJ (1987) Lipid biomarkers in marine ecology. *In*: Sleight MA (ed) *Microbes in the sea*. Ellis Horwood, Chichester, pp 119-138
- Sautour B, Castel J (1999) Grazing activity of mesoplanktonic copepods in a shallow bay during an algal spring bloom (Marennes-Oléron Bay, France). *Journal of the Marine Biological Association of the United Kingdom* 79: 73-84
- Schmidt K, Jónasdóttir SH (1997) Nutritional quality of two cyanobacteria: How rich is 'poor' food? *Marine Ecology Progress Series* 151: 1-10

- Schmidt K, Kähler P, Bodungen Bv (1998) Copepod egg production rates in the Pomerian Bay (southern Baltic Sea) as a function of phytoplankton abundance and taxonomic composition. *Marine Ecology Progress Series* 174: 183-195
- St John MA, Lund T (1996) Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. *Marine Ecology Progress Series* 131: 75-85
- Tang KW, Dam HG, Feinberg LR (1998) The relative importance of egg production rate, hatching success, hatching duration and egg sinking in population recruitment of to species of marine copepods. *Journal of Plankton Research* 20: 1971-1987
- Tiselius P, Jonsson PR (1990) Foraging behaviour of six calanoid copepods: observations and hydrodynamic analysis. *Marine Ecology Progress Series* 66: 23-33
- Turner JT, Tester PA (1989) Zooplankton feeding ecology: nonselective grazing by the copepods *Acartia tonsa* Dana, *Centropages velificatus* De Oliveira, and *Eucalanus pileatus* Giesbrecht in the plum of the Mississippi River. *Journal of Experimental Marine Biology and Ecology* 126: 21-43
- van Beusekom JEE, Mengedoth D, Augustin CB, Schillig M, Boersma M (subm) Phytoplankton and nutrient dynamics in the Bornholm Basin 2002-2003 during the German GLOBEC Project
- Vargas MA, Rodríguez H, Moreno J, Olivares H, Del Campo JA, Rivas J, Guerrero MG (1998) Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. *Journal of Phycology* 34: 812-817
- Veloza AJ, Chu F-LE, Tang KW (2005) Trophic modification of essential fatty acids by heterotrophic protists and its effects on the fatty acid composition of the copepod *Acartia tonsa*. *Marine Biology* 148: 779-788
- Viitasalo, M (1992) Calanoid resting eggs in the Baltic Sea: implications for the population dynamics of *Acartia bifilosa* (Copepoda). *Marine Biology* 114: 397-405.
- Vincent D, Hartmann HJ (2001) Contribution of ciliated microprotozoans and dinoflagellates to the diet of three copepod species in the Bay of Biscay. *Hydrobiologia* 443: 193-204
- Wasmund N, Nausch G, Matthäus W (1998) Phytoplankton spring blooms in the southern Baltic Sea - spatio-temporal development and long-term trends. *Journal of Plankton Research* 20: 1099-1117
- Wasmund N, Pollehne F, Postel L, Siegel H, Zettler ML (2003) Biologische Zustandseinschätzung der Ostsee im Jahre 2002. *Marine Science Reports* 56
- Wasmund N, Uhlig S (2003) Phytoplankton trends in the Baltic Sea. *ICES Journal of Marine Science* 60: 177-186
- Werner I, Auel H (2004) Environmental conditions and overwintering strategies of planktonic metazoans in and below coastal fast ice in the Gulf of Finland (Baltic Sea). *Sarsia* 89: 102-116
- Wiadnyana NN, Rassoulzadegan F (1989) Selective feeding of *Acartia clausi* and *Centropages typicus* on microzooplankton. *Marine Ecology Progress Series* 53: 37-45

## CHAPTER IV

Impact of food quantity and quality on the reproductive success  
of the copepod *Temora longicornis* in the North Sea  
– the role of essential fatty acids

Peters J, Dutz J, Hagen W

submitted to *Marine Ecology Progress Series*



## **IMPACT OF FOOD QUANTITY AND QUALITY ON THE REPRODUCTIVE SUCCESS OF THE COPEPOD *TEMORA LONGICORNIS* IN THE NORTH SEA – THE ROLE OF ESSENTIAL FATTY ACIDS**

### **Abstract**

This field study focused on the transfer of dietary fatty acids (FAs) into the eggs of *Temora longicornis* and aimed to assess their limitation potential for egg production and egg viability. *In situ* egg production rates, hatching success and FA profiles of females as well as eggs were determined and compared to food indicators, i.e. POC, PON, and FAs of size fractionated seston samples. The individual egg production ranged from 14 to 28 eggs per female and day, corresponding to specific egg productions (sEPR) from 0.18 to 0.35. Based on trophic marker FAs *T. longicornis* most likely fed nonselective during our study. FA contents of eggs ranged between 2.6 and 4.3 ng per egg and correlated significantly with the FA content in the seston (size class 1-30µm). Strong similarities in FA profiles of eggs and seston as well as correlations of absolute FA levels indicated an only minor maternal regulation of egg composition. The significant increase of EPR with increasing diatom-food supply, as indicated by correlation with 16:1(n-7) levels in seston (1-30 µm) and eggs, strongly compensated for the tendency to produce less viable eggs on a diatom dominated diet. Egg viability was overall very high (77% to 94%) at all our stations and did not relate with essential FAs levels in the eggs, indicating that lipids were obviously transferred in sufficient quantities. Thus rather food quantity than quality determined the reproductive success of *T. longicornis* during our study. Stoichiometric comparisons between seston and egg composition suggested nitrogen-containing compounds to have a higher potential in limiting egg production during our study than essential FAs.

### **Introduction**

Detailed knowledge of the processes regulating the reproductive success of copepods is a key in understanding and modelling of their population dynamics. Apart from temperature influences (e.g. Huntley and Lopez 1992, Hirst and Bunker 2003), reproductive processes are mainly determined by food quantity (e.g. Peterson and Kimmerer 1994) and quality, such as cell size (Dam and Peterson 1991), digestibility (Sterner and Schulz 1998), toxicity (Ianora et al. 2003) as well as mineral and biochemical composition (Kjørboe 1989, Jónasdóttir et al. 1995).

Several studies found egg production rates to correlate with rough proxies for food quantity such as chl *a* or particulate organic carbon (e.g. Kleppel 1992, Maps et al. 2005, Castellani

and Altunbaş 2006). Others, in contrast, suggested a limitation by specific food components, like essential amino (Helland et al. 2003) or fatty acids (FAs) (Pond et al. 1996, Hazzard and Kleppel 2003). The potential of a single dietary compound to limit egg production implies, at least in times of low availability, a strong maternal regulation of its transfer into the eggs. Consequently this will lead to a decrease in egg production in favour of a composition that meets minimum requirements for a successful egg development (Anderson and Pond 2000). However, the FA composition of eggs was also found to correspond to the composition of the ingested food (Ederington et al. 1995, Støttrup et al. 1999, Lacoste et al. 2001) and failures in egg development were often associated with diet deficiencies in essential FAs or with ratios of specific FAs (e.g. Jónasdóttir 1994, Jónasdóttir et al. 2002, Hazzard and Kleppel 2003, Tang and Taal 2005). Both observations indicate only a minor regulation. These inherently conflictive mechanisms, affecting egg production and hatching success differently, are not easily assessed, since metabolic processes are intricate and a subject to manifold internal and external variables.

The physiological requirements for a successful embryogenesis of marine copepods are complex, yet poorly understood, and depend next to polyunsaturated FAs (Sargent and Henderson 1986, Shin et al. 2003) on a variety of other essential substances, such as amino acids (Kleppel et al. 1998, Guisande et al. 2000) or sterols (Hassett 2004, Corkett and Hassett 2005). The strong importance of FAs lay not only within their high energetic value, they are also indispensable for the maintenance of the structural and functional integrity of cell membranes (Sargent et al. 1999) and serve as precursors for eicosanoids, which are involved in hormonal regulations in marine invertebrates (Rowley et al. 2005). Further complexity arises from potential interactions of substances, since chemical similarities, e.g. of DHA and EPA can lead to a competition in many chemical and physiological reactions (Sargent et al. 1999). Hence not only the dietary levels of these compounds have to be considered, but also their relative amounts. While laboratory experiments offer a good opportunity to evaluate the role of food quantity- versus quality-aspects of food under controlled conditions, the established relationships might be of less relevance in the field due to compensatory effects of dietary heterogeneity and selective feeding.

In order to assess the potential of specific FAs in determining egg production or hatching success in the field, it is essential not only to examine the relation between seston composition and reproductive success, but also to address the required FA amounts for the production of an egg and the coupling between food and egg quality. Our study aims to improve the understanding of the influence of food quantity and quality on the egg production and egg composition of *Temora longicornis*, with a special focus on the role of essential FAs. *T. longicornis* is one of the most abundant copepods in the southern North Sea (Fransz et al. 1991) with reproductive peaks in April and May (Halsband and Hirche 2001, Arendt et al. 2005) and an important grazer of the phytoplankton spring bloom (Dam and Peterson 1993). Food-limited fecundity and growth of *T. longicornis* are frequently observed (e.g. Petersen and Kimmerer 1994, Kiørboe and Nielsen 1994, Maps et al. 2005) and a recent field study in

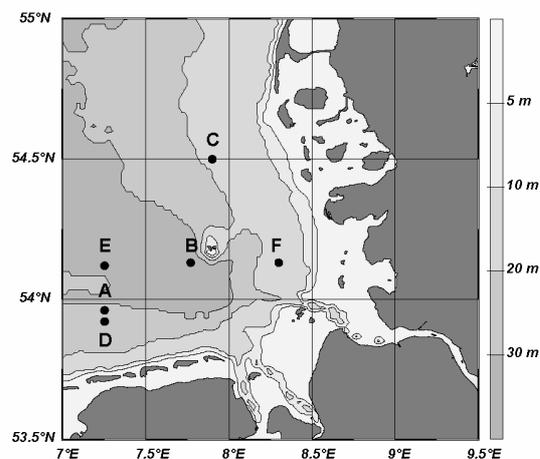
the North Sea emphasised that the hatching success of *T. longicornis* is related with DHA levels and DHA:EPA ratios in the seston (Arendt et al 2005). However, to our knowledge, there are no data on FA profiles of *T. longicornis* eggs and their dietary dependencies.

We therefore determined *in situ* egg production rates, hatching success and FA profiles of *T. longicornis* females as well as eggs and compared them to food indicators, i.e. POC, PON, and FAs. In order to address the diet selection of *T. longicornis* we furthermore used signature FAs in the storage lipids. The use of biomarkers to characterise feeding on different taxonomic groups is well established, e.g. the assignment of 16:1(n-7) and 20:5(n-3) to diatoms and 18:4(n-3) and 22:6(n-3) to dinoflagellates (Daalsgard et al 2003), and can provide information on the recent feeding history of the females. In detail, this study focused on the following questions: (1) does *T. longicornis* feed selectively in order to obtain a diet of high quality, (2) are there indications for a maternal regulation of FA transfer into the eggs, (3) was the egg production limited by food amount or by the abundance of specific FAs, and (4) does the FA composition of the eggs influence egg viability?

## Materials and Methods

### Sampling and experiments

Experiments were performed during two cruises to the southern German Bight, North Sea (17-28 May, 1-8 July 2005) on six stations with no or only low vertical stratification (Fig.1). Zooplankton samples were taken using a WP-2 net equipped with a non-filtering cod end to prevent damage to the animals from 20 m depth to the surface (mesh size 100  $\mu\text{m}$ , vertically towed with 0.2  $\text{m s}^{-1}$ ). When copepod abundance was low, an undulating towed multi-net (mesh size 335  $\mu\text{m}$ , towed with 2.5 knots and 0.2  $\text{m s}^{-1}$ ) was used instead. Immediately on retrieval, the catch was carefully diluted with ambient seawater and kept in a walk-in cooling chamber. Seawater for egg production experiments was collected with 10 l Niskin bottles at 5 m and 10 m or, at vertically stratified stations, at the depth of the thermocline. The water was sieved carefully through a submerged 48  $\mu\text{m}$  net to remove larger zooplankton and copepod eggs and mixed equally. Three to four spawning chambers with an inner compartment equipped with a false bottom made of net gauze of 100  $\mu\text{m}$  mesh size to



**Figure 1.**

Map of investigation area, generated with ODV software (Schlitzer 2005), A-C stations sampled in May, D-F stations sampled in July

prevent females from ingesting their eggs, were filled with the water. Within 10-20 min after capture, actively swimming females of *T. longicornis* were sorted under the stereomicroscope, transferred in groups of 7-10 individuals to the spawning chambers and incubated in a dark temperature-controlled room at *in-situ* temperature (8-9°C in May, 14°C in July). After 24h, incubations were stopped by removing the inner compartment. The pro-some length of females retained on the mesh bottom was measured under a dissecting microscope (Wild M 9.5, 60 x 16 magnification). Eggs collected in the outer compartment were gently concentrated on a submerged sieve of 20 µm, transferred to small Petri dishes and immediately counted under a stereo-microscope.

After counting, eggs from each replicate were transferred to 325 ml bottles containing GF/F filtered ambient seawater and incubated for about 72 h on a rolling apparatus at 1 r.p.m.. Eggs collected from supplementary experiments were incubated in dishes at the same temperature and monitored daily to match the incubation time with hatching of eggs. At the end, bottle contents were concentrated on a 20 µm mesh and fixed with Lugol's solution (2% final concentration). In the laboratory, unhatched eggs, nauplii and empty egg shells were counted under the microscope (Zeiss Axioscope, 200x). Cannibalized eggs accounted for less than 5% of the eggs produced. The egg size was determined in supplementary incubations at each station; at least 30-40 eggs have been measured. Carbon-specific egg production rates were obtained by using carbon-size relationships for eggs and females of *T. longicornis* provided by Dam and Lopes (2003). The body carbon mass of females was calculated from an estimated dry mass assuming that the carbon content is 40% of dry mass. For lipid analyses females were sorted on net gauze and shortly dried by removing the water below the gauze to minimize salt-crysts. Three samples containing each 40-50 females of *T. longicornis* were immediately frozen at -80°C at each station.

To collect sufficient eggs for lipid analyses, approximately 300 females were incubated in 15 (3 x 5 replicates) 1 l containers with a 150 µm screened partition in 48 µm pre-screened water. Every 12 hours in May and 8 hours in July the animals were carefully transferred into new containers and the incubation water was filtered through a submerged sieve of 20 µm to harvest the eggs. The eggs of each series were rinsed into 20 ml glass vials and stored at 2-4°C until further processing to slow down development. After 24 h the eggs were transferred under the stereomicroscope into small vials with GF/F-filtered seawater using an apical thinned-out glass Pasteur pipette. This cleaning procedure was repeated to remove faecal pellets and algal detritus. The purified eggs were then sorted on precombusted (400°C for 12 h) GF/C-filters, which were filmed using a digital camera connected to a computer. The images were used to count the eggs in each sample with an average of 800 eggs per filter. After sorting, the samples were immediately frozen and stored at -80°C.

