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Seasonal changes in food quantity and quality of the common North Sea copepods *Temora longicornis* and *Pseudocalanus elongatus*: a bioassay approach

Marja Koski[1,*], Jörg Dutz[1], Wim Klein Breteler[2], Sebastiaan Rampen[3], Anna Noordeloos[2]

[1]National Institute of Aquatic Resources, Technical University of Denmark, Kavalergården 6, 2920 Charlottenlund, Denmark
[2]Department of Biological Oceanography and [3]Department of Marine Organic Biogeochemistry, Royal Netherlands Institute for Sea Research, PO Box 59, 1790 AB Den Burg, The Netherlands

ABSTRACT: We evaluated the food quantity and quality over a seasonal cycle for the development and egg production of the common North Sea copepods *Temora longicornis* and *Pseudocalanus elongatus*, using a bioassay approach. Seston was sampled from December to October from a well-mixed water column of the Marsdiep (Dutch Wadden Sea) and fed to cultured copepods at a constant temperature of 15°C, thus excluding seasonal effects of temperature, body size, age, and maternal nutrition. Copepod response was evaluated by measuring egg production and juvenile development, while the seston quantity and quality were measured as the concentrations of chl *a*, specific phytoplankton pigments, particulate organic carbon (POC), particulate organic nitrogen (PON), fatty acids, and sterols. The egg production of both copepods was low when feeding on seston collected in winter, but increased to peak values with the seston from the spring bloom in March–April. The juveniles of both species were able to complete their development only in spring experiments. A multiple regression analyses and comparison to a good-quality standard food of the same concentration suggested that, in an annual scale, the egg production and development of *T. longicornis* mainly depended on phytoplankton concentration, while the egg production and development of *P. elongatus* appeared also to benefit from detritus or heterotrophic food sources. The present study did not detect an influence of a specific food quality variable; however, an unexplained high juvenile mortality in summer suggests that all factors are not understood yet.

KEY WORDS: Copepod · Food quantity · Food quality · Seasonal dynamics · Bioassay

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INTRODUCTION

Copepod fecundity and growth in the field can be controlled by factors such as temperature, food quantity, food quality, body size, or different combinations of these. Studies on a global scale identify temperature and chlorophyll *a* (chl *a*) concentration as driving forces influencing copepod fecundity (Calbet & Agustí 1999, Bunker & Hirst 2004), with distinct differences between broadcast spawners and sac-spawners. While fecundity of broadcast spawning species is increasingly dependent on food concentration as the temperature increases, sac-spawning copepods seem to be able to reproduce at low chl *a* concentrations (Bunker & Hirst 2004). In contrast to fecundity, juvenile growth is usually considered to not be limited by food (Hirst & Bunker 2003), but, nevertheless, food limitation of nauplii development has been shown (Bakker & van Rijswijk 1987, Peterson & Kimmerer 1994).

It has long been agreed that chl *a* is not an optimal proxy for food availability of copepods. Instead, copepod fecundity and growth can be related to various

*Email: mak@aqua.dtu.dk

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other variables, such as the amount of nitrogen (Kiørboe 1989), polyunsaturated fatty acids (PUFAs) (Jónasdóttir 1994, Jónasdóttir et al. 1995, Pond et al. 1996, Hazzard & Kleppel 2003), amino acids (Kleppel et al. 1998), sterols (Klein Breteler et al. 2005), or the concentration of heterotrophic food (Ohman & Runge 1994, Dutz & Peters 2008). Theoretical arguments suggest that, since the potential food items are likely to differ in their biochemical components, copepods should be able to overcome limitation due to PUFAs, amino acids, or sterols by selective feeding (Anderson & Pond 2000). In contrast, a frequent limitation by mineral nutrients (e.g. nitrogen) or food quantity could be expected. However, in the field, effects of food quantity and quality are difficult to distinguish (see e.g. Müller-Navarra & Lampert 1996), since many of the quality indicators are related to the quantity. For instance, many of the PUFAs co-vary with the phytoplankton concentration (see e.g. Jónasdóttir et al. 1995), and a correlation between, for example, copepod egg production and concentration of PUFAs thus does not necessarily indicate limitation by food quality, but can simply be due to a limitation by phytoplankton concentration.

