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**Biological processes in the North Sea II. Vertical distribution and reproduction of neritic copepods in relation to environmental factors**

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35 Key words: Copepod, Dogger Bank, reproduction, secondary production, food quantity and quality,  
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## Abstract

We studied the vertical distribution and reproduction of dominant neritic copepod species in the Dogger Bank area and surrounding North Sea to reveal 1) if these species are concentrated in the subsurface chlorophyll maximum layer, 2) if the chlorophyll maximum offers superior food conditions for reproduction compared to surface waters and 3) if the secondary production is thus higher in the frontal areas with a subsurface chlorophyll maximum. In addition, we wanted to 4) identify the most important environmental factors determining the reproduction of neritic copepods in the North Sea. We observed a higher egg production of cultured *Acartia tonsa* when fed with the seston from chlorophyll maximum, but no evidence of a higher copepod abundance in this layer. Secondary production was highest at the station closest to the upwelling of new nutrients, although seasonal differences in environmental variables probably overrode the differences between frontal and stratified stations. Copepod egg production on an annual basis seemed to be best predicted by the body size and specific fatty acids, with a high egg production, but low hatching success associated with a high EPA: DHA ratio. Total secondary production of small copepods seemed mainly related to the species composition, suggesting that factors controlling abundance of specific species rather than reproduction might be more important in determining the secondary production of copepods.

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## Introduction

Coastal areas and many productive frontal areas in the North Sea are dominated by small neritic copepod species, such as *Temora longicornis*, *Pseudocalanus* spp., *Paracalanus parvus*, *Oithona* spp., *Acartia* spp. and *Centropages* spp. (Fransz et al., 1998; Nielsen and Munk, 1998). At the Dogger Bank in the central North Sea, these species can form the majority of zooplankton biomass especially in summer (Fransz et al., 1991; Nielsen et al., 1993), contributing substantially to the diet of larval fish (Nielsen and Munk, 1998). Small copepods are also major grazers of phytoplankton and microzooplankton in the area, with the estimated grazing rates of 15-30% of the daily primary production and a potential to control the biomass of microzooplankton (Nielsen et al., 1993).

The frontal areas of the North Sea, including the Dogger Bank, are characterised by strong subsurface phytoplankton maxima at depths between 15 and 30 m during the stratified season (Richardson et al., 1998; Richardson et al., 2000). These subsurface peaks are suggested to be centres for high zooplankton production, where new production of large phytoplankton (mainly dinoflagellates) is efficiently transferred to copepod production (Richardson et al., 1998). The primary production of subsurface peaks over the year is estimated to exceed that of the spring bloom (Richardson et al., 2000), emphasising the potential importance for small neritic copepod species, with their annual biomass peak in late summer (Nielsen and Munk, 1998). The perceived importance of subsurface chlorophyll maximum for copepod secondary production is based on observations of peak biomass of *Oithona* spp., *Temora longicornis* and *Pseudocalanus* spp. at the pycnocline (Nielsen et al., 1993), as well as a typically higher secondary production at the depth close to the subsurface chlorophyll maxima (Richardson et al., 1998).

Secondary production of copepods is formed by biomass and growth, and mostly measured in terms of egg production. Egg production is influenced by factors such as temperature (Halsband and Hirche, 2001), food concentration (Bunker and Hirst, 2004), composition, mineral quality (Anderson and Pond, 2000) or biochemical quality (Jónasdóttir et al., 1995). Copepod biomass is also influenced by mortality, mainly assumed to be due to predation (Ohman and Wood, 1996). Normal diel vertical migration behaviour of copepods, with an ascent to surface waters for feeding during night time and descent during day time, has been linked to predation risk from visual predators, such as planktivorous fish (Hays et al., 1994 and 1996). As the subsurface maximum layer occurs in the deepest part of the photic zone where light levels are low (Richardson et al.,

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1998), one may assume that vertical migration to greater depths during daytime to avoid visual predators should not be very rewarding. A high copepod biomass in the subsurface chlorophyll maximum layer could thus be due to low predation pressure, while the high production rate could be related to increased food concentration, favourable food composition, superior food quality or low energetic costs of (no) vertical migration.

Our study concentrated on the stratified summer season ~~in the area of the~~ Dogger Bank, although to broaden the range of environmental conditions, we also included transects at other frontal areas in the coastal and central North Sea during different seasons. Dogger Bank is an important feeding ground for planktivorous fish (Daan et al., 1990), and has been studied intensively during the last few decades (Nielsen et al., 1993; Fransz et al., 1998; Richardson et al., 1998), including studies focusing on subsurface phytoplankton blooms (Richardson et al., 2000). We studied the vertical distribution and reproduction of dominant neritic copepod species in this area with 3 questions in mind. First, we wanted to know if these species are concentrated in the chlorophyll maximum layer, and if they perform diel vertical migration. Second, we wanted to know if egg production rates and hatching success are significantly higher in the chlorophyll maximum layer than in the surface. Third, we wanted to identify the most important environmental factors determining the vertical distribution, egg production and hatching success of neritic copepods in the North Sea. To be able to separate effects of temperature, body size and previous feeding history from the effects of food quantity and quality in the subsurface chlorophyll maximum, we included a bio-assay approach (see Müller-Navarra and Lampert, 1996), where cultured copepods were fed with *in situ* water to estimate their reproduction.

## Method

### Sampling stations

The study ~~took place~~ over four years: 2001, 2002, 2003 and 2005. The experiments in 2001 were designed to study the seasonal succession of *in situ* egg production and hatching of small copepods (represented by *Acartia clausi* and *Centropages typicus*) and their dependence on environmental variables (hereafter referred to as 'in situ' experiments), while the sampling in 2002, 2003 and 2005 focused on the late summer vertical distribution and biomass of all dominant neritic copepods. The bio-assay study in 2003 was conducted to evaluate the value of subsurface chlorophyll maximum vs. surface water for copepod (represented by *Acartia tonsa*) reproduction (hereafter referred to as the 'bioassay' experiment). The sampling stations in 2001 were situated

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3 along 4 transects both in the coastal and central North Sea: T1 was situated in the Skagerrak, T4 in  
4 the Dogger Bank area, T5 in the shallow coastal area of the North Sea, and T3 at the transition  
5 between the Skagerrak and North Sea with both shallow and deep stations. The sampling in 2002,  
6 2003 and 2005 was conducted in the Dogger Bank area (Fig. 1). In all years, the water column was  
7 sampled for temperature, salinity and fluorescence profiles (CTD) and microplankton species  
8 composition and biomass. In addition, in 2001 and 2003 fatty acid composition and concentration  
9 was measured. An overview of experiments, measurements and stations is presented in Table I,  
10 while the details of the different methods are described below.

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#### 16 *Hydrography, microplankton and fatty acid analysis*

17 Vertical distributions of temperature, salinity and fluorescence were measured using a  
18 SeaBird SBE11 CTD equipped with an *in situ* fluorometer. Fluorescence profiles were not taken on  
19 the Alkor cruise in September 2001 but standard chlorophyll measurements were made at the  
20 surface and within the well mixed water column. Fluorescence was calibrated against chl-*a*,  
21 measured in water samples from selected depths and stations during each cruise. The linear  
22 regression between fluorescence and chl-*a* typically had an  $R^2$  value > 0.7 (data not shown).

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26 In 2001, microplankton concentration and composition were measured from the water  
27 samples collected at the surface (ca 4 m), in the chlorophyll maximum layer, and close to the  
28 bottom, and integrated over the whole water column. In 2003, phytoplankton was sampled from the  
29 surface and from the chlorophyll maximum layer. The water was sampled using Niskin bottles on  
30 the CTD rosette. Microplankton samples were preserved in 4% acidic Lugol's solution for  
31 phytoplankton and ciliates, and gluteraldehyde for separation of autotrophic versus heterotrophic  
32 organisms. Lugol's samples were allowed to settle in a 50-ml settling chamber (Uttermöhl, 1958;  
33 Hasle, 1979) and gluteraldehyde samples handled according to Haas (Haas, 1982). Phytoplankton  
34 and ciliates were enumerated and geometrical axes of 10 cells per taxon were measured under  
35 inverted microscope (Lugol's samples) or epifluorescence microscope (gluteraldehyde samples).  
36 Volumes were calculated using the software program Planktonsys 3.11 from BioConsult A/S, and  
37 carbon content calculated according to Edler (Edler, 1979) and Mullin et al. (Mullin et al., 1966). A  
38 minimum of 400 cells (in total) were counted from each sample: a minimum of 50 cells sample<sup>-1</sup>  
39 were counted for the most common taxa, while all cells in the 50-ml sample were counted for the  
40 less abundant taxa.

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3 Fatty acid concentration and composition in 2001 were measured only in the  
4 chlorophyll maximum layer, while in 2003 fatty acids were analysed both from the surface and the  
5 chlorophyll maximum layer. In both years 1.5 – 5L of water (depending on seston concentration)  
6 were filtered under a low pressure on precombusted GF/C (Whatmann) filters, folded into cryovials  
7 and immediately frozen at -80 °C in nitrogen atmosphere. In 2001, known water volumes (0.5-60L)  
8 were first size fractionated by successive filtration through 80, 40 and 20 µm mesh nylon gauze  
9 filters to obtain fatty acid composition in different size fractions. Fatty acids were extracted from  
10 filters containing the field collected seston using chloroform:methanol solution (2:1 by volume). A  
11 known amount of the fatty acid C23:0 was added to the sample and used as an internal standard for  
12 quantification of specific fatty acids. After at least 24 hours extraction at -20°C, the chloroform:  
13 methanol phase was collected and the filters were washed three times with chloroform: methanol  
14 solution. The extracts were washed according to the modified Folch method (Hamilton et al., 1993).  
15 After saponification, the samples were transmethylated to fatty acid methyl esters (FAME) using  
16 boron trifluoride (BF<sub>3</sub>) and then stored in air-tight vials in an argon atmosphere at -80°C until GC  
17 analysis. The FAME sample was injected into a gas chromatograph (Hewlett Packard 5809A, with a  
18 30 m omegawax 320 µm column, and equipped with a split/splitless injection system) using helium  
19 as a carrier gas at 1.8 mL min<sup>-1</sup>. Fatty acid methyl esters were identified based on comparison with  
20 retention times of several standards; Larodan PUFA standard, fatty acid from the dinoflagellate  
21 *Prorocentrum minimum* to locate 18:5(n-3), Matreya PUFA-3 and Supelco 18919, resulting in  
22 identification of 42 fatty acids.  
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### 33 *Copepod biomass and vertical distribution*

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34 The sampling in 2002, 2003 and 2005 was conducted at 2-4 stations along one Dogger  
35 Bank transect, with samples taken at depth-intervals of 10 m (2002) or 5 m (2003 and 2005). The  
36 number of daily samplings was one in 2003 and 2005, and nine in 2002. Zooplankton abundance  
37 was estimated from samples collected using a zooplankton pump (ca 1200 L min<sup>-1</sup>), equipped with a  
38 30 µm net, and preserved with Borax buffered formalin (ca 4% final concentration).  
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42 The zooplankton samples were counted from 1/4 - 1/64 fraction of the sample, so that  
43 the counts exceeded 200 individuals for all dominant copepod species (see Results). Copepods were  
44 identified to the genus or species level, and divided into 7 developmental stages (nauplii +  
45 copepodite I-VI). Loose copepod eggs (not in 2005) and egg-sacs were included in the counts.  
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47 During 2002 and 2003 all individuals were separated into size classes (with a 25 µm precision)  
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3 according to prosome (copepodites and adults) or total (nauplii) length, while in 2005 ca 10  
4 individuals of each species and life-stage were measured. The biomass was calculated from the  
5 length-weight regressions of Klein Breteler and Gonzalez (Klein Breteler and Gonzalez, 1982),  
6 Sabatini and Kiørboe (Sabatini and Kiørboe, 1994) and Cohen and Lough (Cohen and Lough, 1981)  
7 for respectively *Temora longicornis*, *Centropages* spp. and *Pseudocalanus* sp., *Oithona* spp. and  
8 *Paracalanus parvus*. For *Microcalanus pusillus*, the length-weight regression of *T. longicornis* was  
9 used. Dry weight was converted to carbon assuming 40% carbon content of the dry weight (Mullin,  
10 1969).