Seston samples were taken with 10 l water sampler bottles and 40-60 l of water from 5 m and 10 m depths, as well as 15 m depths at deep stations (st. B, st. E) were mixed in equal ratios to receive more representative data for the water column. During all subsequent preparation the samples were kept shaded under ambient temperature condition. Size

fractions were produced by using a slow flow-through system of interleaved submerged sieves of 100  $\mu\text{m}$ , 30  $\mu\text{m}$ , 20  $\mu\text{m}$  and 10  $\mu\text{m}$  mesh size to prevent destruction of fragile organisms. After separation the fractions were carefully resuspended in 1-2 l of GF/F filtrated seawater. Aliquots were filtered with low pressure (<100 mbar) on precombusted (400°C for 12h) GF/C filters for carbon, nitrogen and FA analyses with three replicates each. The filters were immediately frozen and stored at -80°C.

### Analytics

After lyophilization dry mass of females was determined using a micro-balance (+/- 2  $\mu\text{g}$ ). During weighing procedure, samples were temporarily stored in a vacuum desiccator to prevent unequal condensation on the tissue. Lipid extraction was performed with minor modifications as described in Folch et al. (1957) using ultrasonic disruption in dichloromethane:methanol (v/v:2/1) and a washing procedure with aqueous KCl solution (0.88%). For quantification of FAs, tricosanoic acid was added as an internal standard prior to extraction. An additional centrifugation step was carried out prior to the washing procedure for the seston and egg samples to remove GF/C filter remains.

Lipid classes of females were separated by solid phase extraction, using 1 ml SiOH glass columns (CHROMABOND®, Macherey-Nagel) on a vacuum manifold. To remove residues the columns were washed with a solvent sequence of acetone, diethylether and hexane:diethylether-mixtures, prior to sample load. After column conditioning with 4 ml of hexane, 4  $\mu\text{l}$  of lipid extract (lipid concentration approx. 5  $\mu\text{g } \mu\text{l}^{-1}$ ) were added. The neutral lipid fraction was washed out with 2.5 ml hexane:diethylether (v:v/95:5) and 2.5 ml hexane:diethylether (v:v/1:1). Polar lipids were eluted with 2.5 ml methanol and subsequently 5 ml of dichloromethane were added. The polar fraction was then washed with 2 ml aqueous KCl solution (0.88%).

For FA analyses subsamples of total lipids and for the females also samples of the neutral and polar lipid fraction were hydrolysed and FAs were converted to their methyl ester derivatives (FAMES) in methanol containing 3% concentrated sulphuric acid at 80°C for 4 h (Kattner and Fricke 1986). After cooling, 2 ml of aqua bidest. were added, and FAMES were extracted three times with 1 ml hexane. Samples were analysed using a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25  $\mu\text{m}$  film thickness) operated with a temperature programme and helium as carrier gas. Samples were injected using a programmable temperature vaporiser injector (solvent vent mode). FAMES and free fatty alcohols were detected by flame ionisation and identified by comparing retention times with those derived from standards of known composition.

Carbon and nitrogen analyses were conducted using an Euro EA (HEKAtech) element analyser.

## Statistics

All statistical analyses were performed using the software SPSS. For statistical operations that require normal distribution, percentage data, i.e. relative FA compositions, were transformed using an arc sine square root transformation. Differences of measured variables were tested for significance using a one-way ANOVA followed by a Dunnett-T3 test for post-hoc comparisons. Bray-Curtis similarities between the relative FA compositions of seston, eggs and storage lipids of females were calculated on arc sine square root transformed data using the PRIMER software.

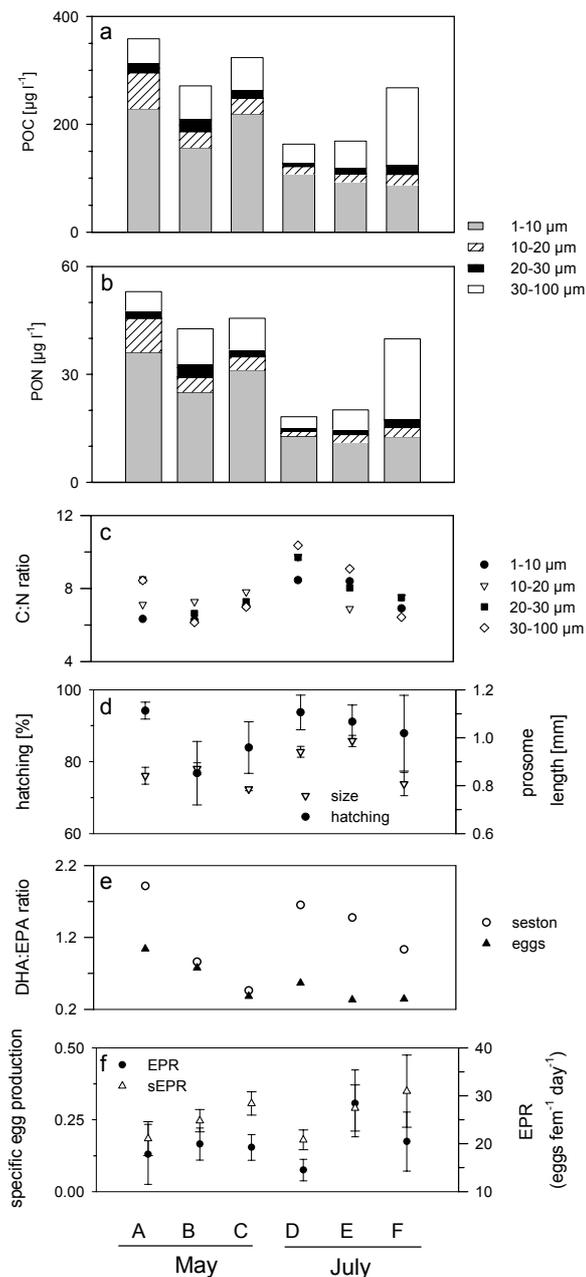
For identification of coherences between egg production, hatching success and FA composition principal component analyses (PCAs) were performed on the correlation matrix, extracting non-rotated components with eigenvalues  $>1$ . Only variables with score values  $>0.6$  or  $<-0.6$  were considered for further interpretations. The vector angles between variables, described by the first 2 extracted components, were calculated as a parameter of correlation. To adjust for temperature differences on the two cruises, the specific egg production rate was standardised to a temperature of  $10^{\circ}\text{C}$  using a  $Q_{10}$  of 3 (Kiørboe and Sabatini 1995). To describe the impact of food quantity, the first PCA was performed on the standardised egg production, the FA concentration and the particulate organic carbon (POC) and nitrogen (PON) contents of the total seston and of the size classes 1-30  $\mu\text{m}$  and 30-100  $\mu\text{m}$ . The influence of egg quality was determined by a second PCA using the relative FA composition of the eggs, the standardised egg production and the hatching success. Since the PCA detects only linear interrelations, correlations were additionally checked using the non-parametric Spearman-Rank test.

## Results

### Seston

The total POC concentration varied between 163 and 359  $\mu\text{g C l}^{-1}$  (Fig. 2a), with maximum amounts in May (st. A and C) and lowest levels in July (st. D and E). Highest carbon contents were generally found in the fraction 1-10  $\mu\text{m}$  (55 to 68% of total POC). Similarly the majority of total PON, ranging from 18  $\mu\text{g l}^{-1}$  (st. D) to 53  $\mu\text{g l}^{-1}$  (st. A), was observed in the fraction 1-10  $\mu\text{m}$  (55-70% of total PON). Only at station F, over 50% of PON and POC was found in the size class 30-100  $\mu\text{m}$ . The C:N ratio varied from 6.4 (st. B) to 8.9 (st. D), corresponding to atomic ratios of 7.9 to 10.4 (Fig. 2c). Lipid content in terms of total FAs and alcohols ranged from 13.8 to 40.8  $\mu\text{g total FAs l}^{-1}$  (Fig.3b) with significant differences between stations and size classes (ANOVA from  $p=0.000$  to  $p=0.004$ ) and strongly correlated with POC ( $r=0.96$ ,  $p=0.002$ ).

The FA composition of total seston was characterised by high amounts of 16:0 (19-27% of total FAs), 18:0 (6-22%), 20:5(n-3) (6-22%) as well as 16:1(n-7) (5-15%). Relative PUFA contents of total seston ranged from 21% of total FAs (st. C and D) up to 37 and 38% of total



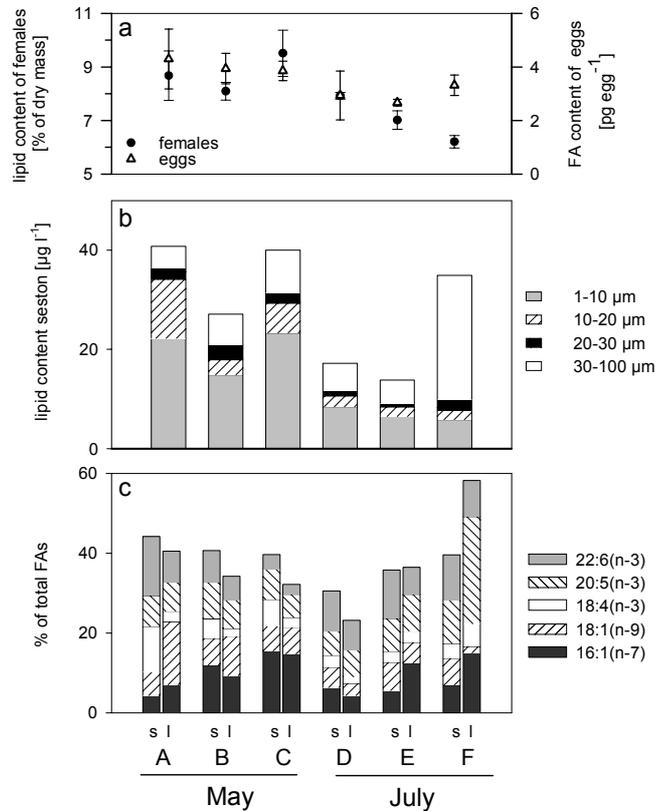
**Figure 2.**

- Concentration of particulated organic carbon (POC) in different size fractions of seston,
- Concentration of particulated organic nitrogen (PON) in different size fractions of seston,
- Weight ratios of POC and PON in different size fractions,
- Hatching success (filled circles) and prosome length of females (open triangles), standard deviation (error bars),
- DHA:EPA ratio of seston (open circles) and eggs (filled triangles),
- Individual daily egg production (EPR, filled circles) and specific egg production (sEPR, open triangles), standard deviation (error bars)

FAs (st. A and F). For some analyses FA data of the different size classes were grouped into the two size fractions 1-30  $\mu\text{m}$  and 30-100  $\mu\text{m}$ , since strongest correlations of *T. longicornis* were found with the size class 1-30  $\mu\text{m}$ , which most likely represented the major food source (see section below). In May elevated levels of the dinoflagellate markers 22:6(n-3) and 18:4(n-3) were observed at station A, comprising together 26% of total FAs in the size class 1-30  $\mu\text{m}$  (Fig.3c) and corresponding to a maximum concentration of  $9.5 \mu\text{g l}^{-1}$  (Fig. 4). The diatom marker 16:1(n-7) became more abundant in this size class at the stations B and C with 12 and 16% of FAs respectively (Fig.3c) and showed highest concentration at station C with  $6.1 \mu\text{g l}^{-1}$  (Fig. 4). In July, seston 1-30  $\mu\text{m}$  was characterised by much lower FA concentrations, while relative proportions of the saturated FAs as well as 22:6(n-3) were elevated. The size fraction 30-100  $\mu\text{m}$  showed generally high amounts of saturated FAs, with a peak of 18:1(n-9) at station A, as well as very high 20:5(n-3) and 16:1(n-7) values at station F in July, reaching together nearly 42% of all FAs.

**Figure 3.**

- a.** Total fatty acid content of females (filled circles) and eggs (open triangles), standard deviation (error bars),
- b.** Total fatty acid concentration in different size fractions of seston,
- c.** Relative fatty acid composition of seston 1-30  $\mu\text{m}$  (s) and 30-100  $\mu\text{m}$  (l), only signature FAs presented



#### *Temora longicornis* females and eggs

The lipid content of *T. longicornis* females ranged from 6.2 to 9.5% of dry mass (Fig. 3A) and correlated significantly with the lipid content of the seston 1-30  $\mu\text{m}$  ( $\rho = 0.89$ ,  $p = 0.019$ ), especially with the size class 1-10  $\mu\text{m}$  ( $\rho = 1$ ).

The FA composition of total lipids of females showed low variability and was dominated by 16:0 (17-19% of total FAs), 20:5(n-3) (24-33%) and 22:6(n-3) (15-24%) (data not shown). These FAs are typical membrane components and together comprised 73-78% of FAs in the polar lipid fraction (data not shown). In contrast, the composition of storage (i.e. neutral) lipids varied more between stations, but basic characteristics were rather constant with 16:0, 16:1(n-7) and 20:5(n-3) as major FAs at all stations composing together 50-61% of total FAs in this lipid fraction (table 1). Most significant differences in the storage lipid composition were found for 16:1(n-7), 18:2(n-6), 18:4(n-3), 20:5(n-3) and 22:6(n-3). In May 18:4(n-3) and 22:6(n-3) reached maximum values at the stations A and B (Fig 4b), whereas station C was characterised by a very high 16:1(n-7) level (25% of total FAs). The FA 20:5(n-3) exhibited maximum percentages in July (st. E and F) with 25% and 31% of total FAs, respectively (Fig. 4).