The effects of natural variations of food quantity and quality on copepod fecundity and growth are obscured by the effects of other environmental variables, such as temperature, body size, feeding history, copepod age, and maternal nutrition. We wanted to exclude these effects by using cultured copepods at a constant temperature as a kind of bioassay for seasonal changes in food quality and quantity. To distinguish between the effect of food quantity and quality, we used the approach suggested by Müller-Navarra & Lampert (1996) where copepod responses to seston are compared to their responses to corresponding concentrations of good-quality control food. The 2 copepod species used were *Temora longicornis* and *Pseudocalanus elongatus*, representing broadcast and sac-spawners, respectively. Potentially these 2 groups should have different weight-specific growth and fecundity rates (Kiørboe & Sabatini 1995) as well as different responses to temperature and food quantity (Klein Breteler & Gonzalez 1986, Hirst & Bunker 2003, Bunker & Hirst 2004). Our aims were to (1) evaluate the degree of food limitation (either by quality or quantity) of these 2 common copepod species, and (2) determine which of the seston components or compounds were most important for copepod egg production and development. We hypothesised that the effect of food alone is sufficient to explain the seasonal changes in copepod egg production and growth/development observed, for example, in the North Sea (i.e. a low egg production during winter, a peak production during the spring bloom, intermediate post-bloom production and low production during the late summer and autumn) (see e.g. Halsband & Hirche 2001), as well as the generally low growth and high mortality of most of the annual cohorts (Peterson & Kimmerer 1994).

MATERIALS AND METHODS

Study area. Monthly sampling was performed at a tidal inlet Marsdiep (53° 00′ 18″ N, 04° 47′ 42″ E; Dutch Wadden Sea), from the pier of the Netherlands Institute for Sea Research, from December 2000 through to October 2001. Sampling was conducted during high tide, so that the collected seston represented the Dutch coastal water on its way to enter the Wadden Sea (Cadée & Hegeman 2002 and references therein). The study area represents a nutrient-rich, coastal, temperate environment throughout the year revealing a high concentration of particulate organic carbon (POC) due to detritus, with a pronounced phytoplankton peak in spring (see e.g. Philippart et al. 2000). The area is well mixed and shallow, with an average depth of ca. 3 m at the sampling point (for the general hydrography of the area, see Postma 1954, 1967). Since the end of 1970s the area is considered eutrophic, with an annual average chl *a* concentration of >9 µg l^{-1} and a spring phytoplankton peak up to 30 µg l^{-1} (Philippart et al. 2000). The spring bloom generally starts in March, consisting mainly of large diatoms, whereas the peak of the bloom occurs in April, with a major contribution from the colonial haptophyte *Phaeocystis* (see e.g. Cadée & Hegeman 2002), followed by an increase in the heterotrophic food web (bacteria, heterotrophic nanoflagellates, and ciliates) (van Boekel et al. 1992). The long-term development in phytoplankton composition suggests a control by the relative and absolute abundances of nitrogen and phosphorus, with occasional switches occurring between N- and P-limitation, and coinciding changes of phytoplankton species composition (Philippart et al. 2000). The dominant zooplankton species in the area are *Acartia clausi*, *Temora longicornis,* and *Pseudocalanus elongatus*: while *A. clausi* is considered a summer species, *T. longicornis,* and *P. elongatus* reach their maximum egg production and abundance in spring and early summer (Fransz & van Arkel 1983, Fransz et al. 1992).