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12 To avoid bias due to low numbers of individuals, the vertical distribution of different  
13 life-stages was examined in detail only for those species which contributed  $\geq 10\%$  to the total  
14 biomass for the given year. To ensure sufficient numbers of individuals all copepodite stages (I-V)  
15 were grouped together. Vertical distribution was expressed both in absolute numbers (ind. m<sup>-3</sup>) and  
16 as a proportion of the population at a given depth (% of total biomass).

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### 21 22 23 *Reproduction*

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25 *In situ* egg production and hatching success of *Acartia clausi* and *Centropages typicus*  
26 were measured along 4 transects during spring (March-April), summer (May/June, July/August)  
27 and autumn (September) 2001. Egg production and hatching experiments were carried out wherever  
28 *A. clausi* and / or *C. typicus* were present, which resulted in a total of 22 and 10 *in situ* egg  
29 production stations for *A. clausi* and *C. typicus*, respectively (Fig. 1, Table I). In addition, *in situ*  
30 egg production of *C. typicus* was measured in one occasion in 2003. The bio-assay in 2003 was  
31 conducted at 4 stations along one transect, which was repeated 6 times, so that each station was  
32 sampled during 6 subsequent days at approximately same time of the day.

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36 *In situ reproduction:* Individuals for the *in situ* experiments were collected by vertical  
37 hauls from below the thermocline, with a 180  $\mu$ m plankton net (WP2) equipped with a 5 L non-  
38 filtering cod end. Healthy active females were placed individually into 250 ml bottles containing 64  
39  $\mu$ m sieved ambient seawater from 4 m depth (15-30 replicates) and the bottles were placed in a dark  
40 temperature-controlled room (6-12°C). The sieving removed any ambient copepod eggs, while parts  
41 of the small size plankton community passed through. After 24 hours, eggs were collected by  
42 filtering the samples gently onto a 20  $\mu$ m mesh and immediately counted using a stereo microscope.  
43 The prosome lengths of the females were measured. Eggs from all replicates from a specific station  
44 were pooled and incubated in 650 mL bottles containing 20  $\mu$ m sieved ambient water, at *in situ*  
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3 temperature (6-12°C) appropriate for the station where they were collected. After an additional 72  
4 hours, the bottle contents were filtered onto a 20 µm mesh, and fixed in 5% Lugol's solution. Eggs,  
5 nauplii and empty egg shells were later counted under a binocular microscope. As the incubation  
6 time in April-May was too short to obtain complete hatching at the low temperature (6-7 °C),  
7 hatching success during these months could not be estimated. Weight-specific egg production was  
8 calculated based on the length-carbon regression of Berggreen et al. (Berggreen et al., 1988),  
9 assuming the carbon content of eggs to be ca 0.04 µg C egg<sup>-1</sup> (Kiørboe and Sabatini, 1995).

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11 *Bioassay:* The bioassay was conducted using *Acartia tonsa*, which have been cultured  
12 in the National Institute for Aquatic Resources (DTU Aqua) for over 100 generations. Typically,  
13 copepods are reared at 18 °C and 33 ‰, and fed *Rhodomonas* sp. in excess. In the experiments,  
14 recently (≤ 3 days before) matured *A. tonsa* were fed with *in situ* water from the chlorophyll  
15 maximum layer and surface, as well as with a saturating concentration (> 400 µg C L<sup>-1</sup>) of  
16 *Rhodomonas* sp. (control for standard egg production), at a constant temperature of ca 15 °C. This  
17 bioassay approach excluded the effects of body size, maternal nutrition and temperature, so that all  
18 the changes in egg production and hatching success were supposedly due to changes in food  
19 conditions, either quantity or quality (Müller-Navarra and Lampert, 1996; Koski et al., 2010).

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21 However, the use of cultured copepods introduces other problems; such as the typically low egg  
22 production, as well as the question of how well do the cultured individuals represent *in situ* animals  
23 (Koski et al. 2010). To reduce these problems, we included the standard food source *Rhodomonas*  
24 sp., to give an estimate of the close-to-maximum egg production of the culture.

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26 The bioassay was run for a total of 6 days, of which the first 24-h period served as an  
27 adaptation time. In the beginning of the experiment ca 15 adult females and 3 adult males were  
28 sorted out from the culture and placed together into each of 5 replicate bottles of ca 0.6 L. After  
29 each 24-h period, the eggs were counted, the copepod condition checked (dead/alive), and the  
30 copepods were thereafter transferred to a new food suspension, which was collected daily. The  
31 mortality during the incubations was relatively low, typically < 10 % d<sup>-1</sup> in both surface and  
32 chlorophyll maximum water. For estimation of hatching success, the eggs from the 5 replicate  
33 bottles were pooled into one 0.3 L hatching bottle, which was left to incubate for ca 3 days. All  
34 bottles were placed on a plankton wheel (ca 1 RPM), in the dark and at ca 16 °C. This was  
35 approximately similar to the surface temperature at the study site. At the termination of the hatching  
36 experiment, the bottle-contents were carefully filtered onto a 50 µm sieve and preserved in 4% acid  
37 Lugol's solution, for later counts of eggs and nauplii.

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### Secondary production

Copepod secondary production was calculated from the depth-integrated carbon biomass and weight-specific egg production, assuming that all stages have equal weight-specific growth rates (Berggreen et al., 1988). This method has many limitations (see e.g., Hirst and Sheader, 1997), and the secondary production rates presented here should thus be taken as rough estimates. To estimate the egg production we used the egg sacs collected with the zooplankton pump hauls for sac-spawning species, and both eggs collected with the pump and egg production measured in incubations for broadcast spawning species. The egg production of the sac-spawners was calculated by dividing the depth integrated egg sac biomass by the biomass of sac-spawning females (egg-ratio method; e.g., Sabatini and Kiørboe, 1994) and temperature-dependent development time of eggs (Andersen and Nielsen, 1997). The egg production of broadcast spawners was first estimated similarly, but since the rates obtained were very low, it seemed likely that the number of eggs in the water column did not accurately represent the egg production (due to e.g., sinking of eggs). We therefore decided to use the regression between egg production and body size (prosoma length) obtained from all *in situ* incubations pooled together to estimate the egg production of broadcast spawning copepods. However, as the egg production of copepods < 1 mm in prosoma length was not related to body size (Fig. 2), we decided to use the average egg production of all individuals < 1 mm for this size class, and only use the significant regression between prosoma length and egg production for the individuals > 1 mm ( $R^2 = 0.84$ ;  $p < 0.0001$ ; Fig. 2).

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### Statistics

Egg production, spawning percentage, brood size, hatching and prosoma length were tested for differences between stations or water layers (only bioassay) using 1- or 2-way analyses of variance (ANOVA); and, after pooling the stations, for differences between months (only *in situ* reproduction). The Tukey HSD *post-hoc* test was used for pairwise comparisons. If the conditions for normality and equal variance were not met, the non-parametric Kruskal-Wallis test and Dunn's pairwise comparison were used. Pearson correlation analysis was used to check for the correlations between egg production / hatching, body size and temperature, as well as between biomass / secondary production and temperature, chl-*a* and microplankton concentration.

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3 To investigate the dependence of egg production and hatching success on  
4 environmental variables, we ran a series of multiple regression analyses. To account for the effects  
5 of body size and temperature ~~on the *in situ* reproduction~~, a multiple regression analysis was first run  
6 for egg production, body size and temperature. As the model showed a contribution of body size  
7 rather than temperature (see Results), we then used the weight-specific egg production in forward  
8 stepwise multiple regression analysis with the environmental variables, without correcting the egg  
9 production for temperature. Finally, we combined the significant factors from both models for a  
10 final model. ~~For *in situ* reproduction~~, temperature and salinity were included as averaged values,  
11 while chl-*a* and phytoplankton concentration / composition were included as integrated values for  
12 the 0-50 m water column. For fatty acids only values from ~~the~~ chlorophyll maximum layer were  
13 available. As *Acartia* spp. in the North Sea appears to be relatively evenly distributed in the upper  
14 40 m of the water column (P. Munk, DTU Aqua; pers. com) we consider this to be a reasonable  
15 assumption.  
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22 The environmental variables ~~used in the multiple regression model~~, were chosen after  
23 data reduction by ~~principal component analysis (PCA)~~. The PCA was run on ~~a correlation matrix~~  
24 with Varimax rotation, first separately for all the fatty acids, and then after combining the fatty  
25 acids which had the highest loading on different PCs with the other environmental variables. ~~The~~  
26 PCA for fatty acids included 22 stations. In short, the PCA grouped the fatty acids according to the  
27 size fractions rather than type, so that most fatty acids in the size fraction ~~0-20  $\mu\text{m}$  had a moderate~~  
28 or significant loading on PC3, most fatty acids in 20-40  $\mu\text{m}$  had a significant loading on PC2, and  
29 most fatty acids in 40-80  $\mu\text{m}$  had a significant loading on PC1 (data not shown). However, the  
30 percentage of PUFA from total fatty acids in 0-20 and 20-40  $\mu\text{m}$  size fractions, and EPA: DHA  
31 ratio in the 40-80  $\mu\text{m}$  size fraction, typically had a high loading on a different PC than the absolute  
32 concentrations. We thus chose the sum of PUFAs in different size classes (0-20, 20-40 and 40-80  
33  $\mu\text{m}$ ), percentage of PUFAs in 0-20 and 20-40  $\mu\text{m}$  size class and DHA: EPA ratio in 40-80  $\mu\text{m}$  size  
34 class for further analysis with the other environmental factors.  
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41 Due to the missing data on phytoplankton biomass, the final PCA only included 11  
42 stations. Roughly, the PCA grouped together diatoms with the total (autotrophic) microplankton  
43 biomass and EPA:DHA in 40-80  $\mu\text{m}$  size fraction (PC1), heterotrophic biomass of all kinds (PC2),  
44 dinoflagellates and chl-*a* (PC3), flagellates represented by a high PUFA percentage in the 0-20  $\mu\text{m}$   
45 fraction (PC4), and temperature with a high percentage of PUFA in 40-80  $\mu\text{m}$  fraction (PC5; Table  
46 IIA). Based on this, we chose EPA:DHA in ~~the~~ 40-80  $\mu\text{m}$  size fraction as a representative of PC1,  
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ciliate biomass as a representative of PC2, chl-*a* as a representative of PC3, percentage of PUFA in the 0-20 µm size fraction as a representative of PC4 and temperature as a representative of PC5, to be used together with the egg production and hatching in the multiple regression model. Chl-*a* was used as a representative of PC3 rather than dinoflagellate biomass, as this allowed more stations (22) to be included in the model.

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As standard copepods from a culture were used in the bioassay experiment, we did not need to account for the effect of body size or temperature, and only a single multiple regression was run. For all analysis we used responses from day 4 only, as this allowed a long adaptation time for the animals combined with simultaneous measurements of all environmental variables; the total number of observations was thus 8 (4 stations with 2 water layers). Similar to the *in situ* reproduction, PCA was used to select for the environmental variables to be included in the model. All fatty acids had the highest loading in PC1, while only the ratio of EPA:DHA was separated with the highest loading on PC2 (data not shown). We thus included total fatty acids and the EPA:DHA ratio for the second PCA. The PCA combining fatty acids with other environmental variables separated flagellates and dinoflagellates together with a high concentration of total fatty acids (PC1) from diatoms and ciliates with a high EPA: DHA ratio (Table IIB). We thus chose total fatty acids as a representative of PC1 and EPA:DHA ratio as a representative of PC2. All statistics were conducted using the SigmaStat 3.1 or SPSS 11.5 statistical packages.