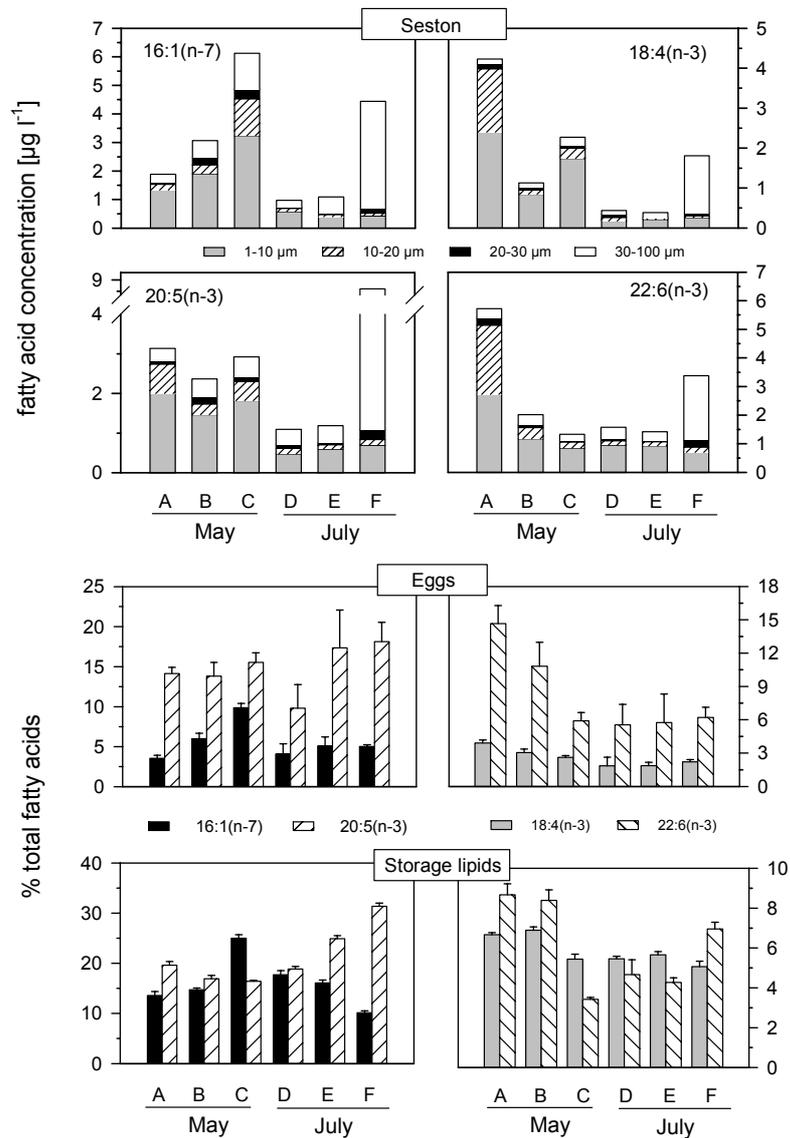
**Table 1.** Fatty acid (FA) composition of storage lipids [% of total fatty acids] of *Temora longicornis* females FA with max. values < 2% not shown, sd = standard deviation

| % of total FA | May  |     |      |     |      |     | July |     |      |     |      |     | ANOVA<br>p |
|---------------|------|-----|------|-----|------|-----|------|-----|------|-----|------|-----|------------|
|               | A    |     | B    |     | C    |     | D    |     | E    |     | F    |     |            |
|               | mean | sd  |            |
| 14:0          | 6.3  | 0.2 | 5.9  | 0.3 | 5.9  | 0.5 | 6.7  | 0.9 | 8.4  | 0.7 | 6.4  | 0.1 | 0.001      |
| 16:0          | 18.2 | 0.3 | 18.7 | 0.4 | 19.3 | 0.5 | 21.2 | 0.5 | 17.6 | 0.3 | 17.1 | 0.5 | 0.000      |
| 18:0          | 3.3  | 0.3 | 3.3  | 0.2 | 2.6  | 0.1 | 3.3  | 0.1 | 2.9  | 0.2 | 3.2  | 0.2 | 0.005      |
| 16:1(n-7)     | 13.5 | 0.8 | 14.7 | 0.4 | 25.0 | 0.7 | 17.7 | 0.8 | 16.1 | 0.6 | 10.1 | 0.4 | 0.000      |
| 18:1(n-7)     | 2.5  | 0.1 | 2.7  | 0.0 | 2.8  | 0.1 | 3.7  | 0.1 | 3.3  | 0.2 | 3.0  | 0.1 | 0.000      |
| 18:1(n-9)     | 3.8  | 0.2 | 4.3  | 0.4 | 3.5  | 0.6 | 3.1  | 0.2 | 2.4  | 0.2 | 2.2  | 0.1 | 0.000      |
| 18:2(n-6)     | 4.2  | 0.1 | 4.4  | 0.0 | 6.3  | 0.3 | 2.0  | 0.0 | 1.2  | 0.0 | 1.7  | 0.0 | 0.000      |
| 18:4(n-3)     | 6.7  | 0.1 | 6.9  | 0.2 | 5.4  | 0.2 | 5.5  | 0.1 | 5.7  | 0.2 | 5.1  | 0.3 | 0.000      |
| 20:5(n-3)     | 19.6 | 0.7 | 16.9 | 0.7 | 16.4 | 0.2 | 18.9 | 0.5 | 24.9 | 0.6 | 31.3 | 0.7 | 0.000      |
| 22:6(n-3)     | 8.7  | 0.5 | 8.4  | 0.5 | 3.4  | 0.1 | 4.7  | 0.7 | 4.3  | 0.2 | 7.0  | 0.3 | 0.000      |

Total FA content of eggs ranged between 2.6 and 4.3 ng per egg (Fig. 3A) and correlated significantly with the total FA content in the seston ( $\rho = 0.83$ ,  $p = 0.042$ ). Based on a mean total FA content of 3.5 ng per egg and assuming polar lipids as the only FA-containing lipid class as well as a cholesterol content of approximately 10% of total lipids as found for *Acartia* sp. (Støttrup et al. 1999), a maximum total lipid content of 6 ng per egg can be estimated. Dry mass is derived from calculated carbon contents of eggs (mean 105 ng C per egg) assuming carbon to be 45% of dry mass as found for *Acartia tonsa* eggs (Kjørboe et al. 1985). This results in a calculated lipid content of approximately 2.5% of dry mass.

In contrast to the lipids of females, the variability in the FA composition of the eggs was much higher, but also showed large amounts of 16:0 and 20:5(n-3) with 19-24% and 10-18% of total FAs, respectively (table 2). Similarly to the FA composition of the females 22:6(n-3) levels were significantly elevated in May (st. A and B) with up to 15% of total FAs, as well as 16:1(n-7) at station C (10% of total FAs) (Fig 4c). The FA 18:0 peaked at station D, whereas the stations E and specifically F showed highest 20:5(n-3) levels similar to the storage lipids of the females. No significant correlation was found between the relative FA compositions of females and eggs, except for 18:2(n-6) ( $\rho = 0.87$ ,  $p = 0.019$ ) and 20:5(n-3) ( $\rho = 0.83$ ,  $p = 0.042$ ), although similar patterns in maximum FAs were observed.

Stronger correlations were found by comparing the FA content of eggs and seston 1-30  $\mu\text{m}$  as well as the quantitative composition of eggs and seston, especially for 16:1(n-7), 18:2(n-6), 18:4(n-3), 20:5(n-3) and 22:6(n-3) (Fig.5). A multivariate comparison of the relative FA composition of eggs and females with the seston revealed strong similarities, with the highest consistence between eggs and seston in the size class 1-30  $\mu\text{m}$  (average Bray-Curtis similarity 91%) (table 3). This strong connection of eggs to the small size class was mainly due to significant correlations of 16:1(n-7) and 18:4(n-3) (Fig.5). In contrast, females showed slightly lower similarities with seston composition and more variability in the attribution to the different size classes.



**Figure 4.** Concentration of signature fatty acids in different size fractions of seston and relative amounts of signature fatty acids in the eggs and storage lipids of *Temora longicornis* females

The individual egg production ranged from 14 to 28 eggs per female and day (Fig. 2d). Due to strong differences in body size (Fig. 2e) the specific egg production (sEPR) varied strongly from 0.18 to 0.35. In both months lowest values of sEPR were observed in the southern part of the study area (st. A and D), while highest production was found in the most northern (st. C) and eastern station (F), respectively. Egg mortality was generally low with on average 77 to 94% of all eggs hatching (Fig. 2e). A negative linear correlation of temperature standardised specific egg production (sEPR<sub>Temp</sub>) with the 22:6(n-3):20:5(n-3)

**Table 2.** Fatty acid [FA] composition of *Temora longicornis* eggs, p = level of significance, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, FA < 4% max = FA with max. values always below 4% of total FAs, sd = standard deviation

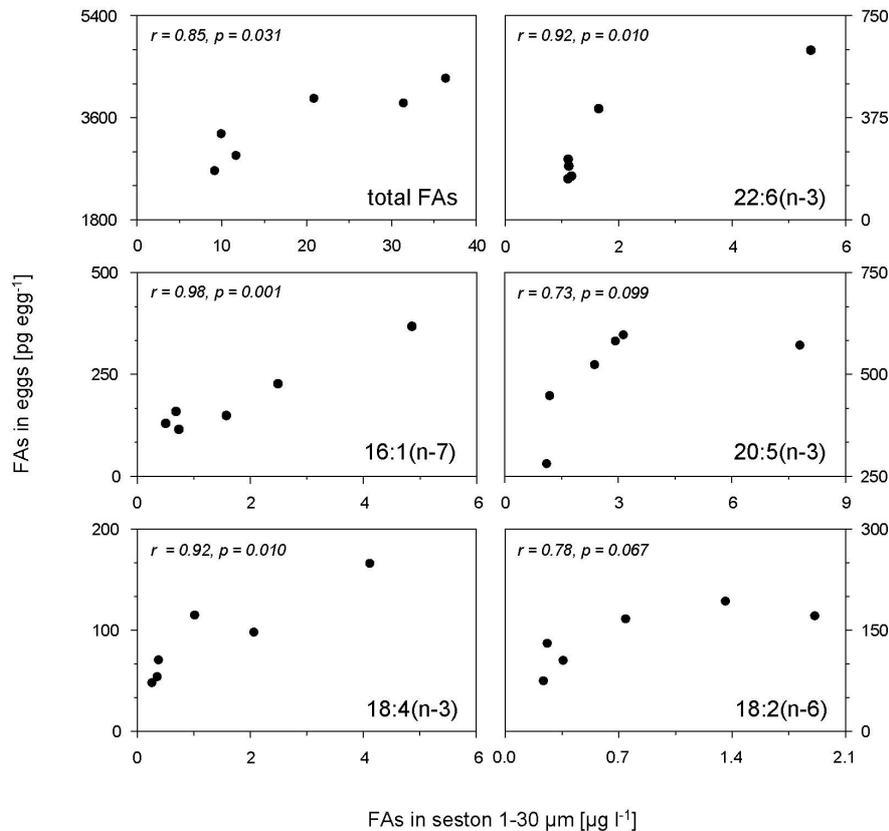
| % of total FA          | May  |     |      |     |      |     | July |     |      |     |      |     | ANOVA<br>p |
|------------------------|------|-----|------|-----|------|-----|------|-----|------|-----|------|-----|------------|
|                        | A    |     | B    |     | C    |     | D    |     | E    |     | F    |     |            |
|                        | mean | sd  |            |
| 14:0                   | 2.7  | 0.7 | 2.8  | 0.3 | 2.9  | 0.4 | 3.3  | 0.3 | 3.0  | 0.5 | 3.6  | 0.4 |            |
| 16:0                   | 19.2 | 0.4 | 21.0 | 1.1 | 20.9 | 0.3 | 24.1 | 0.7 | 22.2 | 1.1 | 20.4 | 0.6 | ***        |
| 18:0                   | 8.7  | 0.8 | 8.8  | 0.5 | 7.1  | 0.8 | 15.3 | 1.4 | 10.3 | 1.7 | 8.5  | 1.4 | ***        |
| 16:1(n-7)              | 3.6  | 0.3 | 6.0  | 0.7 | 9.9  | 0.5 | 4.1  | 1.3 | 5.1  | 1.1 | 5.0  | 0.2 | ***        |
| 18:1(n-7)              | 1.8  | 0.0 | 2.5  | 0.1 | 3.1  | 0.2 | 2.6  | 0.2 | 3.0  | 0.5 | 3.4  | 0.6 | **         |
| 18:1(n-9)              | 5.9  | 0.1 | 7.3  | 0.7 | 7.4  | 0.8 | 7.9  | 3.0 | 8.1  | 1.9 | 10.2 | 5.8 |            |
| 18:2(n-6)              | 4.1  | 0.2 | 4.4  | 0.5 | 5.2  | 0.3 | 3.7  | 0.8 | 3.0  | 0.6 | 4.0  | 1.6 |            |
| 18:4(n-3)              | 3.9  | 0.3 | 3.0  | 0.3 | 2.6  | 0.2 | 1.9  | 0.8 | 1.9  | 0.3 | 2.2  | 0.2 | **         |
| 20:5(n-3)              | 14.1 | 0.8 | 13.8 | 1.7 | 15.5 | 1.2 | 9.8  | 3.0 | 17.4 | 4.7 | 18.1 | 2.4 | *          |
| 22:6(n-3)              | 14.7 | 1.6 | 10.8 | 2.2 | 5.9  | 0.7 | 5.6  | 1.8 | 5.8  | 2.6 | 6.2  | 0.9 | ***        |
| FA < 4% <sub>max</sub> | 21.3 | 0.9 | 19.6 | 4.2 | 19.5 | 0.7 | 21.8 | 3.0 | 20.4 | 3.9 | 18.3 | 2.7 |            |

(DHA:EPA) ratio in the seston 1-30  $\mu\text{m}$  ( $r=-0.87$ ,  $p=0.024$ ), while hatching success was positively rank-correlated with the DHA:EPA ratio ( $\rho=0.94$ ,  $p=0.005$ ) (Fig.2f). However, we did not find any relation of egg production or hatching success with the DHA:EPA ratios of the eggs. No correlations were found with the (n-6):(n-3) ratios in the seston or the eggs.

The first PCA on egg production and quantitative seston constituents revealed a strong dependency of the  $sEPR_{Temp}$  on the POC and PON concentration of total seston, as well as on the content of 16:0, as the major seston component (table 4). Most coherences were found with FAs in total seston, namely 18:4(n-3), 18:1(n-9) and 18:1(n-7), as well as with FAs in the size class 1-30  $\mu\text{m}$ , especially 20:5(n-3) and 16:1(n-7). A second PCA on FA composition of eggs,  $sEPR_{Temp}$  and hatching success revealed a positive relation of egg production with the levels of 18:2(n-6), 16:1(n-7) and 20:5(n-3) in the eggs as well as a negative relation with the FAs 16:0 and 18:0 and with hatching success. Accordingly, levels of 16:1(n-7) ( $\rho=-0.89$ ,  $p=0.019$ ) and 20:5(n-3) in the eggs showed a negative correlation with hatching success (table 5). The absolute amount of 16:1(n-7) in the eggs correlated positively with  $sEPR_{Temp}$  ( $r=-0.93$ ,  $p=0.006$ ) as well with the  $sEPR_{Temp}$  that included only viable eggs ( $r=-0.92$ ,  $p=0.01$ ) (Fig. 6).