Seston composition. Seston was collected using a bucket, transported immediately to the laboratory, and kept in the dark at 15°C. Before use, the seston was carefully filtered through a 140 µm net to remove mesozooplankton. At the start of each experiment, duplicate samples (0.2 to 1 l) were taken for chl *a* in 2 size fractions (total and <5 µm), for phytoplankton pigments, for POC and nitrogen and for fatty acids and sterols (Tables 1 & 2), as these were considered to be the main variables determining the food quantity and

Table 1. Chl *a* (total and <5 µm size fractions), proportion of the dominant algae groups (% biomass; based on specific pigments), particulate organic carbon (POC) and nitrogen (PON) (µg l^{-1}), POC:PON and POC:chl *a* ratios (wt) in Marsdiep at the start of the experiments. Number of replicate development (Dev.) and egg production (EP) experiments that were conducted with *Temora longicornis* and *Pseudocalanus elongatus* are indicated for each month. nd: no data; dia: diatoms; chl: chlorophytes; pry: prymnesiophytes; chr: chrysophytes; pra: prasinophytes; cry: cryptophytes; cya: cyanobacteria; eu: eustigmatophytes

Month	Chl *a* (µg l^{-1})		Dominant algae groups (%)	Mineral nutrients (µg l^{-1})		POC:PON	POC:chl *a*	Expts	
	<5 µm	Total		POC	PON			Dev.	EP
Dec	0.1	1.0	nd	535	78	6.9	520	2	4
Jan	0.2	1.4	nd	406	58	7.0	290	2	4
Feb	0.1	1.5	dia (55), chl (19)	492	64	7.7	340	2	4
Mar	1.2	10.6	dia (63), pry (21), chr (15)	971	173	5.6	91	2	3
Apr	1.1	9.8	dia (61), pry (36)	916	169	5.4	93	0	4
May	1.6	16.7	dia (84)	1111	192	5.8	66	0	4
Jun	0.6	3.0	dia (18), pra (18), cry (17), pry (15), cya (15)	nd	nd	nd	nd	0	3
Jul	nd	10.9	dia (53), eu (21), chr (13)	nd	nd	nd	nd	2	3
Sep	1.2	4.0	dia (66), pry (11)	713	119	6.0	180	2	4
Oct	0.2	2.4	nd	1352	188	7.2	580	0	4

Table 2. Parameters from the factor analysis (PCA with a Varimax rotation) with different seston variables. The total variance explained by each principal component (PC) is indicated in parentheses. The moderate (≥0.5) and strong (≥0.7) loadings are printed in **bold**. The variables selected for the multiple linear regression (MLR) model are underlined. Fuco: fucoxanthin; zea: zeaxanthin; perid: peridin; allo: alloxanthin. SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids

	PC1 (53%)	PC2 (20%)	PC3 (13%)	PC4 (8%)	PC5 (6%)
Chl *a*	**0.786**	0.181	0.582	0.062	0.084
Chl *a* <5 µm	**0.720**	−0.086	0.454	0.493	0.158
Fuco	**0.889**	0.158	0.195	−0.027	0.382
Zea	**0.880**	−0.018	0.179	0.130	−0.421
Perid	**0.938**	0.099	0.163	−0.286	0.031
Chl *c*2 + *c*3	**0.941**	0.143	0.182	−0.026	0.246
Chl *b*	−0.304	−0.308	−0.417	**0.795**	−0.086
Allo	0.072	0.363	0.470	**0.716**	−0.360
POC	0.112	**0.965**	0.121	0.180	−0.091
PON	0.471	**0.825**	0.161	0.267	−0.016
MUFA + SAFA	−0.064	**0.953**	0.177	−0.199	0.128
PUFA	**0.657**	0.222	**0.713**	−0.062	−0.083
C16 PUFA	**0.891**	0.123	0.334	−0.086	−0.267
C18 PUFA	0.097	−0.112	−0.135	−0.129	**0.971**
C20 PUFA	0.409	0.296	**0.863**	−0.028	0.001
C22 PUFA	0.271	0.146	**0.929**	−0.002	−0.206
Sterols	0.080	**0.937**	0.141	−0.234	−0.204

quality of copepods (Jónasdóttir et al. 1995, Pond et al. 1996, Klein Breteler et al. 1999, 2005). The remaining water was used in egg production and development experiments, starting <2 h after the sampling of the water. The seston was kept for a maximum of 3 d, after which new water was collected for the development experiments. Specific pigments in seston were analysed weekly, but the mineral and fatty acid content of the seston was assumed to remain approximately the same between the collections. In further analysis, the start samples were used in connection with the egg production experiments, while the averages of the weekly pigment measurements were used for development experiments.