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## Results

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### Environment

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*Seasonal variation at transect stations:* The water column was well-mixed in March and April, while in May and July a thermocline, accompanied with increased salinity, formed below ca 20 m. There was no strong subsurface chlorophyll maximum layer, but chl-*a* was either equally distributed in the upper 40 m (March and July), increasing slightly with depth (April), or clearly highest in the surface layer (May) (Fig. 3). The average water column temperature (0-50 m) over the year was rather similar at all stations, 4-6 °C during March-April, close to 10 °C in May and 11-15 °C during July-September (Table III). The highest chl-*a* concentrations of ca 5 µg L<sup>-1</sup> (ca 140 mg m<sup>-2</sup>) were observed during spring in March at T3 and May at T5, at other times and transects the average chl-*a* concentrations ranged between 1.6 and 3.5 µg L<sup>-1</sup> (38-102 mg m<sup>-2</sup>). Diatoms dominated the microplankton composition at all stations and times and the proportion of heterotrophic organisms was typically between 10-20% of the total microplankton carbon.

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Polyunsaturated fatty acids (PUFA) were abundant in the layer of maximum chl-*a*, with concentrations up to ca 50  $\mu\text{g L}^{-1}$ , forming between 14 and 36% of the total fatty acids (Table III).

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Year-to-year variation at the Dogger Bank: In all years surface temperatures were high (ca 18 °C) and a thermocline was situated between ~ 25 and 30 m (Fig. 4). Clear chlorophyll subsurface maxima were found just below the thermocline, with peak concentrations of 2-2.5  $\mu\text{g chl-}a \text{ L}^{-1}$ . Also the biomass of heterotrophic microplankton was higher in the chlorophyll maximum layers than at the surface, up to ca 30  $\mu\text{g C L}^{-1}$  (Fig. 4). During the bio-assay study when microplankton composition and fatty acid concentration of seston were measured in more detail, about half of the microplankton was formed by heterotrophic dinoflagellates and ciliates (Table III), with an approximately equal contribution of autotrophic flagellates, dinoflagellates and diatoms.

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Both the absolute ( $\leq 12 \mu\text{g L}^{-1}$ ) and proportional (5-12%) concentrations of PUFA were lower during the bioassay study than on any of the transects sampled for *in situ* reproduction (Table III). Details of the environmental conditions in the sampling area in 2002 are presented by Poulsen and Kiørboe (Poulsen and Kiørboe, 2006), and in 2005 by Koski et al. (Koski et al., 2007) and Jónasdóttir and Koski (Jónasdóttir and Koski, submitted).

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#### Vertical distribution and biomass

No diel vertical migration was observed for any of the copepod species, but the different species seemed to consistently inhabit different depth layers (Fig. 5). While *Pseudocalanus* sp. and *Temora longicornis* seemed to primarily reside below the thermocline, copepod nauplii, *Paracalanus parvus*, *Microcalanus pusillus* and *Oithona* spp. were mainly found above the thermocline. *Centropages typicus* was primarily found above the thermocline in 2002, but in 2005 this species seemed to have a bimodal daytime distribution, with about half of the individuals residing below the thermocline. If the vertical distribution was separated into life-stages it appeared that all life-stages (separated to nauplii, copepodites, males and females) of *Oithona* spp. and *P. parvus* preferred the surface layers. The distribution of other species was more spread with most of the copepodites of *M. pusillus* and *C. typicus* rather evenly distributed in the upper 40 m, and most of the copepodites of *T. longicornis* and *Pseudocalanus* sp. evenly distributed below 20 m. In contrast, adults of these species were most abundant between 30 and 40 m, thus close to the chlorophyll maximum layer, with the exception of *Pseudocalanus* sp. females, which were most abundant close to the bottom (data not shown).

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If the vertical distribution was compared with the temperature and chl-*a* concentration, it appeared that *Oithona* spp., *Paracalanus parvus* and *Centropages typicus* were most abundant in the layer where temperature was > 14 °C, while *Temora longicornis* and *Microcalanus pusillus* were spread in temperatures between 6 and 15 °C and 7 and 18 °C, respectively (Fig. 6A). *Pseudocalanus* spp. had a bimodal distribution with a high proportion of individuals residing either in rather cold (6-8 °C) or rather warm (> 14 °C) water layers. With the possible exception of adult stages of *T. longicornis*, *M. pusillus* and *C. typicus*, none of the species appeared to be strongly associated with the chlorophyll maximum layer, as the peak distribution of most life-stages of all species typically was at ≤ 1 µg Chl-*a* l<sup>-1</sup> (Fig. 6B). Similarly, no effect of salinity was observed for any of the species or life-stages (data not shown).

The depth integrated biomass of small copepods varied threefold, with the lowest values observed in 2002 (90-120 mg C m<sup>-2</sup>), while in 2003 and 2005 the biomass ranged from 130 to 290 mg C m<sup>-2</sup>. Typically, sac-spawners (*Oithona* spp.) dominated the biomass in 2002, while in 2003 all stations were dominated by calanoid broadcast spawning species (*Microcalanus pusillus* and *Centropages typicus*), and in 2005 the dominance switched between broadcast spawners (mainly *Temora longicornis*; station 8) and sac-spawners (*Oithona* spp.; station 5; Table IV, Fig. 5). No significant correlation was observed between the depth-integrated copepod biomass and phytoplankton (average and max. chl-*a* concentration) or microzooplankton concentration, neither between the total biomass and temperature (Pearson  $p > 0.05$ ; data not shown). However, the biomass of sac-spawning copepods was significantly negatively correlated with temperature (Pearson correlation coefficient -0.789;  $p < 0.05$ ).

#### Egg production and hatching

**In situ reproduction:** The egg production rate of *Acartia clausi* was highest in April ( $21 \pm 15$  eggs f<sup>-1</sup> d<sup>-1</sup>; mean ± SD), due to the significantly higher number of eggs produced per brood compared to the other months (Kruskal-Wallis;  $H_3 = 95$ ;  $p < 0.001$ ). However, there were also significant differences in egg production between stations (1-way ANOVA,  $F_6 = 6.7$ ,  $p < 0.001$  for April; Kruskal Wallis,  $H_4 = 45.5$ ;  $p < 0.001$  for June and Kruskal-Wallis,  $H_7 = 64.8$ ,  $p < 0.001$  for July), sometimes with > 10-fold differences in egg production even between the stations on the same transect (Fig. 7A). Hatching success over the year varied between 60 and 75%, but, due to the short incubation time, the spring hatching results had to be omitted. The between-station variation in hatching success was typically less than the variation in egg production, although a few

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3 exceptionally low hatching successes of 26 and 33% were observed at stations 5.7 in June and 3.4  
4 in July, respectively. The female prosome length indicated that the spring, early summer and late  
5 summer individuals belonged to different generations (Kruskal-Wallis;  $H_3 = 252$ ;  $p < 0.001$ ; Table  
6 V). Due to the low female abundance in spring, the high egg production in April did not result in a  
7 high number of nauplii, but the nauplii peak was observed in late summer during the peak  
8 abundance of females (Fig. 7A).

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12 *Centropages typicus* egg production was highest in late summer ( $65 \pm 56$  eggs  $f^{-1} d^{-1}$ ),  
13 intermediate in spring ( $34 \pm 24$  eggs  $f^{-1} d^{-1}$ ) and low in autumn ( $14 \pm 17$  eggs  $f^{-1} d^{-1}$ ), due to the  
14 significantly lower number of eggs per brood in September than in March or July (Kruskal-Wallis;  
15  $H_2 = 70$ ;  $p < 0.001$ ). Similar to *Acartia clausi*, also *C. typicus* egg production was significantly  
16 different between stations, both in July (Kruskal-Wallis;  $H_4 = 17.0$ ;  $p < 0.01$ ) and in September  
17 (Kruskal-Wallis;  $H_3 = 29.8$ ;  $p < 0.001$ ), although with typically lower between-station variation  
18 (Fig. 7B). When measured, the hatching success was relatively low ( $36 \pm 11\%$ ). Based on the  
19 female prosome length, it appeared that the spring individuals belonged to a different generation  
20 than summer and autumn individuals (Kruskal-Wallis;  $H_2 = 24$ ;  $p < 0.001$ ; Table V). Similar to  
21 *Acartia clausi*, the high egg production of spring and summer did not result in a nauplii peak which  
22 only occurred during the high female abundance in September (Fig. 7B).

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24 The egg production rate of *Acartia clausi* was significantly positively correlated with  
25 prosome length, and significantly negatively correlated with temperature (Pearson correlation;  $p <$   
26  $0.01$ ; Fig. 8), while hatching did not correlate with prosome length, temperature or egg production  
27 (Pearson;  $p > 0.5$ ; data not shown). Egg production of *C. typicus* was not significantly related to  
28 body size or temperature (Pearson;  $p > 0.05$ ; Fig. 8). As a multiple correlation analysis with egg  
29 production of *A. clausi* against body size and temperature showed that the variation in egg  
30 production was mostly explained by body size (Table VIA), we decided to use the weight-specific  
31 egg production in the multiple regression analysis with food variables, but not to correct the egg  
32 production for temperature. Based on the PCA, we chose one environmental variable representing  
33 each PC for the multiple correlation analysis with the weight-specific egg production and hatching  
34 success. The analysis showed that PC2 and PC4 represented by EPA: DHA ratio in the size class of  
35 40-80  $\mu m$  and percentage of PUFA from the total fatty acids in the 0-40  $\mu m$  size fraction had a  
36 significant effect on egg production, while only the EPA:DHA ratio in the 40-80  $\mu m$  size class had  
37 a significant effect on hatching success. The model implied an increase of  $0.02 \mu g C (\mu g C)^{-1} d^{-1}$  in  
38 weight-specific egg production and a decrease of 19 % in hatching success with a unit change in  
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EPA: DHA ratio and a decrease of  $0.001 \mu\text{g C} (\mu\text{g C})^{-1} \text{d}^{-1}$  with a unit change in the percentage of 0-40  $\mu\text{m}$  PUFAs (Table VIA). Along with the EPA: DHA ratio in 40-80  $\mu\text{m}$ , also diatoms, total autotrophs and total microplankton concentration had a significant loading on PC2, while flagellates had a significant loading along with the PUFA proportion in PC4 (Table IIA), so that the effect of these factors could not be separated.

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Both the model with body size and the model with food variables, however, only explained  $\leq 60\%$  of the variation in egg production. We thus decided to combine these two and ran the multiple regression analysis with the egg production, body size and EPA:DHA ratio in 40-80  $\mu\text{m}$  size class. The combination of body size and EPA:DHA ratio in 40-80  $\mu\text{m}$  (representing the biomass of diatoms and autotrophic microplankton in general) explained 68% of the variance in egg production, with an increase of  $46 \text{ eggs f}^{-1} \text{d}^{-1}$  with a millimetre increase in prosome length and an increase of  $4.8 \text{ eggs f}^{-1} \text{d}^{-1}$  with a unit increase in EPA:DHA ratio. Due to scarcity of data, a multiple regression analysis was not attempted for *Centropages typicus* egg production or hatching success.

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*Bio-assay:* Egg production of the cultured *Acartia tonsa* fed with *in situ* water was generally low, on average ca 5 and  $1.5 \text{ eggs f}^{-1} \text{d}^{-1}$ , in the chlorophyll maximum and surface layers, respectively (Fig. 9A, Table V). The cumulative egg production (day 3) was significantly different between the stations (2-way ANOVA;  $F_{3,33} = 9.8$ ;  $p < 0.001$ ) and the water layers ( $F_{1,33} = 61.5$ ;  $p < 0.001$ ), with significantly highest egg production at station 8 and in the chlorophyll maximum layer (Tukey;  $p < 0.05$ ). The average egg production in the chlorophyll maximum layer was similar to the egg production on *Rhodomonas* sp. (Tukey HSD;  $p > 0.05$ ). Hatching success was high, respectively 81 and 86% in the surface and chlorophyll maximum layers, and similar to the hatching on *Rhodomonas* sp. (Fig. 9B, Table V). The multiple regression analysis confirmed the positive effect of increasing EPA: DHA ratio (here associated with diatom and ciliate biomasses) on egg production and its negative effect on hatching success, as observed with the *in situ* reproduction. Model coefficients suggested an increase in egg production by  $11.4 \text{ eggs f}^{-1} \text{d}^{-1}$  and a decrease in hatching success by 59.7 % with a unit change in EPA: DHA ratio. However, the model explained only 45% of the egg production and 55 % of the hatching success of *Acartia tonsa* (Table VIB).