**Table 3.** Bray-Curtis Similarity [%] between relative fatty acid composition of eggs, neutral lipids of *Temora longicornis* females and seston (based on square root arcus sinus transformed data)

|                          | May  |      |      | July |      |      |
|--------------------------|------|------|------|------|------|------|
|                          | A    | B    | C    | D    | E    | F    |
| eggs vs seston           |      |      |      |      |      |      |
| < 30 $\mu\text{m}$       | 90.3 | 90.3 | 89.6 | 91.0 | 91.0 | 90.7 |
| > 30 $\mu\text{m}$       | 86.6 | 87.9 | 86.1 | 89.2 | 87.6 | 78.4 |
| neutral lipids vs seston |      |      |      |      |      |      |
| < 30 $\mu\text{m}$       | 88.2 | 90.6 | 89.0 | 81.8 | 77.8 | 84.1 |
| > 30 $\mu\text{m}$       | 82.8 | 83.7 | 82.6 | 77.9 | 84.1 | 89.8 |



**Figure. 5** Correlation between fatty acid (FA) amounts in the eggs and their concentration in the seston size fraction 1-30  $\mu\text{m}$

## Discussion

Recent studies emphasise the high importance of food quality for growth and reproductive success of copepods and special importance is attached to the role of essential FAs (e.g. Jónasdóttir et al. 1995, Jónasdóttir and Kiørboe 1996, Pond et al. 1996, Shin et al. 2003, Arendt et al. 2005). However, the relevance of essential FAs for egg production and hatching success of copepods in marine systems is yet poorly understood and knowledge primarily bases on observed correlations between reproductive success and FA composition of bulk seston measurements. Even though these correlations indicate a nutritional regulation, they do not necessarily provide information on the underlying causal relationships. Further insights on the mechanism that determine the reproductive success might be derived by elucidating the coupling of food composition with egg quality and the consequences for hatching success. Hence, our field study focused on the transfer of dietary FAs into the eggs and aimed to asses their limitation potential for the egg production and egg viability of *Temora longicornis* in the North Sea.

**Table 4.**

|  | seston [ $\mu\text{g l}^{-1}$ ]                | PC1 (51.4%) | alpha |
|--|--|-------------|-------|
| Principal component (PC) analysis, loadings of variables (absolute values of seston) on PC 1, bold = significant spearman rank correlations ( $p < 0.05$ ), sEPRTemp = temperature standardised egg production, alpha = angle between vectors described by PC 1 and PC 2 | sEPR <sub>Temp</sub>                           | 0.73        | -     |
|  | <b>POC total</b>                               | 0.91        | 0     |
|  | <b>PON total</b>                               | 0.87        | 5     |
|  | 18:4(n-3) total                                | 0.82        | 5     |
|  | <b>16:0 total</b>                              | 0.87        | 6     |
|  | 18:1(n-9) 30-100 $\mu\text{m}$                 | 0.92        | 9     |
|  | <b>20:5(n-3) 1-30 <math>\mu\text{m}</math></b> | 0.99        | 19    |
|  | <b>18:1(n-9) total</b>                         | 1.00        | 22    |
|  | <b>18:1(n-7) total</b>                         | 0.97        | 23    |
|  | <b>16:1(n-7) 1-30 <math>\mu\text{m}</math></b> | 0.75        | 24    |
|  | PON 1-30 $\mu\text{m}$                         | 0.98        | 24    |
|  | <b>18:1(n-9) 1-30 <math>\mu\text{m}</math></b> | 1.00        | 26    |
|  | 18:1(n-7) 1-30 $\mu\text{m}$                   | 0.97        | 26    |
|  | 18:2(n-6) total                                | 0.97        | 26    |
|  | 14:0 1-30 $\mu\text{m}$                        | 0.99        | 28    |
|  | 18:4(n-3) 1-30 $\mu\text{m}$                   | 0.89        | 28    |
|  | POC 1-30 $\mu\text{m}$                         | 0.98        | 28    |
|  | 14:0 total                                     | 0.98        | 29    |
|  | 18:2(n-6) 1-30 $\mu\text{m}$                   | 0.95        | 30    |
|  | 16:0 1-30 $\mu\text{m}$                        | 0.96        | 32    |
| 22:6(n-3) 1-30 $\mu\text{m}$   | 0.62   | 32          |       |

Signature FAs were used to analyse the feeding history of *T. longicornis* females and, in order to obtain mostly unaltered signals, we specifically focused on the FA composition of storage, i.e. neutral lipids. Elevated values of 22:6(n-3) and to a minor degree of 18:4(n-3) most likely reflect an ingestion of dinoflagellates or cryptophytes in May, while constantly high levels of 16:1(n-7) and 20:5(n-3) indicate that diatoms contributed strongly to the diet (Daalsgard et al. 2003 and references therein). Although the FA compositions of neutral lipids and eggs were rather uniform, the profiles still differed significantly between stations and displayed strong correlations with seston lipids, emphasising that sampled seston was actually the food used to build up the eggs. Thus diet composition was reflected rather quickly in the eggs, expressing the high physiological turn-over rates in females. Laboratory studies demonstrated that labelled carbon can be detected in oocytes of *T. longicornis* 24 hours after ingestion (Smith and Hall 1980), hence basically similar transfer rates are to be expected for FAs.

A strong dietary influence on the FA composition of eggs and nauplii of *Acartia tonsa* and *Calanus helgolandicus* has been shown in laboratory experiments (Ederington et al. 1995, Støttrup et al. 1999, Lacoste et al. 2001). Comparisons between FA patterns of eggs and food might therefore be used to describe food selectivity. While Pond et al. (1996) did not record high similarities between FA profiles of seston and *C. helgolandicus* eggs and ascribed this to a selective feeding behaviour, similarities were extremely high in our study, especially

| eggs [%]             | PC1 (43.5 %) | alpha |
|----------------------|--------------|-------|
| sEPR <sub>Temp</sub> | -0.76        | -     |
| <b>18:2(n-6)</b>     | -0.68        | 11    |
| 18:4(n-3)            | -0.92        | 58    |
| 22:6(n-3)            | -0.76        | 75    |
| 18:1(n-9)            | 0.65         | 97    |
| 14:0                 | 0.75         | 114   |
| 16:0                 | 0.81         | 148   |
| 18:0                 | 0.76         | 177   |
|                      | PC2 (33.7 %) | alpha |
| sEPR <sub>Temp</sub> | 0.61         | -     |
| 16:1(n-7)            | 0.80         | 30    |
| 20:5(n-3)            | 0.62         | 35    |
| 18:1(n-7)            | 0.89         | 79    |
| 18:1(n-9)            | 0.65         | 97    |
| hatching success     | -0.66        | 161   |
| eggs [%]             | PC2 (33.7 %) | alpha |
| hatching success     | -0.66        | -     |
| 18:1(n-9)            | 0.65         | 103   |
| 18:1(n-7)            | 0.89         | 121   |
| sEPR <sub>Temp</sub> | 0.61         | 161   |
| 20:5(n-3)            | 0.62         | 165   |
| <b>16:1(n-7)</b>     | 0.80         | 170   |

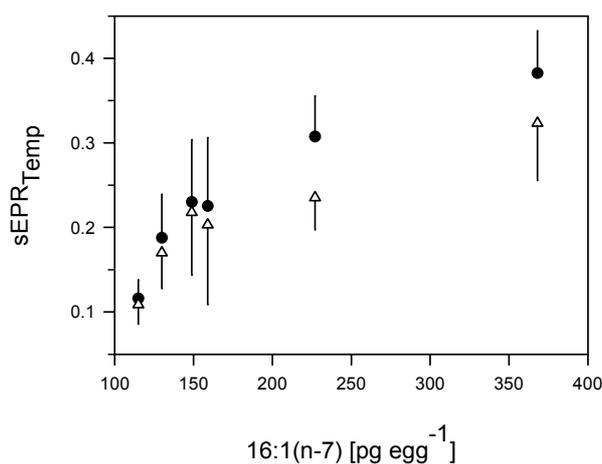
**Table 5.**

Principal component (PC) analysis, loadings of fatty acids in the eggs [% of total fatty acids], standardised egg production rates and hatching success on PC 1 and PC 2 bold = significant spearman rank correlations ( $p < 0.05$ ), sEPR<sub>Temp</sub> = temperature standardised egg production, alpha = angle between vectors described by PC 1 and PC 2

with the FA profiles of 1-30  $\mu\text{m}$ -seston. The correlation observed between the FA content of females and of 1-10  $\mu\text{m}$ -seston, as well as between FA levels in the eggs and in the 1-30  $\mu\text{m}$  seston further indicate a primarily use of the size fraction  $< 30 \mu\text{m}$ . Since the majority of POC was found in these small size fractions and since we detected no preference for larger food particles, *T. longicornis* most likely fed nonselective during our study.

Mean daily egg production ranged from 14 to 28 eggs per female and was similar to other field studies, which reported individual daily rates from 8 to 32 eggs (Kiørboe and Nielsen 1990, Bautista et al. 1994, Jónasdóttir et al. 1995, Tang et al. 1998). Maximum daily rates in the North Sea can increase up to 66 eggs in May (Halsband-Lenk et al. 2004) and 77 eggs in April (Arendt et al. 2005), hence individual egg production rates were rather moderate. In contrast, specific production rates were partly high and varied strongly within the investigation area, but still did not reach maximum values (Halsband and Hirche 2001, Arendt et al. 2005). Therefore, egg production was most likely limited during our study. Specific egg production rates showed similar ranges in both months, although feeding conditions as indicated by POC and PON levels strongly differed. However, if the temperature difference of both months is taken into account, we observed a strong correlation of temperature

standardised egg production ( $sEPR_{Temp}$ ) with POC and PON contents as well as with the levels of 16:0, 18:4(n-3) and 18:1 isomers in the seston. This indicated that the food amount most likely determined productivity of *T. longicornis* during the investigation period.



**Figure. 6** Correlation between 16:1(n-7) and the specific temperature standardised egg production ( $sEPR_{Temp}$ ) (filled circles) and the  $sEPR_{Temp}$  that included only the viable eggs produced (open triangles), standard deviation (error bars)

A positive relation between  $sEPR_{Temp}$  and diatom markers in the 1-30  $\mu\text{m}$ -seston indicated that diatoms provided an important food source for production at that time, as was also reflected in the FA profiles of the eggs. However, not only the proportion of diatom markers in the eggs but, to a lesser degree, also that of dinoflagellate markers were associated with  $sEPR_{Temp}$ . Therefore, increased food signals in the eggs coincided with enhanced egg production. The latter was positively correlated with the relative 18:2(n-6) content in the eggs. This FA was related to the hatching success of *C. helgolandicus* (Pond et al. 1996), suggesting that 18:2(n-6) might play an important role during egg development.

While the DHA:EPA ratio in the seston correlated negatively with egg production, we found a positive relationship with hatching success. Similar correlations between DHA:EPA ratios in the diet and egg viability of *T. longicornis* were reported from a field study in the North Sea (Arendt et al. 2005). However, DHA:EPA ratios in the eggs did not suggest a coherence of both variables. Hence, this commonly used FA-ratio is more likely an indicator of specific feeding conditions in the field, rather than a quality attribute of the diet, that directly affects egg viability. Low DHA:EPA ratios in the seston reflect high proportions of diatoms, which in turn might affect the hatching success of non-selective feeders adversely. The detected negative correlation between the diatom marker 16:1(n-7) in the eggs and their viability

supports this assumption. Previous studies in the North Sea reported similar coherences, where high proportions of 16:1(n-7) in the seston coincided with increased egg mortality in *T. longicornis* (Arendt et al. 2005) and *C. finmarchicus* (Jónasdóttir et al. 2002). However, egg viability was overall very high at all our stations. Since we found no significant correlation of hatching success with changes in total FA content or essential FAs levels in the eggs, lipids were obviously transferred in sufficient quantities or even in excess to their needs. Hence the slight but significant negative influence of diatoms on the hatching success might rather be explained by minor nutritional deficiencies of non-FA-components. Diatoms have been found to provide insufficient sterol amounts in experiments, hence diatom blooms could present conditions under which dietary sterol limitation becomes possible (Hasset 2004). However, the tendency to produce less viable eggs on a diatom dominated diet was clearly compensated by the strong increase in egg production with increasing diatom-food supply. Thus rather food quantity than quality determined the reproductive success of *T. longicornis* during our study.

Despite the overall importance of food quantity, a largely unexplained correlation between dietary FAs and the reproductive success occurred. Whether this correlation reflects a causal relationship, i.e. FA limited the production of *T. longicornis*, might be assessed using a stoichiometric approach. A prediction of food availability from bulk measurements of seston is problematic, since carbon and nitrogen might originate from inaccessible sources like small particles or detritus. However minimum demands for nutritional components can be estimated, assuming a hypothetical 100% accessibility in the seston and a gross growth efficiency (GGE) from literature (Checkley 1980, Petersen and Dam 1996). Calculated N:PUFA ratios (egg nitrogen content according to Dam and Lopes 2003) were found to be much lower in seston (mean ratio = 5) than in eggs (mean ratio = 18). Hence, GGE for PUFAs deduced from nitrogen demands and N:PUFA ratios were extremely low, ranging between 0.02-0.07. Since PUFAs abound in seston compared to nitrogen and are most likely accessible in higher proportions, as they mainly derive from living organisms, we suggest that nitrogen-containing compounds might have had a higher potential in limiting egg production during our study than essential FAs. Similarly, an inefficient transfer of dietary proteins into the eggs of *Acartia tonsa* was suggested (Kleppel and Hazzard 2000). However, as we do not know maximum growth efficiencies of *T. longicornis* for the different nutritional compounds, identified limitation factors based on stoichiometry should be interpreted with caution (e.g. Tang and Dam 1999).

Further indications for the limitation potential of FAs on the egg production might be derived from the transfer of specific FAs into the eggs. FA levels in the eggs were related with their concentrations in the seston, thus indicating an only minor maternal regulation. Hence, rather the hatching success than the egg production should be affected more strongly in times of low availability of essential FAs, unless the regulation would start to increase with increasing dietary deficiencies. Values of 22:6(n-3) previously recorded in the North Sea were more than ten times lower than in the present study and were associated with low

hatching success in *T. longicornis* (Arendt et al. 2005). Still, extremely low 22:6(n-3) values often coincide with low food supply (Arendt et al. 2005) and will consequently lead to lower egg production with lower FA demands.

The generally low FA demand for the production of eggs is associated with the extremely low lipid content. Similar low lipid values were found for *A. tonsa* eggs with only 1.2 ng total FAs per egg in the field (Hazzard and Kleppel 2003) and 6 ng unsaturated FAs per egg in an experimental study (Drillet et al. 2005). Other copepod species are known to transfer large lipid depots into the eggs, e.g. *C. helgolandicus* 35% (Guisande and Harris 1995) and *Paraeuchaeta polaris* (Auel 2004) up to 93% lipid per dry mass. In contrast, eggs of *T. longicornis* obviously do not rely strongly on lipids as energy source. This is conclusive, considering the fast embryonic development of *T. longicornis*, e.g. 2.7 days from egg to nauplius stage II (NII) at 10°C in the North Sea (Halsband-Lenk et al. 2002). The ontogenetically early onset of feeding as NII (Klein Breteler et al. 1990) reduces the need for long-lasting maternal energy supplies. Guisande and Harris (1995) found relatively high levels of carbohydrates in eggs of *C. helgolandicus* (up to 13% of dry mass), which decreased during embryonic development. This energy source can rapidly be used and might therefore be appropriate for fast developing eggs and nauplii. In conclusion, it seems likely that non-FA components like carbohydrates, proteins, glyco-compounds or sterols play an important role in the embryonic development of *T. longicornis* and might thus strongly affect the reproductive success.