Chl *a* samples were first size-fractionated, using a 5 µm membrane filter, for <5 µm fraction and total chl *a*, filtered onto Whatman GF/F filters, extracted in 90% acetone, sonicated, centrifuged, and analysed using a spectrophotometer. Specific phytoplankton pigments were also analysed after filtering onto GF/F filters, using high performance lipid chromatography (HPLC); the relative abundances of different phytoplankton groups were calculated by using the CHEMTAX program (CSIRO Marine Laboratories, Hobart; Mackey et al. 1996, Riegman & Kraay 2001). POC and particulate organic nitrogen (PON) samples were filtered onto combusted GF/F filters and kept frozen (−50°C) until analysis. The POC and PON concentrations were measured with a Carlo Erba CHN analyser. The fatty acid and sterol samples were filtered onto combusted GF/F filters and stored at −50°C under N_2 until analysis. The lipid and sterol analyses were performed as described in Klein Breteler et al. (1999). Due to co-elution, we were unable to quantify all individual fatty acids.

Experiments. Between December 2000 and October 2001, 10 egg production and 6 development experiments were conducted between December 2000 and October 2001 using cultured copepods of *Temora*

longicornis and *Pseudocalanus elongatus*, which were fed seston collected from the Marsdiep (Table 1). All copepods originated from a recently matured generation grown under standard conditions (Klein Breteler 1980), so that all seasonal changes in egg production and/or development response were supposedly due to either food quantity or quality. All experiments were conducted at a constant temperature of 15°C and in dim light. With the exception of *T. longicornis* egg production (see next subsection), all experimental bottles were slowly rotated (ca. 1 rpm) using a rolling table. As a control for similar egg production and development among copepod generations, egg production and development were also measured using a saturating concentration (>400 µg C l^{-1}) (Klein Breteler et al. 1995) of the cryptophyte *Rhodomonas* sp. *Rhodomonas* sp. was chosen as a standard good-quality food due to its high concentration of essential nutritional components (e.g. PUFAs and sterols) (Klein Breteler et al. 2004) and the relatively high egg production and growth/development response of *T. longicornis* and *P. elongatus* feeding on *Rhodomonas* sp., observed in a number of previous studies (e.g. Koski et al. 2006).

The copepod cultures are described in detail in Klein Breteler (1980) and Klein Breteler et al. (1982); the culture conditions of *Rhodomonas* sp. are described, for example, in Koski et al. (2006). Briefly, the copepods were fed with a mixture of *Rhodomonas* sp., the prymnesiophyte *Isochrysis galbana,* and the heterotrophic dinoflagellate *Oxyrrhis marina*, in concentrations >300 µg C l^{-1}. Under these conditions, the development time from egg to adult was ca. 20 d (Klein Breteler et al. 1995), with an egg production rate of ca. 12 and 3% of the body weight (8 and 2 eggs female^{-1} d^{-1}) for *Temora longicornis* and *Pseudocalanus elongatus*, respectively (Koski et al. 2006). *Rhodomonas* sp. was grown in continuous cultures, using f/2 medium (Guillard 1975) and a dilution rate of ca. 0.2 d^{-1}. The cell concentration of *Rhodomonas* sp. was measured using an Elzone electronic particle counter (Particle Data) and converted to carbon assuming a carbon content of 36 pg cell^{-1} (Koski et al. 2006).