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### Secondary production

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The secondary production of dominant neritic copepods in July-August was typically highest at the shallow station 8 ( $18\text{-}19 \text{ mg C m}^{-2} \text{d}^{-1}$ ). The secondary production at this station was

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3 mostly formed by broadcast spawning calanoid species. At most other stations the secondary  
4 production ranged from 8 to 14 mg C m<sup>-2</sup> d<sup>-1</sup>, with the exception of station 4/5 which in 2003 had a  
5 high secondary production of 19 mg C m<sup>-2</sup> d<sup>-1</sup>, but in 2002 and 2005 a low secondary production of  
6 5-8 mg C m<sup>-2</sup> d<sup>-1</sup>. The high secondary production in 2003 was associated with a high biomass of  
7 broadcast spawning copepods, while in 2002 and 2005 sac-spawning copepods dominated the  
8 biomass at this station (Table IV). Secondary production thus did not always correlate with the  
9 biomass, but a high proportion of broadcast spawning species tended to lead to a high secondary  
10 production, likely due to their higher weight-specific egg production (Table IV). No significant  
11 correlations were observed between secondary production and phytoplankton (average or maximum  
12 chl-*a*) or microzooplankton biomass (Pearson, *p* > 0.05; data not shown), but secondary production  
13 was significantly positively correlated with the average water column temperature (Fig. 10).  
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## 20 Discussion

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22 In summary, our results showed that the vertical distribution of small copepods was  
23 not determined by subsurface chlorophyll maximum, but only temperature seemed to have an effect  
24 on the distribution of (some) species. Secondary production, however, was highest at the most  
25 productive station 8 on top of the Dogger Bank, and egg production seemed to benefit from the  
26 chlorophyll maximum particularly at this station. Secondary production appeared to be governed  
27 mostly by the copepod species composition and reproductive rate, as the broadcast spawning  
28 calanoid species had a substantially higher weight-specific reproduction than e.g., *Oithona* spp.  
29 While reproduction seemed to be controlled by the changes in body size and qualitative aspects of  
30 food (such as the EPA: DHA ratio), temperature appeared to have an influence on species  
31 composition (broadcast vs. sac-spawning species). The seasonal differences in temperature (body  
32 size) and food quality likely overrode the potential effects of food quantity, which otherwise could  
33 have manifested in a high reproduction at productive frontal stations. These findings and their  
34 relevance are discussed in detail below.  
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## 41 Secondary production on Dogger Bank

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43 It has been suggested that the frontal zones in the North Sea are hot spots of biological  
44 activity, where production, food-web structure and diversity change within short distances (Nielsen  
45 and Munk, 1998). In these areas the elevated chlorophyll concentration would support high  
46 zooplankton production, with an associated biomass peak of copepods in the chlorophyll maximum  
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layer (Nielsen et al., 1993; Richardson et al., 2000). On the Dogger Bank, stations 5 and 8 could be considered the most productive, as they were located closest to the area with the upwelling of new nutrients (Richardson et al., 2000). Our study confirmed the high secondary production at these stations, though at station 5 only when the small copepods were dominated by broadcast-spawning calanoids. The other potential frontal stations, 5.1, 5.2 and the stations in the transect 1 (see Fig. 1), did not show a clear subsurface chlorophyll maximum, but the chl-*a* values were low throughout the summer. Consequently, reproduction was not elevated at these stations (Fig. 7), and reproduction on an annual scale appeared controlled by other factors than chl-*a*.

The highest depth integrated biomass and secondary production at stations 5 and 8 were similar to those which have been reported previously for the small copepods in the frontal areas of the North Sea, but lower than the typical biomass in the coastal areas where the small copepod species dominate (Nielsen and Munk, 1998). It appeared that the high biomass did not directly lead to a high secondary production (see Table IV), but species composition (broadcast vs. sac-spawners) and weight-specific reproduction determined the secondary production. Neither biomass nor secondary production were connected to chlorophyll concentration, but temperature seemed to have an effect on dominance of specific groups. It seemed that the biomass of sac-spawning copepods was negatively related to temperature, while the total secondary production increased with increasing temperature, with a highest depth-integrated production occurring at temperatures > 13 °C (Fig. 10). This was likely due to the high abundance of two out of three dominant calanoid broadcast-spawning species (*Centropages typicus* and *Microcalanus pusillus*) at the warm temperatures of ca 18 °C (Fig. 6), although temperature could also have a direct influence on the reproduction rate (e.g., Koski & Kuosa, 1999; Table VI). If the secondary production of small copepods was compared to that of *Calanus* spp. (Jónasdóttir and Koski, submitted) it appeared that the small copepods dominated the secondary production at station 8, while at stations 3 and 5 further away from the Dogger Bank secondary production of *Calanus* spp. was ca 2-6 times higher than that of small copepods. However, there was a large between-year variation in secondary production of both *Calanus* spp. and small copepods at these stations, with a 2-3 times higher secondary production of small copepods in 2002, but 2-6 times higher secondary production of *Calanus* spp. in 2005 (Jónasdóttir and Koski, submitted).

*The effect of subsurface chlorophyll maximum on vertical distribution and reproduction*

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Our results for the vertical distribution of dominant small copepod species did not support the observations of highest biomass in the subsurface chlorophyll maximum (Fig. 5), but were more similar to the studies showing maximum abundances below or above the pycnocline (Harris, 1988; Hays et al., 1996). It appeared that only adult stages of some copepod species (*Temora longicornis*, *Centropages typicus* and *Microcalanus pusillus*) resided in or close to the chlorophyll maximum layer, while juvenile stages and all stages of other species preferred layers above or below the chlorophyll maximum.

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In principal the choice of the habitat layer of copepods should represent the best choice between maximum food abundance and minimum predation risk (Fossheim and Primicerio, 2008). Although visual predators (such as planktivorous fish) mainly forage in the surface layers, invertebrate predators can be abundant in the subsurface chlorophyll maximum (Fossheim and Primicerio, 2008). Some of the main invertebrate predators are chaetognaths, which have been shown to feed selectively on some small copepods, such as *Pseudocalanus* sp., and may control their biomass in late summer and autumn (Tønnesson and Tiselius, 2005). Although no significant connection between the abundance of different copepod species in the chlorophyll maximum and chaetognath abundance was observed in the study area (data not shown), it appeared that in 2003 the maximum abundance of chaetognaths (ca 50 ind. m<sup>-3</sup>) was located close to the surface, while in 2002 and 2005 when copepods were typically either above or below the chlorophyll maximum layer, chaetognath abundances were at their maximum (20-50 ind. m<sup>-3</sup>) in the chlorophyll maximum. It could be that the vertical distribution of invertebrate predators contributes to the vertical distribution of small copepods, along with food availability, temperature and salinity. However, in the present study we only observed weak suggestions of the effects of temperature and invertebrate predators and no effects of salinity or subsurface chlorophyll maximum.

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Harris (Harris, 1988) suggested that, although copepods rarely reside in the subsurface chlorophyll maximum, they fill their guts and produce maximum amount of eggs while swimming through it during vertical migration. This behaviour was shown with *Calanus helgolandicus*, while in the study of Harris (Harris, 1988) *Acartia* spp. was the only copepod species which was permanently associated with the chlorophyll maximum layer. The subsurface chlorophyll maximum layer could thus be especially profitable for these species, although other neritic copepods would neither migrate through nor reside in the chlorophyll maximum layer (see Fig. 5).

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The egg production of *Acartia tonsa* was always higher when incubated in chlorophyll maximum water than in surface water at all stations. It has been suggested that the abundant

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dinoflagellates in the subsurface chlorophyll blooms would be profitable for copepod production (Richardson et al., 1998). However, this was not case in our study (Table VIA), although on average 55% of the phytoplankton biomass in the subsurface maximum was composed of dinoflagellates. The most abundant dinoflagellates during our study were various *Ceratium* species, which due to their large size and spiny morphology are typically not ingested by *Acartia* spp., although larger copepods such as *Calanus* spp. might graze on them (Jansen et al., 2006).

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It has also been shown that phytoplankton in the subsurface chlorophyll maximum is less nutrient limited than phytoplankton in the surface layer (Richardson et al., 1998 and 2000). As exponentially growing algae are generally of a better quality food than senescent ones (Jónasdóttir, 1994), a positive effect of food quality could be expected due to a higher nutrient concentrations in the chlorophyll maximum layer. If we assume that most of the phytoplankton diet of *Acartia tonsa* was formed by diatoms and flagellates (thus not *Ceratium*), and plot the egg production rate as a function of the concentration of these groups for the two layers, it indeed appears that the egg production was generally higher while feeding on chlorophyll maximum algae than on the corresponding concentration of surface algae (Fig. 11A). This apparent higher quality could have been related to the proportional PUFA content, which was higher in the chlorophyll maximum ( $12 \pm 6\%$ ) than in the surface ( $5.3 \pm 0.7$ ), the high EPA: DHA ratio as suggested by the multiple regression analysis, or possibly to e.g., lower C:N ratio of the seston, which was not measured in the present study. Whatever the reason for improved quality, it did not affect the hatching, as no difference between the layers was observed for the hatching success (Fig. 11B).

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#### Factors controlling reproduction

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We observed both seasonal (between months) and spatial (between stations) variation in egg production. As observed earlier, *Acartia clausi* egg production was positively related to prosome length and negatively related to temperature, likely reflecting the negative effect of temperature on body size (e.g., Halsband and Hirche, 2001). As the body size or temperature rarely differed between the sampling stations (during the same month), the effect of body size on egg production was mainly relevant at an annual scale. If the effect of body size was removed by using the weight-specific egg production, the egg production appeared to be dependent on the qualitative aspects of the food, such as proportional PUFA content and EPA: DHA ratio. This effect of food could have been due to both spatial and seasonal patchiness in the food environment and could thus

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3 affect the egg production both at short and long (annual) time scales. The beneficial effect of a high  
4 EPA: DHA ratio was also suggested by the bioassay experiments.

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6 EPA:DHA ratio has been observed to correlate with egg production in several studies,  
7 sometimes positively and sometimes negatively (reviewed by Jónasdóttir et al., 2009). The reason  
8 for the correlation between egg production and EPA:DHA ratio is unclear, although it is assumed  
9 that it has to do with the chemical similarity of these compounds and the following competition in  
10 chemical and physiological reactions (Sargent et al., 1999). However, in our study the beneficial  
11 effect of a high EPA:DHA ratio and a high percentage of PUFA likely reflected a positive effect of  
12 autotrophic food sources in general (diatoms in particular), as a high EPA:DHA ratio also reflected  
13 high concentrations of diatoms and total autotrophic microplankton (Table II). This suggested that  
14 the body size (likely controlled by temperature) and specific fatty acids abundant in phytoplankton  
15 were the main factors controlling egg production. Our results are thus in agreement with previous  
16 field studies demonstrating a combined effect of temperature (via body size) and food (Calbet &  
17 Agusti 1999, Bunker & Hirst 2004, Maps et al. 2005), although EPA: DHA ratio (or diatom  
18 biomass) rather than bulk chl-*a* appeared to be a better predictor of egg production.

22 As observed earlier, egg production and hatching success were controlled by different  
23 factors; in fact while the high EPA: DHA ratio was beneficial for egg production, it appeared to  
24 have a negative effect for hatching success. Typically, high hatching success correlates with a high  
25 ingestion of DHA, while a negative correlation is often observed between hatching success and  
26 EPA: DHA ratio (Jónasdóttir et al., 2009). High EPA concentration of seston is mostly associated  
27 with a high biomass of diatoms, which typically promotes high egg production but low hatching  
28 success (e.g., Peters et al., 2007). As diatoms often dominate the phytoplankton in the coastal areas  
29 of the North Sea, particularly during the spring bloom, a low hatching success, together with a low  
30 female abundance, can lead to low recruitment irrespective of the high egg production rate.