### Acknowledgements

The study was funded by the German Federal Ministry for Education and Research within the GLOBEC GERMANY project (03F0418C). We wish to thank the crews and scientific parties of the RV *Heincke* and *Poseidon* for the excellent support during the field phase. We also thank B. Niehoff and C. v. Waldthausen for C:N-measurements. The valuable discussions on this data with H. Auel and D. Stübing improving the manuscript are gratefully acknowledged.

### References

- Anderson TR, Pond DW (2000) Stoichiometric theory extended to micronutrients: comparison of the roles of essential fatty acids, carbon, and nitrogen in the nutrition of marine copepods. *Limnol Oceanogr* 45: 1162-1167
- Arendt KE, Jónasdóttir SH, Hansen PJ, Gärtner S (2005) Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. *Mar Biol* 146: 513-530
- Auel H (2004) Egg size and reproductive adaptations among Arctic deep-sea copepods (Calanoida, *Paraeuchaeta*). *Helgol Mar Res* 58: 147-153

- Bautista B, Harris RP, Rodriguez V, Guerrero F (1994) Temporal variability in copepod fecundity during two different spring bloom periods in coastal waters off Plymouth (SW England). *J Plankton Res* 16: 1367-1377
- Castellani C, Altunbas Y (2006) Factors controlling the temporal dynamics of egg production in the copepod *Temora longicornis*. *Mar Ecol Prog Ser* 308: 143-153
- Checkley Jr. DM (1980) The egg production of a marine planktonic copepod in relation to its food supply: laboratory studies. *Limnol Oceanogr* 25: 430-446
- Crockett EL, Hasset RP (2005) A cholesterol-enriched diet enhances egg production and egg viability without altering cholesterol content of biological membranes in the copepod *Acartia hudsonica*. *Physiol Biochem Zool* 78: 424-433
- Daalsgard J, St. John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. *Advances in Mar Biol* 46: 225-340
- Dam HG, Lopes RM (2003) Omnivory in the calanoid copepod *Temora longicornis*: feeding, egg production and egg hatching rates. *J Exp Mar Biol Ecol* 292: 119-137
- Dam HG, Peterson WT (1991) *In situ* feeding behaviour of the copepod *Temora longicornis*: effects of seasonal changes in chlorophyll size fractions and female size. *Mar Ecol Prog Ser* 71: 113-123
- Dam HG, Peterson WT (1993) Seasonal contrasts in the diel vertical distribution, feeding behaviour, and grazing impact of the copepod *Temora longicornis* in Long Island Sound. *J Mar Res* 51: 561-594
- Drillet G, Iversen MH, Sørensen TF, Ramløv H, Lund T, Hansen BW (2005) Effect of cold storage upon eggs of a calanoid copepod *Acartia tonsa* (Dana) and their offspring. *Aquaculture DOI*. 10.1016/j.aquaculture.2005.11.018
- Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. *Limnol Oceanogr* 40: 860-867
- Folch J, Lees M, Sloane-Stanley GH (1957) A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* 226: 497-509
- Fransz HG, Colebrook JM, Gamble JC, Krause M (1991) The zooplankton of the North Sea. *Neth J Sea Res* 28: 1-52
- Guisande C, Harris R (1995) Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. *Limnol Oceanogr* 40: 476-482
- Guisande C, Riveiro I, Maneiro I (2000) Comparisons among the amino acid composition of females, eggs and food to determine the relative importance of food quantity and food quality to copepod reproduction. *Mar Ecol Prog Ser* 202: 135-142
- Halsband C, Hirche HJ (2001) Reproductive cycles of dominant calanoid copepods in the North Sea. *Mar Ecol Prog Ser* 209: 219-229
- Halsband-Lenk C, Hirche H-J, Carlotti F (2002) Temperature impact on reproduction and development of congener copepod populations. *J Exp Mar Biol Ecol* 271: 121-153
- Halsband-Lenk C, Carlotti F, Greve W (2004) Life-history strategies of calanoid congeners under two different climate regimes: a comparison. *ICES J Mar Sci* 61: 709-720
- Hasset RP (2004) Supplementation of a diatom diet with cholesterol can enhance copepod egg production rates. *Limnol Oceanogr* 49: 488-494

- Hazzard SE, Kleppel GS (2003) Egg production of the copepod *Acartia tonsa* in Florida Bay: role of fatty acids in the nutritional composition of the food environment. *Mar Ecol Prog Ser* 252: 199-206
- Helland S, Nejstgaard JC, Humlen R, Fyhn HJ, Båmstedt U (2003) Effect of season and maternal food on *Calanus finmarchicus* reproduction, with emphasis on free amino acids. *Mar Biol* 142: 1141-1151
- Hirst AG, Bunker AJ (2003) Growth of marine planktonic copepods: global rates and patterns in relation to chlorophyll *a*, temperature, and body weight. *Limnol Oceanogr* 48: 1988-2010
- Huntley ME, Lopez MDG (1992) Temperature-dependent production of marine copepods: a global synthesis. *Am Nat* 140: 201-242
- Ianora A, Poulet SA, Miralto A (2003) The effects of diatoms on copepod reproduction: a review. *Phycologia* 42: 351-363
- Jónasdóttir SH (1994) Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations. *Mar Biol* 121: 67-81
- Jónasdóttir SH, Kiørboe T (1996) Copepod recruitment and food composition: do diatoms affect hatching success? *Mar Biol* 125: 743-750
- Jónasdóttir SH, Fields D, Pantoja S (1995) Copepod egg production in Long Island Sound, USA, as a function of chemical composition of seston. *Mar Ecol Prog Ser* 119: 87-98
- Jónasdóttir SH, Gudfinnsson HG, Gislason A, Astthorsson OS (2002) Diet composition and quality for *Calanus finmarchicus* egg production and hatching success off south-west Iceland. *Mar Biol* DOI 10.1007/s00227-002-0782-0
- Kattner G, Fricke HSG (1986) Simple gas-liquid chromatography method for simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *J Chrom* 361: 263-268
- Kiørboe T (1989) Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. *Mar Ecol Prog Ser* 55: 229-234
- Kiørboe T, Nielsen TG (1990) Effects of wind stress on the vertical water column structure, phytoplankton growth and productivity of planktonic copepods. In: Barnes M, Gibson RN (eds) *Trophic relationships in the marine environment*. Aberdeen University Press, Aberdeen, pp 28-40
- Kiørboe T, Nielsen TG (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 1. Copepods. *Limnol Oceanogr* 39: 493-507
- Kiørboe T, Sabatini M (1995) Scaling of fecundity, growth and development in marine planktonic copepods. *Mar Ecol Prog Ser* 120: 285-298
- Kiørboe T, Møhlenberg F, Hamburger K (1985) Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar Ecol Prog Ser* 26: 85-97
- Klein Breteler WCM, Schogt N, Gonzalez SR (1990) On the role of food quality in grazing and development of life stages, and genetic change of body size during cultivation of pelagic copepods. *J Exp Mar Biol Ecol* 135: 177-189
- Kleppel GS (1992) Environmental regulation of feeding and egg production by *Acartia tonsa* off southern California. *Mar Biol* 112: 57-65
- Kleppel GS, Hazzard SE (2000) Diet and egg production of the copepod *Acartia tonsa* in Florida Bay. II. Role of the nutritional environment. *Mar Biol* 137: 111-121

- Kleppel GS, Burkart CA, Houchin L (1998) Nutrition and regulation of egg production in the calanoid copepod *Acartia tonsa*. *Limnol Oceanogr* 43: 1000-1007
- Lacoste A, Poulet SA, Cueff A, Kattner G, Ianora A, Laabir M (2001) New evidence of the copepod maternal food effects on reproduction. *J Exp Mar Biol Ecol* 259: 85-107
- Maps F, Runge JA, Zakardjian B, Joly P (2005) Egg production and hatching success of *Temora longicornis* (Copepoda, Calanoida) in the southern Gulf of St. Lawrence. *Mar Ecol Prog Ser* 285: 117-128
- Peterson WT, Dam HG (1996) Pigment ingestion and egg production rates of the calanoid copepod *Temora longicornis*: implications for gut pigment loss and omnivorous feeding. *J Plankton Res* 18: 855-861
- Peterson WT, Kimmerer WJ (1994) Processes controlling recruitment of the marine calanoid copepod *Temora longicornis* in Long Island Sound: egg production, egg mortality, and cohort survival rates. *Limnol Oceanogr* 39: 1594-1605
- Pond D, Harris R, Head R, Harbour D (1996) Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of *Calanus helgolandicus* in coastal waters off Plymouth, UK. *Mar Ecol Prog Ser* 143: 45-63
- Rowley AF, Vogan CL, Taylor GW, Clare AS (2005) Prostaglandins in non-insectan invertebrates: recent insights and unsolved problems. *J Exp Biol* 208: 3-14
- Sargent JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SCM (eds) *The biological chemistry of marine copepods*. Clarendon Press, Oxford, pp 59-108
- Sargent J, McEvoy L, Estevez A, Bell G, Bell M, Henderson J, Tocher D (1999) Lipid nutrition of marine fish during early development: current status and future directions. *Aquaculture* 179: 217-229
- Shin K, Jang M-C, Jang P-K, Ju S-J, Lee T-K, Chang M (2003) Influence of food quality on egg production and viability of the marine planktonic copepod *Acartia omorii*. *Progr Oceanogr* 57: 265-277
- Smith SL, Hall BK (1980) Transfer of radioactive carbon within the copepod *Temora longicornis*. *Mar Biol* 55: 277-286
- Sterner RW, Schulz K (1998) Zooplankton nutrition: recent progress and reality check. *Aquat Ecol* 32: 261-279
- Støttrup JG, Bell JG, Sargent JR (1999) The fate of lipids during development and cold-storage of eggs in the laboratory-reared calanoid copepod, *Acartia tonsa* Dana, and in response to different algal diets. *Aquaculture* 176: 257-269
- Tang KW, Dam HG (1999) Limitation of zooplankton production: beyond stoichiometry. *Oikos* 84: 537-542
- Tang KW, Taal M (2005) Trophic modification of food quality by heterotrophic protists: species-specific effects on copepod egg production and egg hatching. *J Exp Mar Biol Ecol* 318: 85-98
- Tang KW, Dam HG, Feinberg LR (1998) The relative importance of egg production rate, hatching success, hatching duration and egg sinking in population recruitment of to species of marine copepods. *J Plankton Res* 20: 1971-1987

## CHAPTER V

Trophic upgrading of nutrient limited algae by heterotrophic protists:  
effects on the reproduction of *Acartia longiremis*

Augustin, C, Schilling M, Peters J, Boersma M

planned for submission to *Journal of Plankton Research*.



## TROPHIC UPGRADING OF NUTRIENT LIMITED ALGAE BY HETEROTROPHIC PROTISTS: EFFECTS ON THE REPRODUCTION OF *ACARTIA LONGIREMIS*

### Abstract

The role of heterotrophic protists as a potential trophic upgrader of suboptimal algal food for marine copepods is still not completely understood. Previous investigations have shown that nitrogen limitation in the prey can influence copepod egg production. However, it has also been shown that a trophic link through heterotrophic protozooplankton may upgrade the nutritional quality of phytoplankton as food for copepods. Here, we investigated the reproduction of the common Baltic Sea copepod *Acartia longiremis* feeding on *Rhodomonas* sp. grown under nitrogen sufficient and depleted conditions, in the presence and absence of two heterotrophic protists, *Oxyrrhis marina* (dinoflagellate) and *Strombidium conicum* (ciliate). When heterotrophic protists were present in the food, the egg production of *A. longiremis* was significantly higher than when feeding on *Rhodomonas* sp. alone. There was no significant difference in egg production between diets containing either the heterotrophic dinoflagellate or the ciliate. *Rhodomonas* sp. cultured in nitrogen depleted media showed an increase in saturated and mono-unsaturated fatty acids compared to a nitrogen sufficient environment. In general, the relative content of polyunsaturated fatty acids remained equal but single fatty acids like EPA and DHA decreased under nitrogen limitation. The high egg production rate of *A. longiremis* fed on the mixture of *Rhodomonas* sp. and *Oxyrrhis marina* or with *Strombidium conicum*, could be not correlated to a single fatty acid or nitrogen condition of the food. Our results show that both types of microzooplankton species may compensate for deficiencies in the algae but this is not due to the nutrition or fatty acid composition. Hence the role of the trophic upgrading by heterotrophic protists for copepods appears to be complex and in need of more investigation.

### Introduction

Heterotrophic protists are an important trophic link between phytoplankton and zooplankton in marine foodwebs. Many heterotrophic protists show strong seasonal variation in abundance (Riegman et al. 1993, Calbet et al. 2003, Hansen et al. 2003, Setaelae & Kivi 2003, Bojanic et al. 2005) and may control phytoplankton blooms (Maar et al. 2002, Johansson et al. 2004, Tillmann 2004, Zhang et al. 2006). They are an important component of the diet of many marine copepods (Stoecker & Capuzzo 1990, Calbet & Saiz 2005). Copepods have been shown to feed selectively on heterotrophic protists such as ciliates and dinoflagellates (Jonsson & Tiselius 1990, Turner et al. 2001, Sommer et al. 2005). These organisms on the other hand graze on bacteria and phytoplankton at relatively high rates

(Hansen et al. 1997, 2000), which results in rapid regeneration and cycling of nutrients (Flynn & Fielder 1989). In general, under circumstances of nitrogen limitation of phytoplankton, protists might be a particularly rich source of proteins and amino acids, compared to phytoplankton and detritus (Stoecker & Capuzzo 1990).