Egg production: Experiments started with a period of adaptation (48 h), followed by a 24 h (*Temora longicornis*) or 48 h (*Pseudocalanus elongatus*) experimental period. This difference was made because all *P. elongatus* females were without egg sacs in the beginning of the incubations, and it was assumed that forming a new egg sac would take more than 24 h. The length of the incubation period also ensured that most of the copepod eggs that were possibly included in the seston (passing through the 140 µm sieve) had time to hatch and thus did not bias the egg production measurements.

At the start of the adaptation period, ca. 15 recently moulted *Temora longicornis* or 10 *Pseudocalanus elongatus* females from the stock culture were placed into 3 to 4 replicate 1 l beakers with a loose net (200 µm) separating eggs and females (*T. longicornis*) or 0.6 l bottles (*P. elongatus*), either containing the <140 µm filtered natural seston or *Rhodomonas* sp. After the experiment, the numbers of eggs were counted and the condition of the females (dead/alive) was checked. Mortality of the females during the experiments was low (max. 15%), with generally all the individuals surviving to the end of the incubation. For a comparison between species, the egg production was calculated as weight-specific rates, assuming the carbon content of *T. longicornis* females and eggs to be 6 µg C female^{-1} and 0.09 µg C egg^{-1}, respectively, and the carbon content of *P. elongatus* females and eggs to be 11.5 µg C female^{-1} and 0.19 µg C egg^{-1}, respectively (Koski et al. 2006). Weight-specific egg production was tested for differences among months and species with a 2-way analysis of variance (ANOVA), followed by a Tukey's HSD post hoc test. If the conditions for ANOVA were not met, the data was log- or square root-transformed, or Kruskal-Wallis ANOVA on ranks was used.

A high concentration of copepods as used in our experiments could have induced food limitation during incubation. In *Acartia tonsa,* maximum filtration rates were recorded at a food concentration of ca. 150 µg C l^{-1} (Kiørboe et al. 1985). At a similar concentration (190 µg C l^{-1}) of *Rhodomonas* sp., adult *Temora longicornis* and *Pseudocalanus elongatus* from our stock cultures had filtration rates of 5.2 ± 1.3 and 11.4 ± 2.0 ml ind.$^{-1}$ d^{-1}, respectively (M. Koski unpubl. data). Using these high filtration rates, *T. longicornis* and *P. elongatus* individuals would have, respectively, cleared ca. 8 and ca. 40% of the available food during the experiments. However, at the generally high POC concentration of the seston (see 'Results'), the copepod filtration rates and, hence, their impact on the seston concentration, will have been even lower. We thus assume that food limitation did not seriously bias the results of the egg production experiments.

Development: Experiments were conducted as described in Koski et al. (1998, 2006). Briefly, a high number of early nauplii (NI-II) were collected from the stock culture and placed into replicate 1 l bottles (ca. 1000 nauplii bottle^{-1}), containing the <140 µm filtered seston. For comparison, we also include development in a high concentration of *Rhodomonas* sp. and filtered seawater (starvation), which were conducted at the same time as the current experiments (Koski et al. 2006). The bottles were sampled 3 times a week to count the nauplii and to determine their stage of development. The sample volume was adjusted to keep the copepod biomass in the bottles constant at ca. 70 (±58) µg C bottle^{-1}, but, at the same time, to provide a sufficient number of nauplii for analysis (at least 10, but

typically >30 ind. sample^{-1}). Simultaneously with sampling, ca. 80% of the food suspension was changed. The experiments were terminated either when late copepodite stages (C IV–V) started to appear or when all individuals had died. Assuming a weight-specific filtration rate of *Temora longicornis* nauplii of 1.98 ml (µg C)$^{-1}$ d^{-1} as observed on diatom diets at ca. 200 µg C l^{-1} (Koski et al. 2008), the total filtration due to the nauplii would have amounted to ca. 300 ml (ca. 25% of the bottle) between the water exchanges. This was considered acceptable, and, as with the adult stages, we thus assumed that the experimental conditions did not induce food limitation in the experiments.