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32 In the present study the maximum egg production of *Acartia clausi* occurred in April,  
33 and was similar to the rate previously recorded for this species (Halsband and Hirche, 2001;  
34 Wesche et al., 2007). Due to the low number of females in the water column, the high egg  
35 production in the spring was, however, not followed by an increase in nauplii abundance, which  
36 only peaked in the summer. Similarly, the peak egg production of *Centropages typicus* was highest  
37 in July-August, but the peak abundance of nauplii first occurred 2 months later in September.  
38 Although the irregular sampling and advection did not allow a detailed analysis of population  
39 dynamics; it appeared clear that the high egg production in spring (*Acartia clausi*) or summer  
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3 (*Centropages typicus*) did not lead to an increase in biomass. For *A. clausi* it is also likely that the  
4 female peak in June did not originate from April eggs, but rather March eggs: even in optimal food  
5 conditions the generation time of *A. clausi* at the 6-7 °C would be > 60 days (Huntley and Lopez,  
6 1992; Klein Breteler and Schogt, 1994), while the time between the sampling events was ca 40  
7 days. The April cohort thus more or less disappeared from the water column. It seems that the  
8 success of spring generations of small copepods can be extremely variable, with high mortality rates  
9 following from for example, predation and cannibalism (Peterson and Kimmerer, 1994), food  
10 concentration (Koski et al., 2010) or temperature affecting the timing of the spring bloom (Dutz et  
11 al., 2010).  
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## Table and figure legends

Table I. Overview of the number of sampling stations, copepod species and measured variables. For the location of stations, see Fig. 1. (T) Temperature, (FA) fatty acids.

Table II. Rotated component matrix (PCA with Varimax rotation) showing the loadings of different environmental variables in (A) 2001 and (B) 2003. The significant high loadings ( $> 0.7$ ) for each principal component (PC) are printed in bold, the significant moderate loadings ( $> 0.5$ ) are printed in italics; the variables which were chosen for the multiple regression analysis are underlined. (T) Temperature, (S) salinity, (Chl) chlorophyll *a*, (Flag) flagellates, (aut dino) autotrophic dinoflagellates, (het dino) heterotrophic dinoflagellates, (dino) total dinoflagellates, (diat) diatoms, (cil) ciliates, (aut tot) total autotrophs, (het tot) total heterotrophs, (micropl tot) total microplankton, (PUFA) polyunsaturated fatty acid concentration, (PUFA%) percentage of PUFAs from total fatty acids. All microplankton variables are biomass in carbon ( $\mu\text{g L}^{-1}$ ).

Table III. Average temperature, salinity (‰) and integrated chl-*a* concentration ( $\text{mg m}^{-2}$ ) for the 0-50 m water column ( $\pm$  SD), list of dominant algae groups with the number of stations in which the group dominates in parenthesis, integrated concentration of heterotrophic and total microplankton biomass ( $\mu\text{g C L}^{-1}$ ) with the percentage of heterotrophs in parenthesis, concentration of polyunsaturated fatty acids ARA (18:3n3), DHA (22:6n3), EPA (20:5n3) and total PUFA ( $\mu\text{g L}^{-1}$ ; with the percentage of PUFA from total FA in parenthesis), as well as the DHA: EPA ratio ( $\mu\text{g:}\mu\text{g}$ ) in the chlorophyll maximum layer averaged over the different transects in March-September (T1-5) and August (Bio-assay study; S: surface water, M: chl-max. layer). For the location of transects and the number of stations per transect, see Fig. 1. and Table I (-) Missing data. Other abbreviations as in Table II.

Table IV. Depth-integrated biomass (Bm;  $\text{mg C m}^{-2}$ ) and secondary production (Sp;  $\text{mg C m}^{-2} \text{d}^{-1}$ ) of broadcast and sac-spawning copepods, as well as the average egg production (Ep; % body weight  $\text{d}^{-1}$ ) used to calculate the secondary production, and the species dominating the biomass of small copepods (*Acartia* spp., *Temora longicornis*, *Pseudocalanus* sp., *Centropages typicus*, *Microcalanus pusillus*, *Paracalanus parvus* and *Oithona* spp.) in 2002, 2003 and 2005 (see Fig. 5). The egg production of sac-spawning copepods was calculated using the egg ratio method; the egg

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production of broadcast spawners was based on the significant regression between prosome length and egg production (Fig. 2; Methods). For station localities, see Fig. 1.

Table V. Average egg production (eggs  $f^{-1} d^{-1}$ ), percentage of females producing eggs (%  $day^{-1}$ ) and brood size (no of eggs brood $^{-1}$ ), hatching success (%) and female prosome length (mm) of *Acartia clausi* and *Centropages typicus* in March – September (*in situ* reproduction), and *Acartia tonsa* in August 2003 (bioassay reproduction; mean  $\pm$  SD). The number in parenthesis after the month indicates the number of stations, the number after hatching success the number of observed eggs. The letters group the months which are not significantly different from each other ( $p > 0.05$ ). (Blank) no experiment, (-) missing data.

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Table VI. Multiple regression coefficients and their significance for the models describing the factors influencing (A) the *in situ* egg production and hatching of *Acartia clausi* and (B) the bioassay egg production and hatching of *Acartia tonsa*. In (A) [Egg production = constant + (C1 body size) + (C2 temperature)], [Weight-specific egg production / hatching = constant + (C1 PUFA % in the 0-40  $\mu m$  size fraction) + (C2 EPA: DHA in the 40-80  $\mu m$  size fraction) + (C3 T) + (C4 Chl-*a*) + (C5 ciliate biomass)] and [Egg production = constant + (C1 body size) + (C2 EPA:DHA in 40-80  $\mu m$ )]. In (B) [Egg production / hatching = constant + (C1 EPA: DHA) + (C2 total fatty acid concentration)]. The values represent the best fit of a multiple linear regression for egg production, body size and temperature (*A. clausi*) and the best fit of a forward stepwise regression for the weight-specific egg production and environmental variables (*A. clausi*) and for all regressions with *A. tonsa*. Weight-specific egg production is expressed as  $\mu g C (\mu g C)^{-1} d^{-1}$ , egg production in number of eggs  $f^{-1} d^{-1}$ , body size as length in mm, chl-*a* and total fatty acids as  $\mu g L^{-1}$ , ciliate biomass as  $\mu g C L^{-1}$ , PUFA as a percentage from total fatty acids, and EPA:DHA as a weight ration ( $\mu g : \mu g$ ). The coefficients describe the expected increase / decrease in egg production or hatching per a unit change of the independent variable. (\*) Denotes the significant coefficients at the  $p < 0.05$  level, (\*\*) significant coefficients at the  $p < 0.01$  level.

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Fig. 1. Map of the study area showing transects and sampling stations in (A) 2001 and (B) 2002, 2003 and 2005. The stations in 2001 were situated along 4 transects in the coastal and central North Sea and sampled in March-September, while stations in 2002-2005 were all situated at the Dogger Bank area and sampled in July-August. (Cross) stations with *Acartia* spp. reproduction and

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abundance (2001; white crosses for March), (circle) stations with *Centropages typicus* reproduction and abundance (2001; open squares for March), (star) Dogger bank stations with *A. tonsa* bioassay (2003) and (open circle) sampling for vertical distribution and biomass (2002, 2003 and 2005). In 2001, the first number refers to the transect, the second number to the station (e.g., 3.3 is the station 3 on the transect 3).

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Fig. 2. Egg production (eggs  $f^{-1} d^{-1}$ ) as a function of prosome length (mm) in all incubations. (Solid circles) *Acartia clausi*, (open circles) *Centropages typicus*. The dashed line indicates the significant linear regression between the egg production and body size for  $> 1$  mm copepods (see Methods). As the egg production of *C. typicus* in March and September was exceptionally low (five open circles in the lower right-hand corner of the graph), and clearly out of range in relation to body size, it was excluded from the regression. The regression statistics and the average egg production of  $< 1$  mm copepods are indicated in the figure.

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Fig. 3. Example of a typical vertical distribution of temperature ( $^{\circ}C$ ; open circles), salinity ( $\text{‰}$ ; solid triangles) and chl-*a* ( $\mu\text{g L}^{-1}$ ; solid circles) in March (T3), April (T5), May (T5) and July (T5). For location of the transects, see Fig. 1.

Fig. 4. Vertical distribution of temperature ( $^{\circ}C$ ; lines) and chl-*a* ( $\mu\text{g L}^{-1}$ ; symbols) at Dogger Bank in 2002, 2003 and 2005. (Squares and solid line) station 2, (diamonds and medium dashed line) station 4, (downward triangles and long dashed line) station 1, (circles and dotted line) station 3, (upward triangles and solid line) station 5 and (stars and dash-dot line) station 8. The numbers indicate concentration of microzooplankton and heterotrophic flagellates ( $\mu\text{g C L}^{-1} \pm \text{SD}$ ) in surface and in chlorophyll maximum layer. For locations of the stations, see Fig. 1.

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Fig 5. Vertical distribution of copepod nauplii, *Pseudocalanus* sp., *Centropages typicus*, *Paracalanus parvus*, *Microcalanus pusillus*, *Temora longicornis* and *Oithona* spp. at the Dogger Bank in (A) 2002, (B) 2003 and (C) 2005 ( $\text{ind. m}^{-3}$ ; average of all stations  $\pm$  SE). The shaded area indicates the chlorophyll maximum layer, the number indicates the proportion of the species from total biomass of small copepods (average of all stations  $\pm$  SE). In 2002 black columns represent night samples (average of 4 sampling times), light grey columns day samples (average of 5 sampling times). Note different scales of the x-axis.

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Fig. 6. The proportional distribution of different life stages (% from total) as a function of (A) temperature, and (B) chl-*a*. (Open symbols with a cross) nauplii, (open symbols) copepodite I-V, (solid symbols) females, (grey symbols) males. (Triangles) 2002, (circles) 2003, (squares) 2005. Only species and years representing  $\leq 9\%$  of the total biomass of small copepods are included in the figure (see Fig. 5).

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Fig. 7. Female abundance ( $\text{ind. m}^{-2}$ ), *in situ* egg production ( $\text{eggs f}^{-1} \text{d}^{-1}$ ; mean  $\pm$  SE) and nauplii abundance ( $\text{ind. m}^{-2}$ ) of (A) *Acartia clausi* and (B) *Centropages typicus* along the North Sea transects in March-September. The transect / station numbers as in Fig. 1 and Table III. The calculated nauplii abundance refers to the amount of nauplii estimated based on female abundance, egg production and hatching success, while the observed abundance is the number of nauplii in zooplankton (pump) samples (see Methods). (MD) Missing data.

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Fig. 8. Egg production ( $\text{eggs f}^{-1} \text{d}^{-1}$ ; mean  $\pm$  SE) of *Acartia clausi* and *Centropages typicus* as a function of prosome length (mm; mean  $\pm$  SE) and temperature ( $^{\circ}\text{C}$ ). The significant correlation coefficients from the Pearson correlation analysis are shown in the figure.

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Fig. 9. (A) Cumulative egg production during the 5 subsequent days of incubation ( $\text{eggs f}^{-1}$ ) and (B) average hatching success (%) of cultured *Acartia tonsa* incubated in water collected from chlorophyll maximum (solid symbols, black columns) and surface (open symbols, white columns) layers at the four Dogger Bank stations, as well as in *Rhodomonas* sp. (grey symbols and columns; mean  $\pm$  SE). In (B), 1, 3, 5 and 8 refer to the stations numbers, M and S to chlorophyll maximum and surface layers, respectively.

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Fig. 10. Secondary production ( $\text{mg C m}^{-2} \text{d}^{-1}$ ) of small copepods as a function of average water column temperature. The Pearson correlation statistics are included in the figure.

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Fig. 11. (A) Egg production ( $\text{eggs f}^{-1} \text{d}^{-1}$ ) and (B) hatching success (%) of *Acartia tonsa* as a function of concentration of diatoms and flagellates ( $\mu\text{g C L}^{-1}$ ) in the bio-assay experiment (mean  $\pm$  SE). Only the range of concentrations occurring both in the surface and chlorophyll maximum layer are included. (Filled circles) Surface layer, (open circles) chlorophyll maximum layer.