Heterotrophic protists feed on phytoplankton and is itself ingested by secondary consumers such as copepods. They may improve the food value for these animals. This so called trophic upgrading is not only attributed to more suitable sizes of the protists prey for copepods, but also to changes in the composition of essential fatty acids (EFA) in the heterotrophic protists prey, compared to the phytoplankton (Klein Breteler et al. 1999, Tang & Taal 2005). The content of fatty acids is often reported as one of the main factors influencing food quality in phytoplankton-zooplankton interactions (Müller-Navarra 1995a, Müller-Navarra 1995b). Recent literature has shown that among the EFA mainly essential longchained n-3 polyunsaturated fatty acids like docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) influence the reproduction of copepods (Kleppel et al. 1998, Koski et al. 1998, Hazzard & Kleppel 2003, Shin et al. 2003, Tang & Taal 2005). At the same time, algae grown under nutrient limitation are often a food of lower quality for zooplankters, as not only their nutrient content changes, but also the relative content of many essential fatty acids. Hence, given the observation by Stoecker and Capuzzo (1990) that protists are potentially a food high in nitrogen, and upgrade certain fatty acids, in this study we combined nutrient limitations in primary producers with the addition of heterotrophic protists and fed these to copepods. This has not been done to date.

In the Baltic Sea nitrogen becomes limiting for phytoplankton growth across wide areas in summer (Thomas et al. 2003, Nausch et al. 2004). At the same time after the spring bloom several species of the heterotrophic protists such as *Strombidium* sp. and *Strobilidium*, sp. (ciliate) can become abundant and act as important nutrient regenerators (Johansson et al. 2004). Therefore it is particularly relevant to study the interactions between copepods and smaller heterotrophic protists under nutrient limitation, especially since several studies in the Baltic Sea have shown that densities of microzooplankton and mesozooplankton are often closely correlated (Smetacek 1981, Heiskanen et al. 1996, Kivi et al. 1996, Uitto 1996).

Most laboratory experiments on the heterotrophic protists copepod link have been carried out with *Rhodomonas* sp. and *Oxyrrhis marina* (Dujardin 1841). These species are also found in the brackish waters of the Baltic Sea. In the same area *Acartia* spp. are abundant calanoid copepods, often found in the surface layer throughout the year (Adrian et al. 1999, Johansson et al. 2004). *Acartia longiremis* (Lilljeborg, 1853) is widely distributed across the Baltic Sea and even found in subarctic areas (Norrbin 2001). Here, we investigate the effect of nitrogen depleted food on the reproduction of the common Baltic Sea copepod *A. longiremis*. Females were fed different combinations of the microzooplankton, *O. marina* or *Strombidium conicum* (Lohmann, 1908), and one phytoplankter, *Rhodomonas* sp. grown under nitrogen sufficient and depleted conditions.

## Materials and methods

Feeding experiments were performed using the unicellular autotrophic algae *Rhodomonas* sp. (Cryptophyta), and two species of heterotrophic protists, the dinoflagellate *Oxyhris marina* and the ciliate *Strombidium conicum*. Phytoplankton was grown in batch cultures, under two different nutrient conditions, f/2 Media (Guillard 1975), and N-depleted modified f/2 media, as described in (Augustin & Boersma 2006). The dinoflagellate *O. marina* (Göttingen culture collection, Strain B21.89) was adapted from a salinity of 30 down to 10. The ciliate *S. conicum* was sampled at the Baltic Sea GLOBEC-Germany cruise in September 2002 at the central sampling station of the Bornholm Sea. After transport of the samples to the laboratory, individuals were isolated by dilution of the natural sample and pipetting under a microscope. *O. marina* and *S. conicum* were cultured under saturated concentrations of nitrogen sufficient *Rhodomonas* sp. and maintained in 0.45 µm filtrated North Sea water diluted to a salinity of 10.

*A. longiremis* females were collected at the same station as the ciliates. Copepods were sampled using a WP2-Plankton net equipped with a 10 l cod end (mesh size 200 µm, vertically towed from the bottom to the surface with 0.2 m s<sup>-1</sup>), immediately sorted into filtered sea water and kept at *in situ* temperatures in gently aerated beakers. After transportation to the laboratory the copepods were cultured in temperature controlled rooms (10°C, light:dark cycle of 16:8 h) in 0.45 µm filtrated North Sea water, which was diluted to a salinity of 10. The culture was maintained with *Rhodomonas* sp. as single food. For each experiment, females from a single generation of *A. longiremis* were collected from the culture. The experiments were conducted in 1 l glass bottles (Schott) on a plankton wheel at 1 RPM, at the same temperature and light conditions as for the algae cultures. Every 24 hours the food suspension was renewed, and the copepod eggs were sampled from the old suspension and counted.

### Experiment 1 and 2

To investigate the impact of different microzooplankton species on the egg production one microzooplankton species was added in combination with *Rhodomonas* sp. in each experiment. Each treatment was conducted in three replicates with 11 to 12 females and 2 males, in each. The total target food concentration was adjusted to 500 µg C l<sup>-1</sup> based on cell counts. Egg production rates were quantified from eggs sampled every 24 hours, and hatching success was estimated after 48 h incubation. All cell concentrations were measured with a CASY counter (Schärfe GmbH).

### Experiment 3

Batch cultures of *Rhodomonas* sp. were grown under nitrogen depleted and sufficient conditions. During growth, cell number and fatty acid composition were measured daily. Both microzooplankton species *O. marina* and *S. conicum*, fed either on nitrogen depleted or

sufficient *Rhodomonas* sp., were used as food in this experiment (Table 1). *Rhodomonas* sp. as single food was fed in two different concentrations. The low concentration of  $250 \mu\text{g C l}^{-1}$  was similar to the amount of *Rhodomonas* sp., which was in the mixture with microzooplankton. The high food concentration ( $500 \mu\text{g C l}^{-1}$ ) corresponds to the total carbon amount of food per treatment. At the beginning of the experiment samples were taken from the different stock cultures to measure fatty acid content, CN concentration and cell abundance. Carbon and nitrogen content were estimated with a Fison EA 1108 CHN analyser after filtering of 30 to 200 ml of the algal culture onto a combusted Whatman GF/C filter and storing at  $-20^{\circ}\text{C}$ .

**Table 1.** Species of algae and heterotrophic protists used in this study.

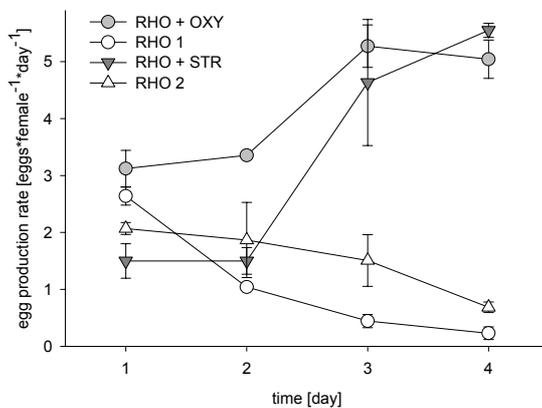
|                            | Nitrogen treatment | Size ( $\mu\text{m}$ ) | Carbon content ( $\text{pg C cell}^{-1}$ ) |
|----------------------------|--------------------|------------------------|--|
| <i>Rhodomonas</i> sp.      | sufficient         | $8.73 \pm 0.1$         | 42.3                                       |
|                            | depleted           | $9.09 \pm 0.2$         | 58.3                                       |
| <i>Strombidium conicum</i> | sufficient         | $13.65 \pm 0.1$        | 1086.0                                     |
|                            | depleted           | $14.09 \pm 0.2$        | 1004.8                                     |
| <i>Oxyrrhis marina</i>     | sufficient         | $19.97 \pm 0.3$        | 996.4                                      |
|                            | depleted           | $18.27 \pm 0.0$        | 1327.2                                     |

Fatty acid samples were taken by filtrating 50-100 ml on pre-combusted GF/C filters (Whatman) at low pressure and stored at  $-80^{\circ}\text{C}$  until further analysis. Lipid extraction was performed with minor modifications as described in Folch et al. (1957) using ultrasonic disruption in dichloromethane:methanol (v/v:2/1) and a washing procedure with aqueous KCl solution (0.88%). For quantification of fatty acids, tricosanoic acid was added as an internal standard prior to extraction. An additional centrifugation step was carried out prior to the washing procedure to remove GF/C filter remains. For fatty acid analyses sub-samples of total lipids were hydrolyzed and fatty acids were converted to their methyl ester derivatives (FAME) in methanol containing 3% concentrated sulphuric acid at  $80^{\circ}\text{C}$  for 4 h (Kattner and Fricke 1986). After cooling, 2 ml of aqua bidest. was added, and FAMEs were extracted three times with 1 ml hexane. Samples were analysed using a gas chromatograph (HP 6890A) equipped with a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25  $\mu\text{m}$  film thickness) operated with a temperature program and helium as the carrier gas. Samples were injected using a programmable temperature vaporizer injector (solvent vent mode). The FAMEs and alcohols were detected by flame ionization and identified by comparing retention times with those derived from standards of known composition.

Results were tested with one way ANOVA followed by Tukey test.

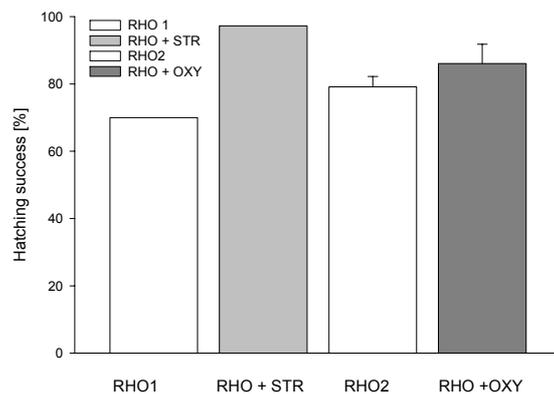
## RESULTS

In the first two experiments the egg production rates of *A. longiremis* were measured for 4 days (Fig. 1). Egg production rates at day 1 varied between 2 and 3 eggs female<sup>-1</sup> day<sup>-1</sup>. In both experiments the females produced more eggs when they were fed a mixed diet including heterotrophic protists than with the phytoplankton itself. The egg production rate after 4 days feeding on *Rhodomonas* sp. mixed with *S. conicum* or *O. marina* increased to 5 eggs female<sup>-1</sup> day<sup>-1</sup>. In contrast, a decrease to 1 egg female<sup>-1</sup> day<sup>-1</sup> was observed in the treatment solely with *Rhodomonas* sp.. However, there was no difference between the two heterotrophic protists. The hatching success (Fig. 2) after feeding on *Rhodomonas* sp. and *S. conicum* was with 70 to 90% in a similar range compared to eggs produced after feeding on *Rhodomonas* sp. and *O. marina* with 80 to 90%.



**Figure 1.**

*A. longiremis*. Egg production rates over time at different food either containing single *Rhodomonas* sp. (open symbols) or *Rhodomonas* sp. and *O. marina* (filled symbols); Different shapes of the symbols present different experiments; error bars indicate standard error of the mean.

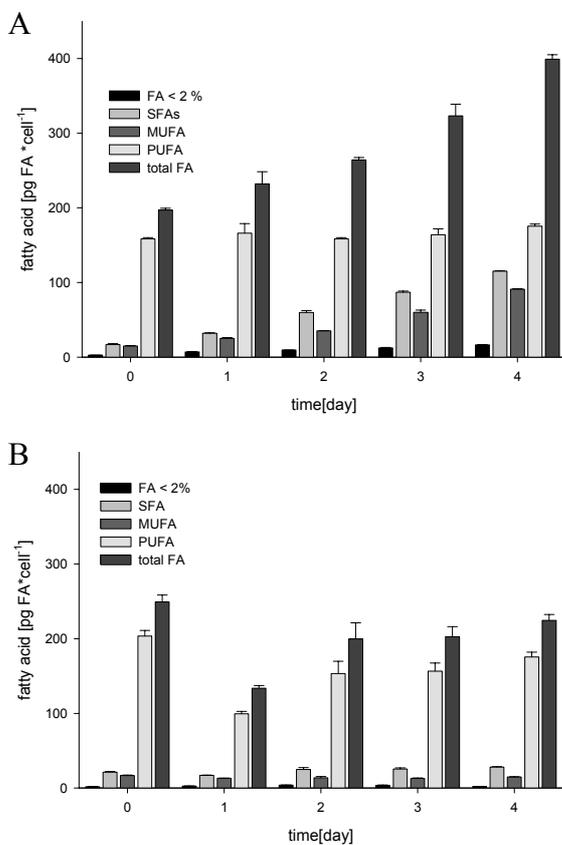


**Figure 2.**

*A. longiremis*. Hatching success after incubation with single *Rhodomonas* sp. (open bars), *Rhodomonas* sp. and *O. marina* (dark grey bars) or *Rhodomonas* sp. and *S. conicum* (light grey bars); Error bars indicate standard error of the mean.

The fatty acid contents of *Rhodomonas* sp. from the different nitrogen treatments differed significantly ( $p < 0.01$ , ANOVA). The amount of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) in the cells increased from 20 to 115 and 200 to 400 pg fatty acids cell<sup>-1</sup>, respectively compared to nitrogen sufficient phytoplankton (Fig. 3A, B). The content of polyunsaturated fatty acids (PUFAs) remained at nearly the same level in

both treatments (Fig. 3A, B). On the other hand, the percentage of EPA and DHA from the total fatty acid content decreased rapidly when the phytoplankton became nitrogen depleted (Fig. 4). In the presence of heterotrophic protists the percentage of EPA and DHA on total fatty acids was different (Fig. 5). In the mixture of *S. conicum* and *Rhodomonas* sp. the DHA content remained at 7% for both nitrogen conditions, while the EPA was significantly higher under depleted condition (28% of total fatty acids) than under sufficient conditions (17% of total fatty acids). On the other hand in *O. marina* the percentage of DHA was high in nitrogen depleted (24% of total fatty acids) and low in the sufficient environment (19% of total fatty acids). The EPA was low in both treatments at nearly 5% of total fatty acids (Table 2).

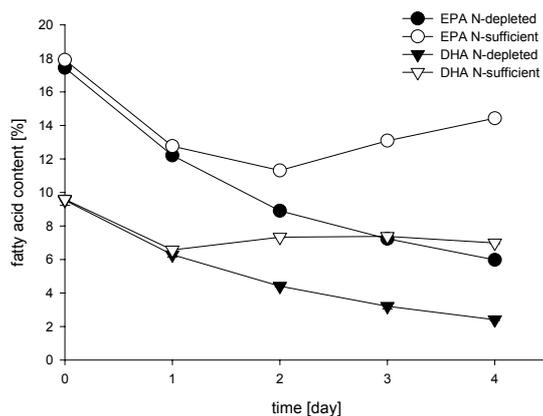


**Figure 3.**

Fatty acid content ( $\text{pg cell}^{-1}$ ) of *Rhodomonas* sp. over the time during growing in either nitrogen depleted (A) or nitrogen sufficient (B) media

The molar C:N ratio (Fig. 6) confirmed that the nitrogen depleted *Rhodomonas* sp. treatments were in fact nitrogen limited. Nitrogen sufficient food had a molar C:N ratio of 4-5, while the nitrogen depleted phytoplankton reached a higher molar C:N ratio from 7 to 9. The mixture of the nitrogen depleted *Rhodomonas* sp. and heterotrophic protists also showed relatively high C:N ratios.