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Table I. Overview of the number of sampling stations, copepod species and measured variables.

<i>Measurement</i>	<i>Species</i>	<i>No of stations</i>	<i>Background</i>
<i>In situ</i> reproduction	<i>A. clausi</i>	22 (2001)	T, Chl- <i>a</i> , microplankton, FA, depth-integrated copepod biomass (day)
	<i>C. typicus</i>	10 (2001)	
	<i>C. typicus</i>	1 (2003)	
Bioassay reproduction	<i>A. tonsa</i>	4 (2003); chl max and surface	Chl- <i>a</i> , microplankton, FA
Vertical distribution and biomass	Dominant neritic species	2 (2002): 5 depths, 9 times a day	T, Chl- <i>a</i> , microplankton
		3 (2003): 11 depths	
		4 (2005): 11 depths	

Table II. Rotated component matrix (PCA) showing the loadings of different environmental variables in (A) 2001 and (B) 2003.

	A) Components in 2001					B) Components in 2003	
	PC 1 (27%)	PC 2 (23%)	PC 3 (16%)	PC 4 (15%)	PC 5 (11%)	PC 1 (80%)	PC 2 (16%)
T	0.0054	-0.108	0.222	0.514	<b>0.784</b>		
Chl	-0.519	-0.445	0.561	0.391	0.166	0.557	0.721
S	-0.492	0.518	0.482	0.254	-0.183		
Flag	0.219	-0.376	-0.151	<b>0.835</b>	-0.111	<b>0.867</b>	0.443
Het dino	0.336	-0.029	<b>0.892</b>	-0.146	-0.017	<b>0.929</b>	0.348
Aut dino	-0.218	-0.101	<b>0.912</b>	0.069	0.121	<b>0.970</b>	0.223
Dino	0.080	-0.069	<b>0.988</b>	-0.048	0.054	<b>0.962</b>	0.253
Diat	<b>0.983</b>	-0.011	-0.048	-0.071	-0.005	0.031	<b>0.995</b>
Cil > 50 µm	0.132	<b>0.810</b>	-0.116	-0.003	0.493		
Cil < 50 µm	-0.130	<b>0.890</b>	-0.265	-0.045	-0.255		
Cil tot	-0.082	<b>0.939</b>	-0.252	-0.039	-0.108	0.375	<b>0.905</b>
Aut tot	<b>0.973</b>	-0.097	-0.017	0.115	-0.020	<b>0.965</b>	0.248
Het tot	0.137	<b>0.886</b>	0.332	-0.131	-0.115	0.664	0.742
Micropl tot	<b>0.978</b>	0.004	0.020	0.099	-0.033	<b>0.886</b>	0.461
PUFA 0-20 µm	0.628	0.329	0.057	0.562	-0.125		
PUFA % 0-20 µm	-0.044	0.142	-0.038	<b>0.928</b>	-0.154		
PUFA 20-40 µm	0.306	-0.266	-0.080	-0.334	0.666		
PUFA % 20-40 µm	-0.339	-0.396	0.136	0.641	0.369		
PUFA 40-80 µm	-0.256	0.021	0.081	-0.206	<b>0.878</b>		
EPA:DHA 40-80 µm	<b>0.961</b>	0.008	0.140	-0.145	0.027		
FA tot						<b>0.931</b>	0.201
<b>EPA:DHA tot</b>						0.339	<b>0.924</b>





Table V. Average egg production, percentage of females producing eggs and brood size, hatching success and female prosome length of *Acartia clausi* and *Centropages typicus* in March – September (*in situ* reproduction), and *Acartia tonsa* in August (bioassay).

	Egg production			Hatching	Length
	Eggs f <sup>-1</sup> d <sup>-1</sup>	% prod	Eggs brood <sup>-1</sup>	%	mm
<i>A. A. clausi</i>					
March (1)	<sup>a,b</sup> 8.2 ± 7.0	<sup>a</sup> 67	<sup>b</sup> 11 ± 6	-	<sup>b,c</sup> 0.9 ± 0.1
April (7)	<sup>a</sup> 21 ± 15	<sup>a</sup> 82 ± 17	<sup>a</sup> 25 ± 6	-	<sup>a</sup> 1.1 ± 0.07
May-June (5)	<sup>b</sup> 8.1 ± 7.2	<sup>a</sup> 71 ± 36	<sup>b</sup> 9 ± 5	<sup>a</sup> 60 ± 22 (320)	<sup>b</sup> 1.0 ± 0.08
July-August (9)	<sup>b</sup> 6.1 ± 6.9	<sup>a</sup> 52 ± 33	<sup>b</sup> 9 ± 4	<sup>a</sup> 75 ± 18 (205)	<sup>c</sup> 0.9 ± 0.06
<i>B. C. typicus</i>					
March (1)	<sup>a</sup> 34 ± 24	<sup>a</sup> 82	<sup>a</sup> 42 ± 19	-	<sup>a</sup> 1.35 ± 0.05
July-August (5)	<sup>a</sup> 65 ± 56	<sup>a</sup> 75 ± 34	<sup>a</sup> 76 ± 13	36 ± 11 (2643)	<sup>b</sup> 1.27 ± 0.07
September (4)	<sup>b</sup> 14 ± 17	<sup>a</sup> 63 ± 36	<sup>b</sup> 19 ± 7	-	<sup>b</sup> 1.26 ± 0.05
August 2003 (1)	39 ± 29	87	45 ± 26	-	1.20 ± 0.04
<i>C. A. tonsa</i>					
Chl-max (4)	<sup>a</sup> 4.7 ± 4.2			<sup>a</sup> 81 ± 8 (534)	
Surface (4)	<sup>b</sup> 1.4 ± 1.3			<sup>a</sup> 86 ± 13 (158)	

Table VI. Multiple regression coefficients and their significance for the models describing the factors influencing A) the egg production and hatching of *Acartia clausi* and B) the egg production and hatching of *Acartia tonsa*.

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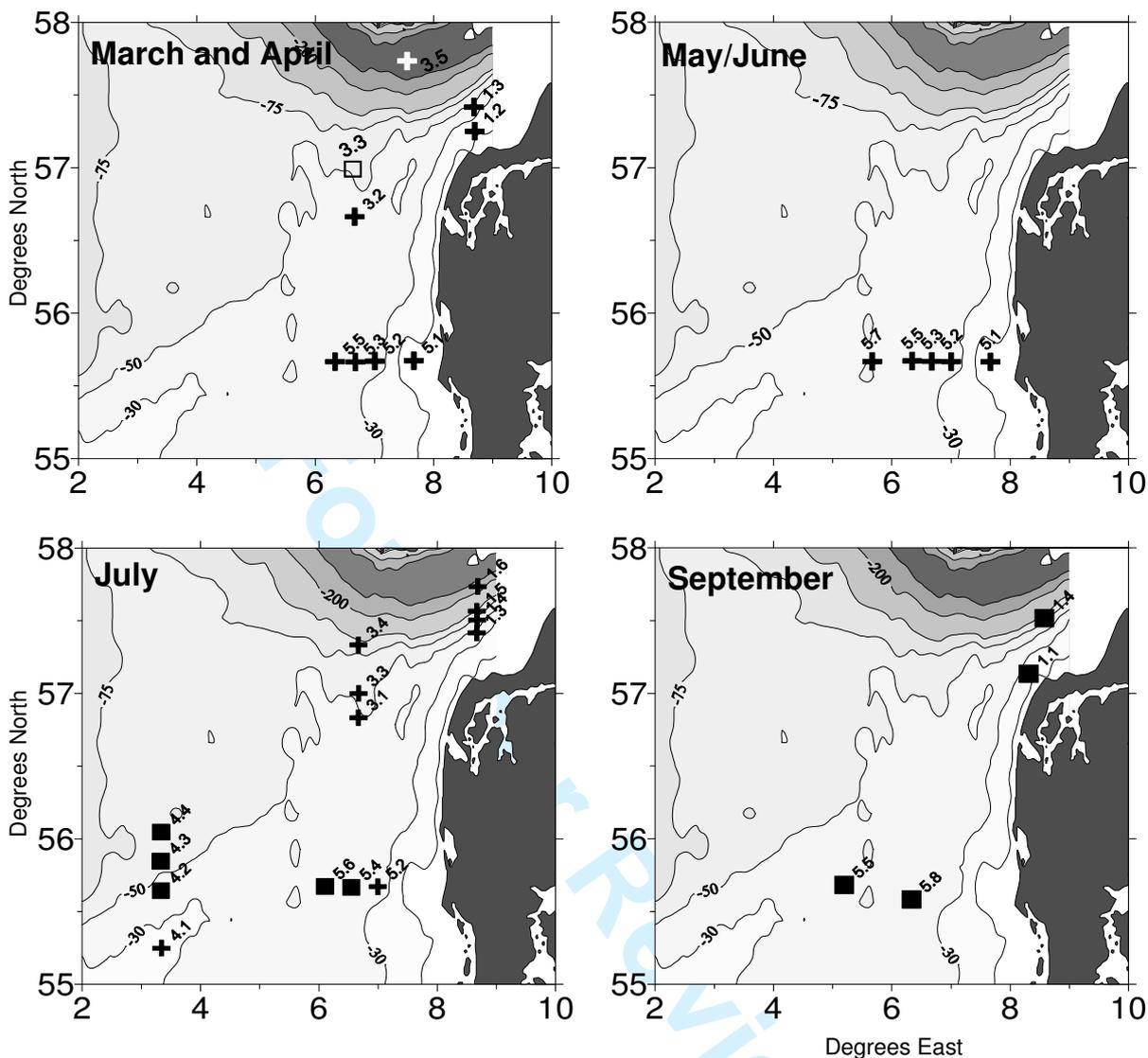
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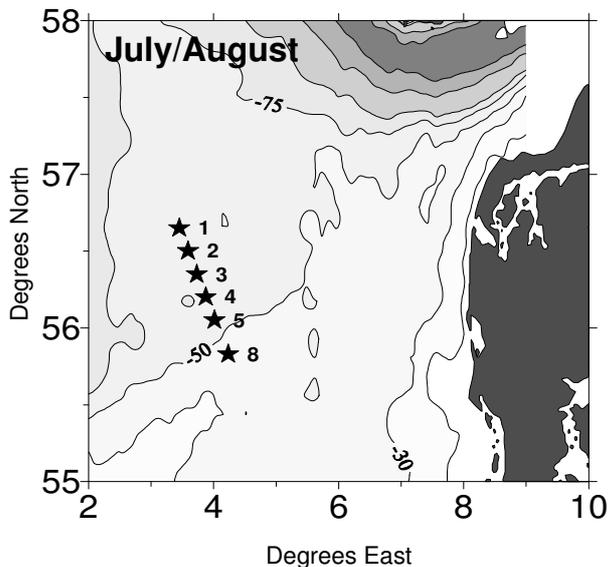
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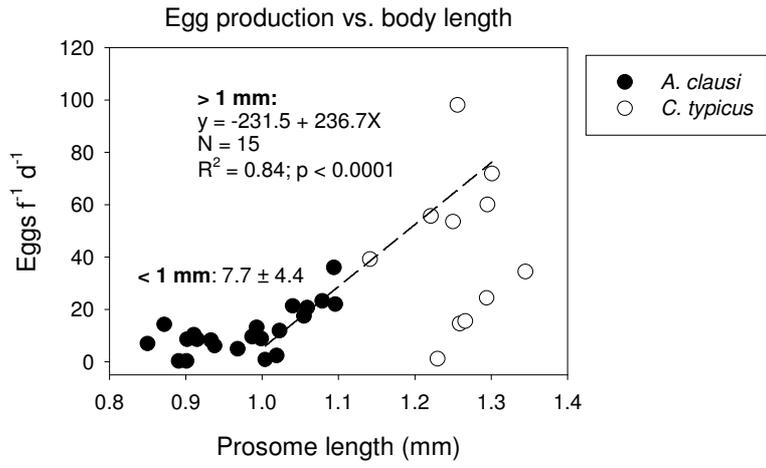
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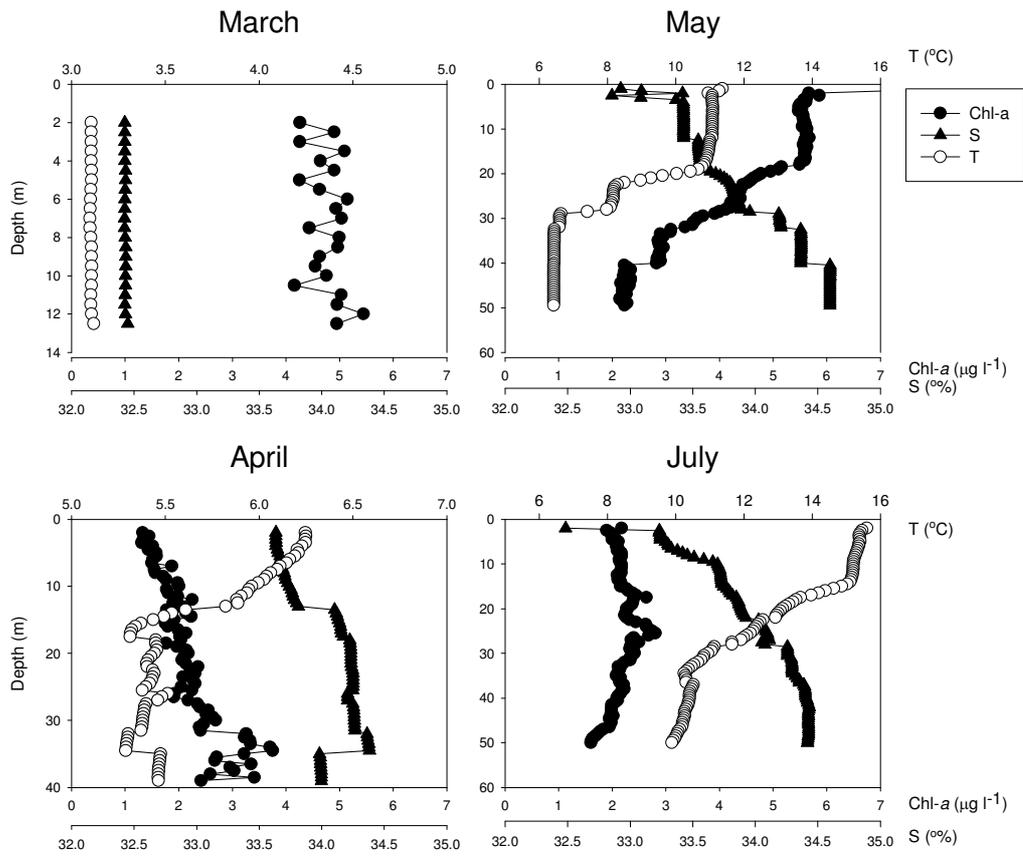
(B)



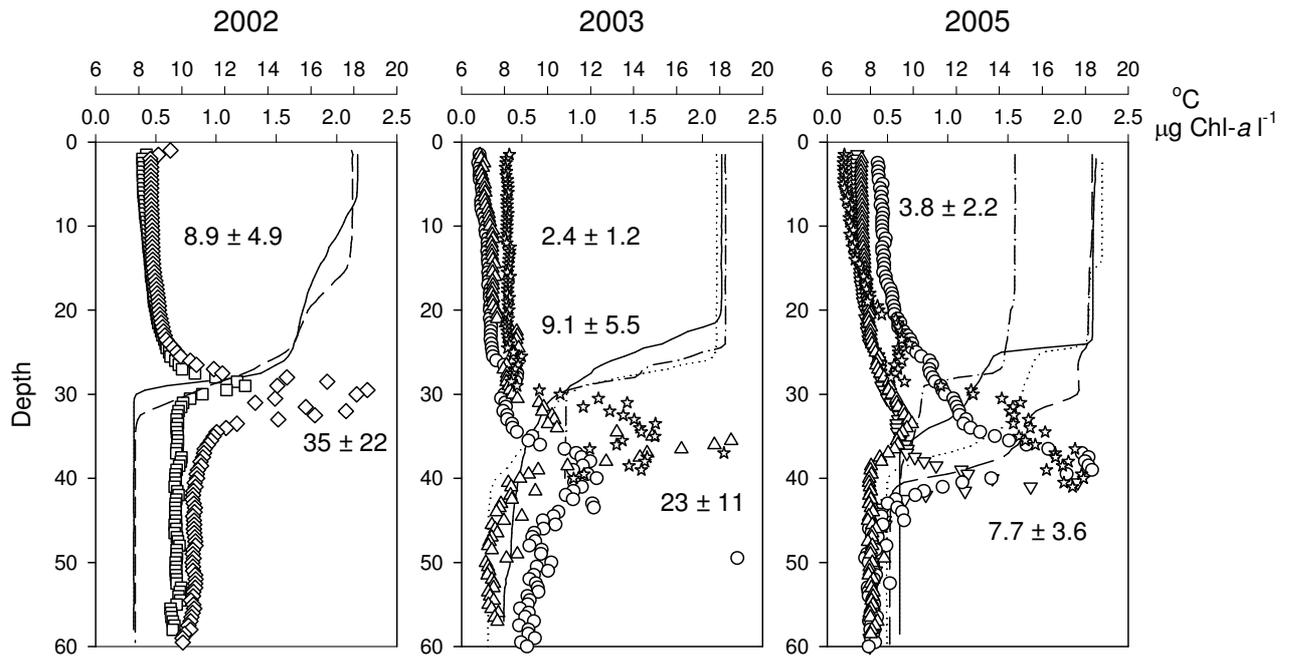
- + *Acartia* 2001 (22)
- *Centropages* 2001 (10)
- ★ *Acartia* bioassay, 2003 (4)  
vertical distribution and biomass  
of all copepods,  
2002 (2), 2003 (3) 2005 (4)



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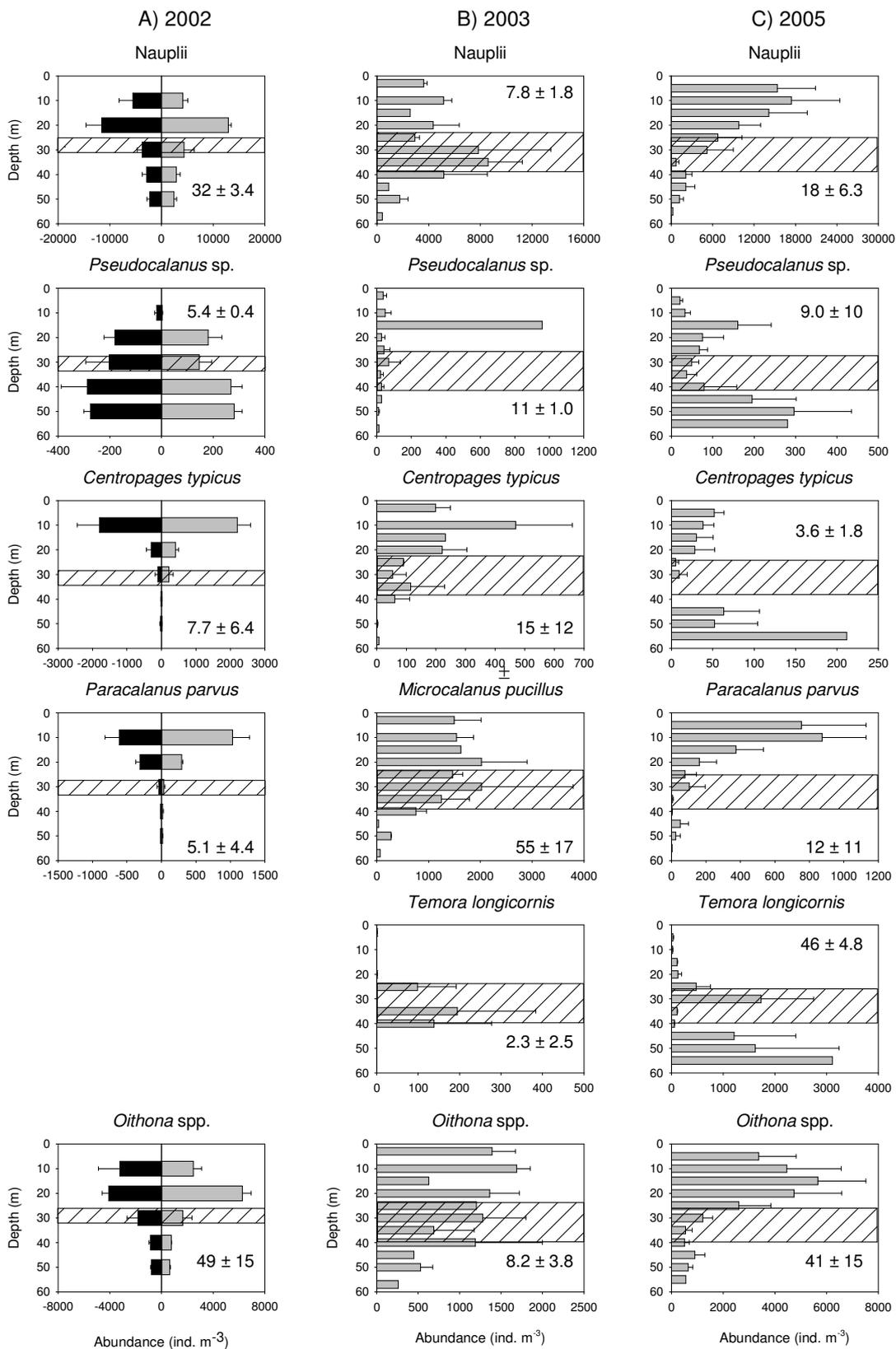


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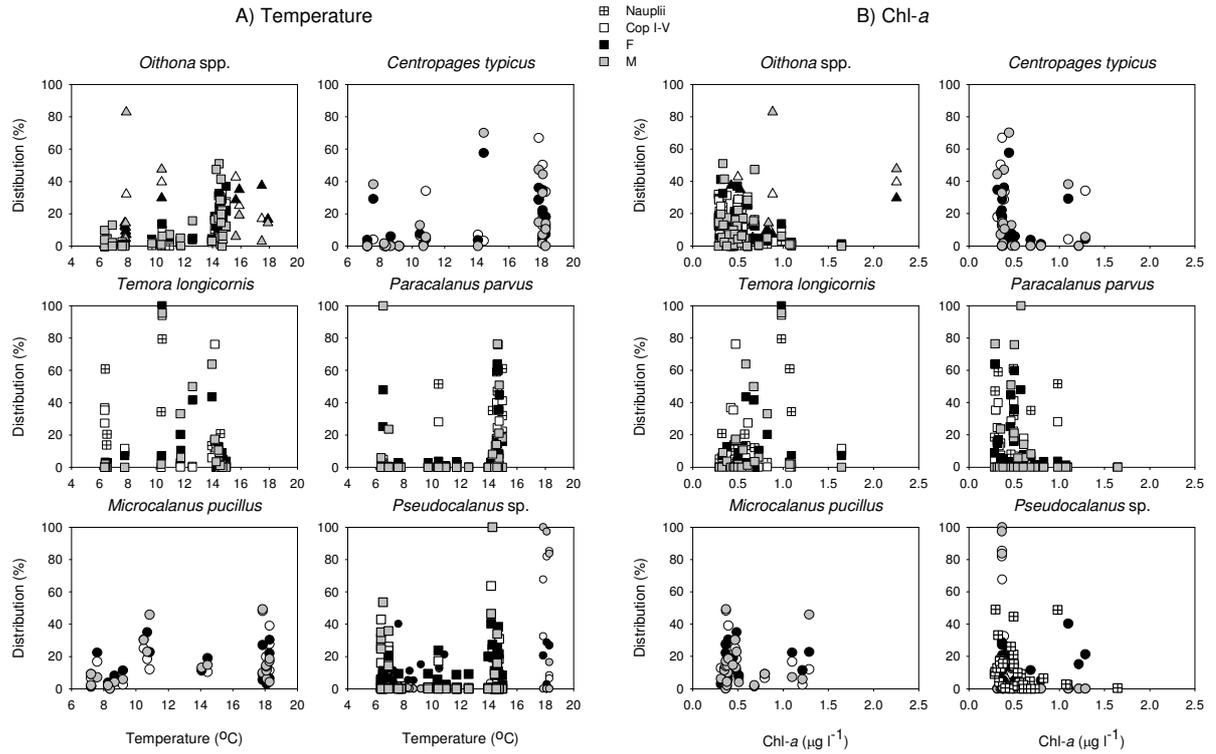