The presence of the heterotrophic protists *S. conicum* or *O. marina* resulted in a significantly higher egg production ( $4 \text{ egg female}^{-1} \text{ day}^{-1}$ ,  $p < 0.01$  ANOVA) (Fig. 7) compared to  $1 \text{ egg female}^{-1} \text{ day}^{-1}$  yielded from feeding on monocultures of *Rhodomonas* sp. in different concentrations. There was no significant difference between the two different microzooplankton types ( $p = 0.45$  ANOVA). No significant effect of nitrogen status was found on egg production, but we observed a marginally significant interaction between limitation and heterotrophic addition (Table 3).



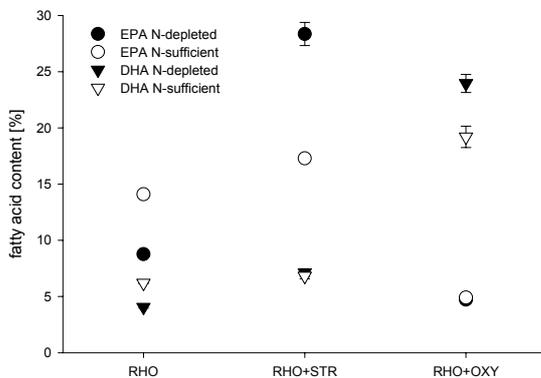
**Figure 4.**

EPA and DHA contents (% total fatty acids) of *Rhodomonas* sp. over the time during growth in either nitrogen depleted or nitrogen sufficient media; error bars represent standard error of the mean.

## Discussion

Our results show that the presence of heterotrophic protists can support higher egg production rates in *A. longiremis* compared to saturated concentrations of *Rhodomonas* sp. alone. The highest egg production rate ( $5 \text{ eggs female}^{-1} \text{ day}^{-1}$ ) that was reached during these laboratory experiments was also found in the field (Peterson et al. 2002). We observed no differences in hatching success between the different treatments, and hatching success was similar to other studies (Tang & Taal 2005). Differences in hatching success have been attributed to the fatty acid content as a food quality factor (Broglia et al. 2003, Arendt et al. 2005), but this cannot be corroborated by our results.

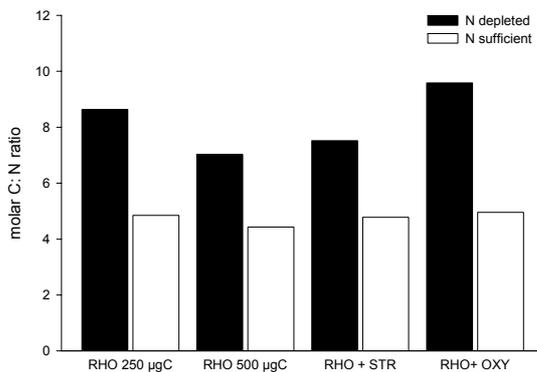
When phytoplankton becomes nitrogen depleted several aspects of algal cells such as carbon content and fatty acid composition change (Koski et al. 1998, Ahlgren & Hyenstrand 2003, Klein Breteler et al. 2005). This change can be so strong that it affects copepod egg production (Kuijper et al. 2004, Augustin & Boersma 2006). It is well known that overall food concentration may strongly control copepod egg production (Kjørboe et al. 1985, Calbet & Alcaraz 1996, Maps et al. 2005). Further, larger prey size (such as for the heterotrophic protists used here) compared to *Rhodomonas* sp. may cause higher feeding rates in *Acartia* (Berggreen et al. 1988, Tang & Taal 2005), or a higher efficiency. Alternatively, heterotrophic protists, like those used here, may also act as trophic upgraders of the food quality for copepods (Klein Breteler et al. 1999), but it is still unclear which specific factor of the ingested prey influences the copepod reproduction the most.



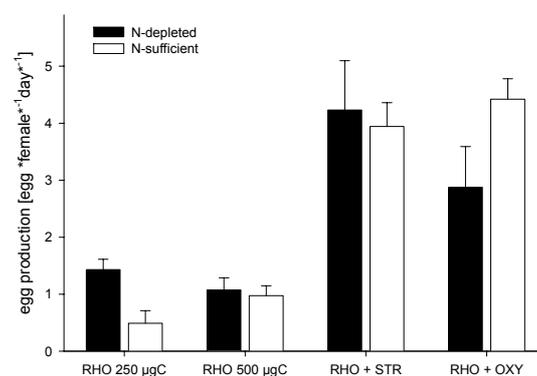
**Figure 5.**

EPA and DHA contents (as% total fatty acid) under nitrogen depleted (filled bars) and nitrogen sufficient (open bars) condition; Error bars represent standard error of the mean.

In our study, the egg production rate of copepods fed on *O. marina* and *S. conicum* were not significantly different from each other but higher than for the single *Rhodomonas* diets. In concert with these results, it has been shown that the presence of heterotrophic protists affects the fatty composition and especially the content of EPA and DHA. Recent studies have shown that *O. marina* is able to trophically transfer or modify biochemical compounds such as DMS (Wolfe et al. 1997), toxins (John et al. 2002) or fatty acids (Kleppel & Burkart 1995, Klein Breteler et al. 1999, Stevens et al. 2004). *O. marina* seems also to have the ability to enhance the food quality by providing essential fatty acids to higher trophic levels, even when fed on fatty acid deficient diet (Kleppel & Burkart 1995, Klein Breteler et al. 1999, Tang & Taal 2005, Veloza et al. 2006). In contrast, a recent study reported lower EPA contents in *O. marina* even when fed on *Rhodomonas* sp. compared to *Rhodomonas* sp. itself (Veloza et al. 2006). But in the same studies *O. marina* contained the highest lipid content. However, when *O. marina* was fed with bacteria no upgrading was observed (Ederington et al. 1995). Several studies, using *Strombidium sulcatum* as a microzooplankton link also fail to show trophic upgrading of the food quality for copepods (Broglia et al. 2003, Klein Breteler et al. 2004).



**Figure 6.** Molar C:N ratios of the different treatments at the beginning of experiment 3; Treatments under nitrogen depleted (filled bars) and nitrogen sufficient (open bars) condition.



**Figure 7.** *A. longiremis*. Egg production rates after incubation in either nitrogen depleted (filled bars) and nitrogen sufficient food (open bars); error bars represent standard error of the mean.

**Table 2.** Fatty acid content (percentage of the total fatty acid) of the used food for *Acartia longiremis*; (R) mean feed on *Rhodomonas* sp.

| Species                            | <i>Rhodomonas</i> sp. |          | <i>Srombidium conicum</i> (R) |          | <i>Oxyrrhis marina</i> (R) |          |
|------------------------------------|-----------------------|----------|-------------------------------|----------|----------------------------|----------|
|                                    | sufficient            | depleted | sufficient                    | depleted | sufficient                 | depleted |
| <b>Saturated fatty acids</b>       |                       |          |                               |          |                            |          |
| 14:0                               | 6.74                  | 8.66     | 3.31                          | 3.72     | 10.5                       | 6.36     |
| 16:0                               | 11.19                 | 16.95    | 9.47                          | 7.83     | 19.07                      | 21.08    |
| 18:0                               | 0.17                  | 1.42     | 1.98                          | 0.93     | 0.9                        | 0.64     |
| <b>Monounsaturated fatty acids</b> |                       |          |                               |          |                            |          |
| 14:1(n-5)                          | -                     | -        | 1.78                          | 1.57     | 1.23                       | 2.16     |
| 16:1(n-7)                          | 0.72                  | 1.2      | 3.28                          | 2.55     | 5.66                       | 2.06     |
| 18:1(n-7)                          | 4.63                  | 3.99     | 5.33                          | 3.06     | 7.63                       | 6.96     |
| 18:1(n-9)                          | 2.67                  | 7.21     | 1.77                          | 1.96     | 2.7                        | 5.04     |
| <b>Polyunsaturated fatty acids</b> |                       |          |                               |          |                            |          |
| 16:2(n-4)                          | 0                     | 0        | 1.26                          | 3.63     | 0                          | 0        |
| 18:2(n-6)                          | 2.73                  | 5.2      | 1.38                          | 2.38     | 1.54                       | 2.93     |
| 18:3(n-3)                          | 18.45                 | 19.37    | 10.42                         | 13.8     | 9.1                        | 10.57    |
| 18:4(n-3)                          | 30.36                 | 21.51    | 30.41                         | 22.02    | 15.18                      | 9.67     |
| 20:5(n-3)                          | 14.09                 | 8.77     | 17.3                          | 28.35    | 4.91                       | 4.72     |
| 22:6(n-3)                          | 6.2                   | 4.06     | 6.82                          | 7.12     | 19.2                       | 23.96    |
| FA < 2%                            | 2.05                  | 1.65     | 5.5                           | 1.07     | 2.4                        | 3.86     |

Based on the assertion of Stoecker and Capuzzo (1990) that heterotrophic protists can be a rich source of nitrogen, we expected that the C:N ratio of the samples of *Rhodomonas* sp. and heterotrophic protists would be lower than the C:N ratios of nitrogen limited *Rhodomonas* cultures. This was not the case. From this we can conclude that nitrogen as such is not limiting egg production in animals fed on nitrogen limited algae.

Further, during nitrogen depletion the content of PUFAs did not change compared to the increasing content of SFAs and MUFAs, but certain PUFAs such as EPA and DHA varied in an opposite manner compared to the total fatty acid content. But also in the heterotrophic protists no clear picture of increased amounts of highly unsaturated fatty acids was observed. Hence, neither the nitrogen content of the heterotrophic protists nor their fatty acid profile can explain the higher egg production when fed mixtures of autotrophes and heterotrophes.

**Table 3.** *Acartia longiremis*; statistical results for comparison of egg production rate (one-way ANOVA);  $p < 0.05$  indicates significant differences

|                           | SS     | Degrees of Freedom | Mean square | F ratio | p        |
|---------------------------|--------|--------------------|-------------|---------|----------|
| Intercept                 | 133.22 | 1                  | 133.22      | 234.64  | <b>0</b> |
| treatment                 | 49.23  | 3                  | 16.41       | 28.9    | <b>0</b> |
| nitrogen effect           | 0.02   | 1                  | 0.02        | 0.03    | 0.872    |
| treatment*nitrogen effect | 4.77   | 3                  | 1.59        | 2.8     | 0.076    |
| Error                     | 8.52   | 15                 | 0.57        |         |          |

Nevertheless, even if we do not know the mechanisms it is clear that copepods may increase their reproductive output by feeding on heterotrophic protists. In the Baltic Sea ciliates like *Strombidium* sp. are found in higher numbers than flagellates like *O. marina* (Setaelae & Kivi 2003, Högländer 2005). Copepods further show high feeding rates on ciliates, and the mesozooplankton could potentially obtain up to 70% of their carbon demand by consuming ciliates in the Baltic Sea (Johansson et al. 2004). The ciliates are seen as important nutrient regenerators (Johansson et al. 2004). But possibly, a demand for carbon, rather than for nitrogen drive the copepods to increase the feeding efficiency by feeding on heterotrophic protists when they are present. *Acartia tonsa* showed a slightly higher ingestion rate for *O. marina* comparing to feeding on *Rhodomonas* sp. (Tang & Taal 2005). Thus, it seems that different heterotrophic protists may compensate and therefore upgrade the nutritional value of the phytoplankton food, at least in some cases, but further experiments have to be done to determine how heterotrophic protists in general act as trophic upgraders for mesozooplankton in the Baltic Sea and elsewhere under nutritional depleted conditions.

### Acknowledgements

We are most grateful to Karin Bickmeyer, for assistance with CN measurements. We thank the crew of the RV “Alkor“ for their brilliant support during the cruises. We also thank Jörg Dutz for helping and establishing at all the practical methods. This study was funded by the GLOBEC-Germany program by the German Federal Ministry of Education and Research (BMBF).

### References

- Adrian R, Hansson S, Sandin B, De Stasio B, Larsson U (1999) Effects of food availability and predation on a marine zooplankton community - A study on copepods in the Baltic Sea. *Int Rev Hydrobiol* **84**:609-626
- Ahlgren G, Hyenstrand P (2003) Nitrogen limitation effects of different nitrogen sources on nutritional quality of two freshwater organisms, *Scenedesmus quadricauda* (Chlorophyceae) and *Synechococcus* sp. (Cyanophyceae). *J Phycol* **39**:906-917

- Arendt KE, Jónasdóttir SH, Hansen PJ, Gaertner S (2005) Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. *Mar Biol* **146**:513-530
- Augustin CB, Boersma M (2006) Effects of nitrogen stressed algae on different *Acartia* species. *J Plankton Res* **28**:429-436
- Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra, ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. *Mar Biol* **99**:341-352
- Bojanic N, Solic M, Krstulovic N, Sestanovic S, Marasovic I, Nincevic Z (2005) Temporal variability in abundance and biomass of ciliates and copepods in the eutrophicated part of Kastela Bay (Middle Adriatic Sea). *Helgol Mar Res* **59**:107-120
- Broglio E, Jónasdóttir SH, Calbet A, Jakobsen HH, Saiz E (2003) Effect of heterotrophic versus autotrophic food on feeding and reproduction of the calanoid copepod *Acartia tonsa*: relationship with prey fatty acid composition. *Aquat Microb Ecol* **31**:267-278
- Calbet A, Alcaraz M (1996) Effects of constant and fluctuating food supply on egg production rates of *Acartia grani* (Copepoda: Calanoida). *Mar Ecol Prog Ser* **140**:1-3
- Calbet A, Saiz E (2005) The ciliate-copepod link in marine ecosystems. *Aquat Microb Ecol* **38**:157-167
- Calbet A, Vaque D, Felipe J, Vila M, Sala MM, Alcaraz M, Estrda M (2003) Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate *Alexandrium minutum*. *Mar Ecol Prog Ser* **259**:303-309
- Ederington MC, McManus GB, Harvey HR (1995) Trophic transfer of fatty acids, sterols, and a triterpenoid alcohol between bacteria, a ciliate, and the copepod *Acartia tonsa*. *Limnol Oceanogr* **40**:860-867
- Folch, J, Lees, M, and Sloane-Stanley, GH (1957) A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* **226**: 497-509.
- Flynn KJ, Fielder J (1989) Changes in intracellular and extracellular amino acids during the predation of the chlorophyte *Dunaliella primolecta* by the heterotrophic dinoflagellate *Oxyrrhis marina* and the use of the glutamine/glutamate ratio as an indicator of nutrient status in mixed populations. *Mar Ecol Prog Ser* **53**:117-127
- Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. Plenum Press, New York
- Hansen AS, Nielsen TG, Levinsen H, Madsen SD, Thingstad TF, Hansen BW (2003) Impact of changing ice cover on pelagic productivity and food web structure in Disko Bay, West Greenland: a dynamic model approach. *Deep-Sea Res* **50**:171-187
- Hansen PJ, Bjørnsen PK, Hansen BW (1997) Zooplankton grazing and growth: scaling within the 2-2,000- $\mu$ m body size range. *Limnol Oceanogr* **42**:687-704
- Hansen PJ, Bjørnsen PK, Hansen BW (2000) Zooplankton grazing and growth: scaling within the 2-2,000- $\mu$ m body size range (ERRATUM). *Limnol Oceanogr* **45**:1891
- Hazzard SE, Kleppel GS (2003) Egg production of the copepod *Acartia tonsa* in Florida Bay: role of fatty acids in the nutritional composition of the food environment. *Mar Ecol Prog Ser* **252**:199-206
- Heiskanen AS, Tamminen T, Gundersen K (1996) Impact of planktonic food web structure on nutrient retention and loss from a late summer pelagic system in the coastal northern Baltic Sea. *Mar Ecol Prog Ser* **145**:195-208
- Höglander H (2005) Studies of Baltic Sea plankton - spatial and temporal patterns. Dissertation; Bibliography, Stockholm Univ. (Sweden)

- Johansson M, Gorokhova E, Larsson U (2004) Annual variability in ciliate community structure, potential prey and predators in the open northern Baltic Sea proper. *J Plankton Res* **26**:67-80
- John U, Tillmann U, Medlin LK (2002) A comparative approach to study inhibition of grazing and lipid composition of a toxic and non-toxic clone of *Chrysochromulina polyepsis* (Prymnesiophyceae). *Harmful Algae* **1**:45-57
- Jonsson PR, Tiselius P (1990) Feeding behaviour, prey detection and capture efficiency of the copepod *Acartia tonsa* feeding on planktonic ciliates. *Mar Ecol Prog Ser* **60**:35-44
- Kattner, G, and Fricke, HSG (1986) Simple gas-liquid chromatography method for simultaneous determination of fatty acids and alcohols in wax esters of marine organisms. *J Chrom* **361**: 263-268.
- Kjørboe T, Moehlenberg F, Hamburger K (1985) Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar Ecol Prog Ser* **26**:85-97
- Kivi K, Kuosa H, Tanskanen S (1996) An experimental study on the role of crustacean and microprotozoan grazers in the planktonic food web. *Mar Ecol Prog Ser* **136**:1-3
- Klein Breteler WCM, Koski M, Rampen S (2004) Role of essential lipids in copepod nutrition: no evidence for trophic upgrading of food quality by a marine ciliate. *Mar Ecol Prog Ser* **274**:199-208
- Klein Breteler WCM, Schogt N, Baas M, Schouten S, Kraay GW (1999) Trophic upgrading of food quality by protozoans enhancing copepod growth: role of essential lipids. *Mar Biol* **135**:191-198
- Klein Breteler WCM, Schogt N, Rampen S (2005) Effect of diatom nutrient limitation on copepod development: role of essential lipids. *Mar Ecol Prog Ser* **291**:125-133
- Kleppel GS, Burkart CA (1995) Egg production and the nutritional environment of *Acartia tonsa*: the role of food quality in copepod nutrition. *ICES J Mar Sci* **52**:297-304
- Kleppel GS, Burkart CA, Houchin L (1998) Nutrition and the regulation of egg production in the calanoid copepod *Acartia tonsa*. *Limnol Oceanogr* **43**:1000-1007
- Koski M, Breteler WK, Schogt N (1998) Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida). *Mar Ecol Prog Ser* **170**:169-187
- Kuijper LDJ, Anderson TR, Kooijman S (2004) C and N gross growth efficiencies of copepod egg production studied using a dynamic energy budget model. *J Plankton Res* **26**:213-226
- Maar M, Nielsen TG, Richardson K, Christaki U, Hansen OS, Zervoudaki S, Christou ED (2002) Spatial and temporal variability of food web structure during the spring bloom in the Skagerrak. *Mar Ecol Prog Ser* **239**:11-29
- Maps F, Runge JA, Zakardjian B, Joly P (2005) Egg production and hatching success of *Temora longicornis* (Copepoda, Calanoida) in the southern Gulf of St. Lawrence. *Mar Ecol Prog Ser* **285**:117-128
- Müller-Navarra DC (1995a) Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. *Arch Hydrobiol* **132**:297-307
- Müller-Navarra DC (1995b) Biochemical versus mineral limitation in *Daphnia*. *Limnol Oceanogr* **40**:1209-1214
- Nausch M, Nausch G, Wasmund N (2004) Phosphorus dynamics during the transition from nitrogen to phosphate limitation in the central Baltic Sea. *Mar Ecol Prog Ser* **266**:15-25

- Norrbin F (2001) Ultra-structural changes in the reproductive system of overwintering females of *Acartia longiremis*. *Mar Biol* **139**:697-704
- Peterson WT, Gómez-Gutiérrez J, Morgan CA (2002) Cross-shelf variation in calanoid copepod production during summer 1996 off the Oregon coast, USA. *Mar Biol* **141**:353-365
- Riegman R, Kuipers BR, Noordeloos AAM, Witte HJ (1993) Size-Differential control of phytoplankton and the structure of plankton communities. *Neth J Sea Res* **31**:255-265
- Setaelae O, Kivi K (2003) Planktonic ciliates in the Baltic Sea in summer: distribution, species association and estimated grazing impact. *Aquat Microb Ecol* **32**:287-297
- Shin K, Jang MC, Jang PK, Ju SJ, Lee TK, Chang M (2003) Influence of food quality on egg production and viability of the marine planktonic copepod *Acartia omorii*. *Prog Oceanogr* **57**:265-277
- Smetacek V (1981) The annual cycle of protozooplankton in the Kiel Bight. *Mar Biol* **63**:1-11
- Sommer U, Hansen T, Blum O, Holzner N, Vadstein O, Stibor H (2005) Copepod and microzooplankton grazing in mesocosms fertilised with different Si: N ratios: no overlap between food spectra and Si: N influence on zooplankton trophic level. *Oecologia* **142**:274-283
- Stevens CJ, Deibel D, Parrish CC (2004) Copepod omnivory in the North water polynya (Baffin Bay) during autumn: spatial patterns in lipid composition. *Deep-Sea Res (I Oceanogr Res Pap)* **51**:1637-1658
- Stoecker DK, Capuzzo JM (1990) Predation on protozoa: its importance to zooplankton. *J Plankton Res* **12**:891-908
- Tang KW, Taal M (2005) Trophic modification of food quality by heterotrophic protists: species-specific effects on copepod egg production and egg hatching. *J Exp Mar Biol Ecol* **318**:85-98
- Thomas H, Pempkowiak J, Wulff F, Nagel K (2003) Autotrophy, nitrogen accumulation and nitrogen limitation in the Baltic Sea: a paradox or a buffer for eutrophication? *Geophys Res Lett* DOI:10.1029/2003GL017937.
- Tillmann U (2004) Interactions between planktonic microalgae and protozoan grazers. *J Eukaryot Microbiol* **51**:156-168
- Turner JT, Levinsen H, Nielsen TG, Hansen BW (2001) Zooplankton feeding ecology: grazing on phytoplankton and predation on protozoans by copepod and barnacle nauplii in Disko Bay, West Greenland. *Mar Ecol Prog Ser* **221**:209-219
- Uitto A (1996) Summertime herbivory of coastal mesozooplankton and metazoan microplankton in the northern Baltic. *Mar Ecol Prog Ser* **132**:47-56
- Veloza AJ, Chu FLE, Tang KW (2006) Trophic modification of essential fatty acids by heterotrophic protists and its effects on the fatty acid composition of the copepod *Acartia tonsa*. *Mar Biol* **148**:779-788
- Wolfe GV, Steinke M, Kirst GO (1997) Grazing-activated chemical defence in a unicellular marine alga. *Nature* **387**:894-897
- Zhang W, Li H, Xiao T, Li C, Sun S, Zhang J (2006) Impact of microzooplankton and copepods on the growth of phytoplankton in the Yellow Sea and East China Sea. *Hydrobiologia* **553**:357-366



**FIRST AUTHOR PRESENTATIONS ON INTERNATIONAL CONFERENCES**

Peters J, Renz J, Hagen W (2003) Seasonality of lipid composition: indication of food quality and trophodynamics in Baltic Sea copepods? 3rd International Zooplankton Production Symposium, Gijon, Spain, 20.-23. May 2003

Peters J, Dutz J, Hagen W (2004) Seasonal changes in the food quality of seston and its implication for the production of the copepods in the Bornholm Basin (Baltic Sea). ASLO/TOS Ocean Research Conference, Honolulu, Hawaii, USA, 15.-20. February 2004

Peters J, Dutz J, Voss R, Baumann H, Temming A, Renz J, Hagen W (2004) On the lipid composition and nutritional condition of the trophic level components seston, copepods and sprat larvae. ICES Annual Science Conference, Vigo, Spain, 22.-25. September 2004

Peters J, Renz J, van Beusekom J, Boersma M, Hagen W (2005) Trophodynamics and life cycle strategies of *Pseudocalanus acuspes* – evidence from lipid composition. ASLO Summer Meeting, Santiago de Compostela, Spain, 19.-24. June 2005

Peters J, Dutz J, van Beusekom J, Boersma M, Hagen W (2006) Trophodynamics of the copepod *Temora longicornis* and its implication for the reproductive success – a fatty acid marker perspective. ASLO Summer Meeting, Victoria, Canada, 4.-9. June 2006

**CO-AUTHOR PRESENTATIONS ON INTERNATIONAL CONFERENCES**

Dutz J, Mohrholz V, Peters J, Renz J, Alheit J (2004) Identification of critical stages in the population dynamics of a key copepod species in the Bornholm Basin (Baltic Sea): potential linkages to physical forcing and climate variability ICES Annual Science Conference, Vigo, Spain, 22.-25. September 2004

Dutz J, Peters J, Hansen F (2006) The importance and quality of ‘large’ microzooplankton for the reproduction of *Acartia clausi* in the North Sea, Germany. ASLO Summer Meeting, Victoria, Canada, 4.-9. June 2006



Es ist vollbracht und nun kommt der schönste Teil der Arbeit. Nun kann ich endlich Danke sagen für all die Unterstützung, die ich in den letzten Jahren von vielen Seiten erfahren habe.

Ein ganz herzliches Danke geht an.....

...Prof. Wilhelm Hagen für die Betreuung dieser Arbeit und die Freiheit, die Sie mir in den letzten Jahren gewährt haben, und natürlich für die Bereitschaft meine Arbeit sogar am anderen Ende der Welt zu begutachten.

...Prof. Sigrid Schiel für die Begutachtung dieser Arbeit, für die aufmunternden Worte und dass Du nicht eine Sekunde gezögert hast, Dich auch im Urlaub meiner Arbeit anzunehmen.

...Holger Auel, für die konstruktive, geduldige und spontane Hilfe in den letzten Wochen und Jahren und dafür, dass Deine Tür immer offen steht für die vielen kleinen und grossen Fragen! Was hätte ich bloss ohne Dich gemacht?

...Dorothee Stübing für all die Antworten auf die „kurzen Fragen“, für die „schnellen“ Blicke auf die Daten und den Spass, den wir im gemeinsamen Büro haben! Bald hast Du eine entspanntere Zimmergenossin, versprochen.

...Silke Laakmann, für die gemeinsame schöne Zeit und die vielen, vielen aufmunternden Worte, wenn der Frust die Überhand gewonnen hat, aber natürlich auch für das fleissige Korrekturlesen, das nächtliche Ausharren in der heissen Phase und überhaupt.

...Petra Wencke für die tolle Unterstützung und die vielen gemeinsamen Stunden am zerlegten GC. Es hat uns zwar graue Haare gekostet, Petra, aber wir haben es geschafft!

...Britta Grote für die Motivation in den letzten Wochen und die Mühe, sich durch meine ganze Arbeit zu lesen – vielen, vielen Dank!

...Stephanie Borchardt für die tolle Unterstützung bei den Lipidanalysen!

...Martin Peters für all die Unterstützung in den letzten Jahren. Was hätte ich bloss ohne Deine Hilfe gemacht, ohne Planktonräder, spontan geschriebene Fettsäure-Auswerteprogramme, den ständigen rund-um-die-Uhr Computersupport und vor allem ohne den Blick eines klugen Nicht-Biologen auf die Geheimnisse des Planktons?! Danke für alles und noch viel viel mehr!

...Jasmin Renz für die letzten Jahre, in denen wir versucht haben, den kleinen Biestern auf die Schliche zu kommen, die geduldige Versorgung mit Literatur, unsere gemeinsamen Tage und Nächte im Kühlraum und für Dein immer offenes Ohr!

...Jörg Dutz für unsere vielen anregenden Diskussionen rund um die Wissenschaft und die gemeinsamen Tage im Kühlraum – es hat wirklich viel Spass gemacht mit Dir zu arbeiten.

- ...alle GLOBECs für die vielen Wasserschöpfer und Netze, die nette Unterstützung bei langen Sortiernächten im Kühllabor und für viele, viele schöne Erinnerungen!
- ...die Schiffsbesatzung, die ein reibungsloses und entspanntes Arbeiten an Bord erst möglich gemacht hat.
- ...Axel Temming für die geistige und tatkräftige Unterstützung bei der Modellentwicklung.
- ...Conny v. Waldthausen und Barbara Niehoff für die Kohlenstoff- und Stickstoff-Messungen.
- ...Martin Graeve für die GC-MS Analysen.
- ...alle weiteren, noch nicht genannten aus unserer Arbeitsgruppe, Tobias Kreibich, Hendrik Wessels, Dirk Elvers und Daniela Böttjer, es macht viel Spass mit euch zu arbeiten. Danke für die letzten Jahre, in denen immer wieder schön war in die Uni zu kommen.
- ...meine Freunde für die viele Geduld. Ich verspreche, dass ich mich jetzt auch mal wieder öfter bei euch melde!
- ...meine Eltern, für eure uneingeschränkte Unterstützung und dass ihr mir beigebracht habt, dass man seine Träume auch verwirklichen kann.
- ...Mark Rulffs, dafür, dass Du alle Höhen und Tiefen der letzten Jahre mitgemacht und letztere immer gelassen ertragen hast, immer hinter mir stehst und für so vieles mehr.
  
- .... alle die ich, zu nächtlicher Stunde, vielleicht vergessen haben sollte.

## **Eidesstattliche Erklärung**

(Gem. § 6(5) Nr. 1-3 PromO)

Hiermit versichere ich, dass ich die vorliegende Arbeit

1. Ohne unerlaubte, fremde Hilfe angefertigt habe
2. keine anderen, als die von mir im Text angegebenen Quellen und Hilfsmittel benutzt habe,
3. die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Bremen, 14. Juli 2006

Janna Peters