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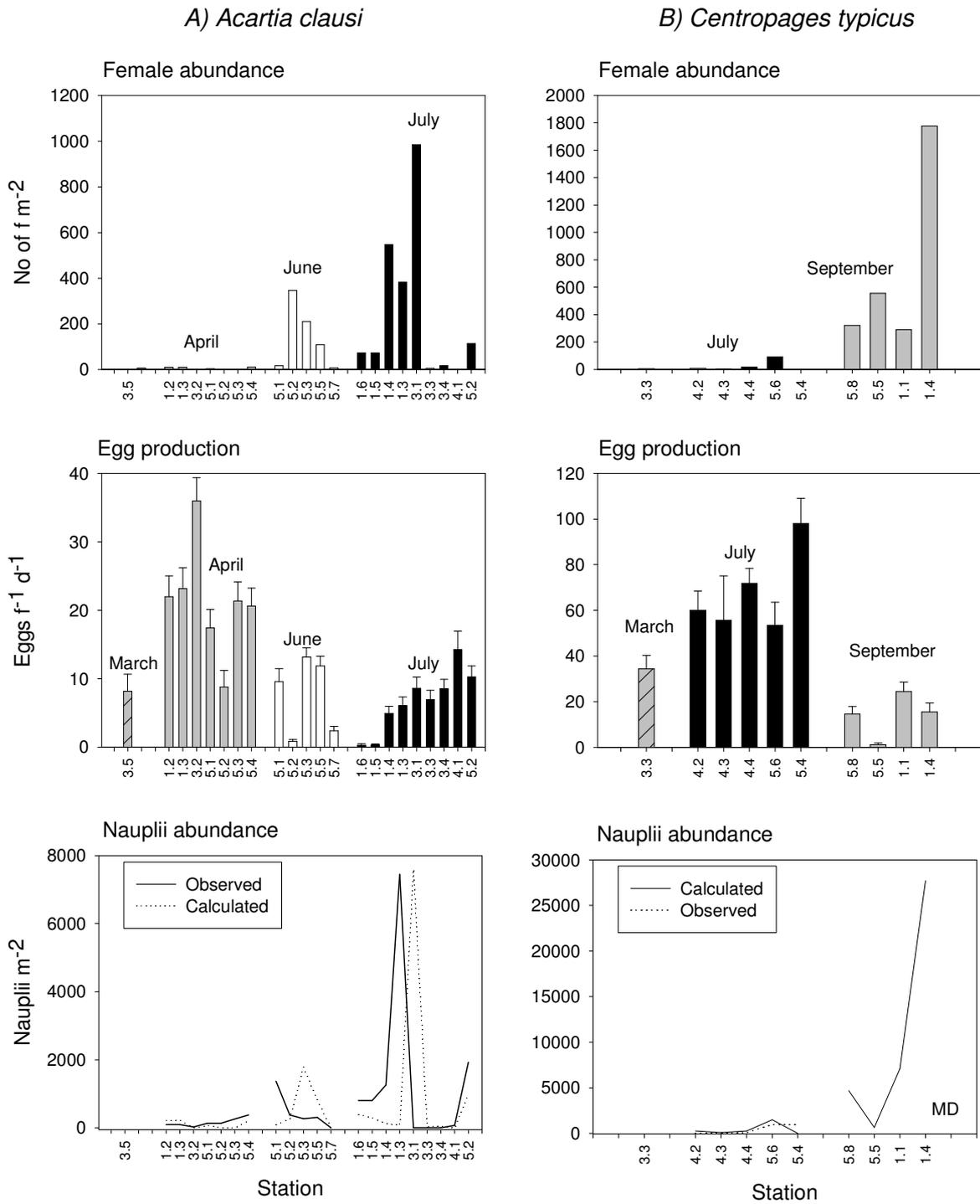


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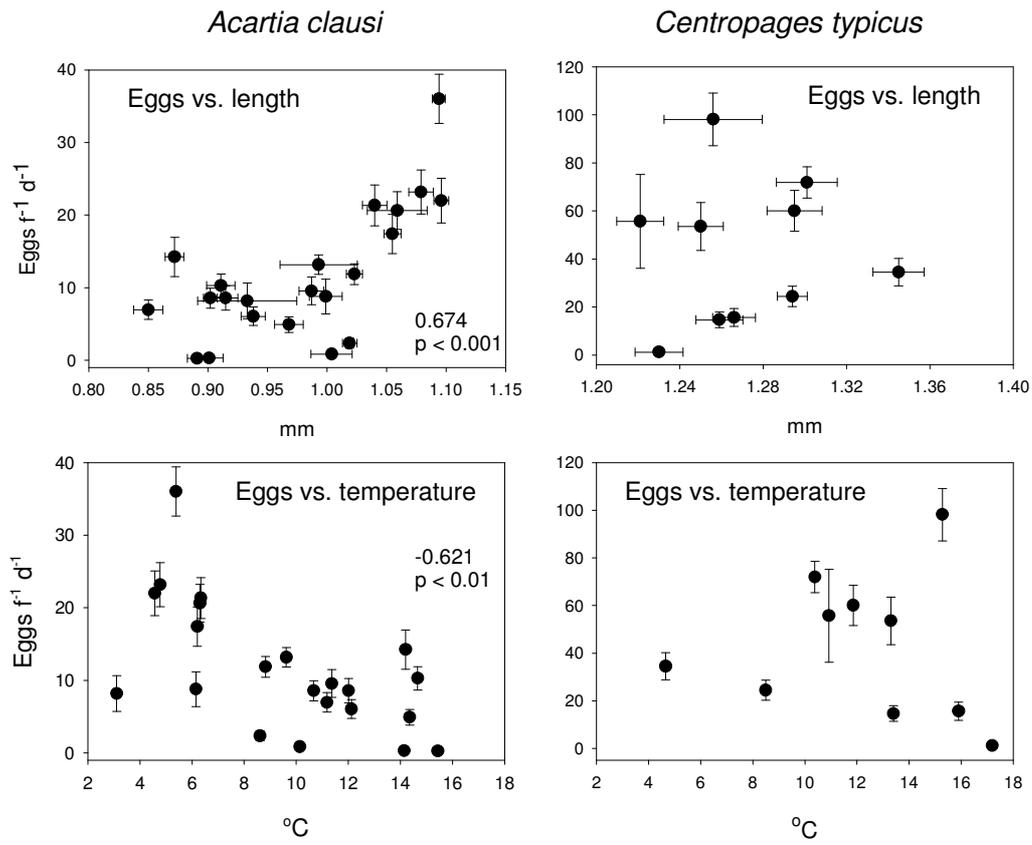
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Review



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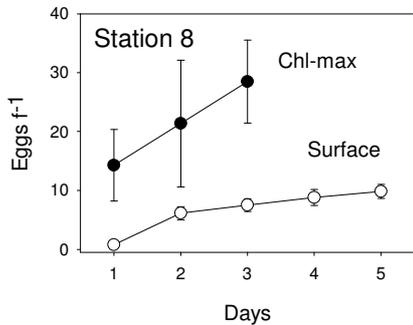
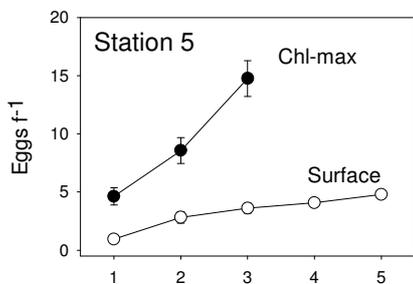
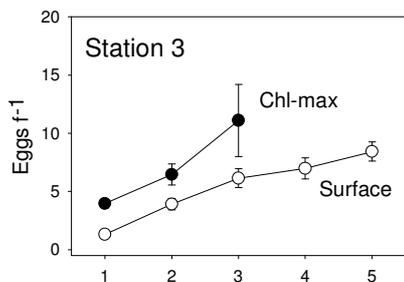
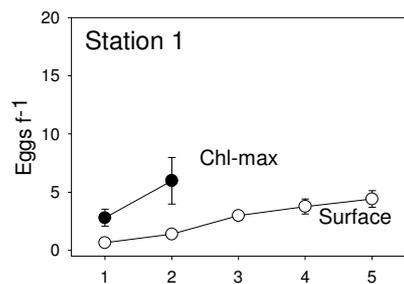
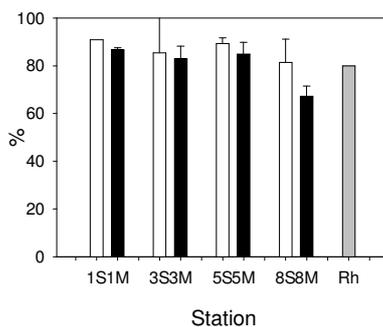
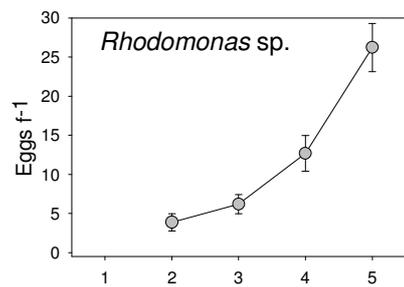
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6 Fig 8

A) Cumulative egg production

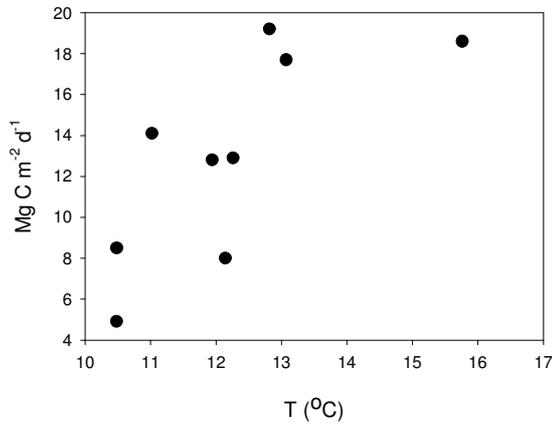
B) Hatching success



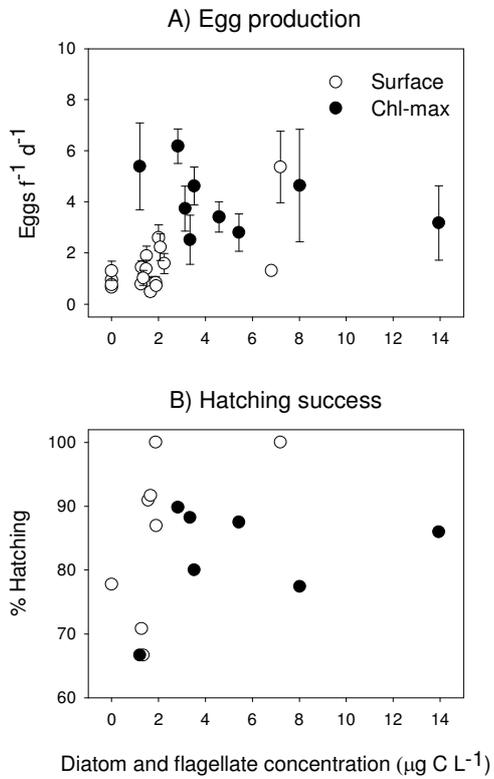
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Secondary production vs. temperature



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10 Fig 10



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12 Fig 11