The success of the juvenile copepods was assessed based on the stage distribution in the final day of the incubation (the length of the incubation period depending on mortality) and on the estimated rates of growth and mortality. The instar weight was derived from the linear regression between log carbon weight and development stage obtained from copepods feeding on a high concentration of *Rhodomonas* sp. (r = 0.85 and 0.95 for *Temora longicornis* and *Pseudocalanus elongatus*, respectively; M. Koski unpubl. data). However, since the development on natural seston during most of the months was suboptimal (see 'Results'), the stage-specific weight of the nauplii was also likely to be less than on a *Rhodomonas* sp. diet. Therefore, the obtained growth rates should be considered maximum estimates. Instantaneous rates of mortality during development were calculated after correcting for sampling mortality according to Klein Breteler et al. (2004).

Effects of food quantity and quality. We used 2 different methods to evaluate which of the seston variables were most important for copepod development and egg production. First, to distinguish between quantity versus quality limitation, following Müller-Navarra & Lampert (1996), we established functional response curves of egg production and growth on natural seston in comparison to a good-quality standard food. Any deviation from the response curve with standard food is assumed to be due to the quality of the food. To create a functional response curve for standard food, we measured the egg production of *Temora longicornis* and *Pseudocalanus elongatus* using *Rhodomonas* sp. as food at concentrations ranging from 0 (filtered sea water) up to ca. 1600 µg C l^{-1} (for methods, see 'Egg production'), and completed the data with previous results (Koski et al. 2006, Dutz et al. 2008). For the functional response of growth on *Rhodomonas* sp., we used data from Koski et al. (1998, 2006, 2008), Dutz & Koski (2006), and unpublished observations (M. Koski & J. Dutz; National Institute for Aquatic Resources, Denmark). Food concentration was expressed as POC (representing the total seston including detritus) and chl *a* (phytoplankton). The carbon concentrations were converted to chl *a* by assuming a chl *a*:carbon ratio of 0.011, viz. the average of the 3 ratios measured in Le Floc'h et al. (2002).

Second, since many of the variables describing food quantity and quality co-varied, we used multiple linear regressions (MLRs) to evaluate which of the seston variables explained the largest part of the variation in copepod egg production and growth. To reduce the number of variables and covariability among these, we first performed a principal component analysis (PCA) with a Varimax rotation method, first for all the specific fatty acids (data not shown), then combining the selected fatty acids with the other seston variables (Table 2). Based on the loadings of different variables, for each PC we chose the sum of the dominant variables (indicated in bold in Table 2) for use in the MLR model. We interpreted the first PC as phytoplankton concentration and chose the dominant pigments (sum of fucoxanthin, zeaxanthin, peridin, and chl *c*2 + *c*3) as its representative. Similarly, we interpreted the second PC as total food concentration and chose POC as its representative. The other 3 PCs were interpreted as specific PUFAs (sum of C20 + C22), specific pigments (sum of chl *b* and alloxanthin) and C18 PUFAs. The MLR models were run using a stepwise backward method, separately for the egg production and growth of both copepod species. Due to the missing data, June and July were excluded from the analysis. For growth, only the first 3 PCs were used. All data were tested for normality and constant variance, as well as for influential outliers. All statistical analyses were performed with the Sigma Stat 3.1 or SPSS 11.5 statistical packages (Systat Software).

RESULTS

Food environment

The chl *a* concentration followed a seasonal succession typical for the coastal North Sea, with relatively low values during the winter (1 to 2.5 µg l^{-1}), high values (10 to 35 µg l^{-1}) during the spring bloom in March–May as well as during a secondary bloom in July–August, and moderate values (3 to 10 µg l^{-1}) during the rest of the summer and autumn, with large short-term fluctuations (Fig. 1, Table 1). Based on the phytoplankton pigments, it appeared that diatoms (fucoxanthin) dominated the phytoplankton during most of the year, with an exception of June, when the composition was more diverse. Besides diatoms, prymnesiophytes (chl *c*2 and chl *c*3) contributed substantially to the spring bloom. For most of the year, >85% of the chl *a* was formed by cells >5 µm. Similar to the

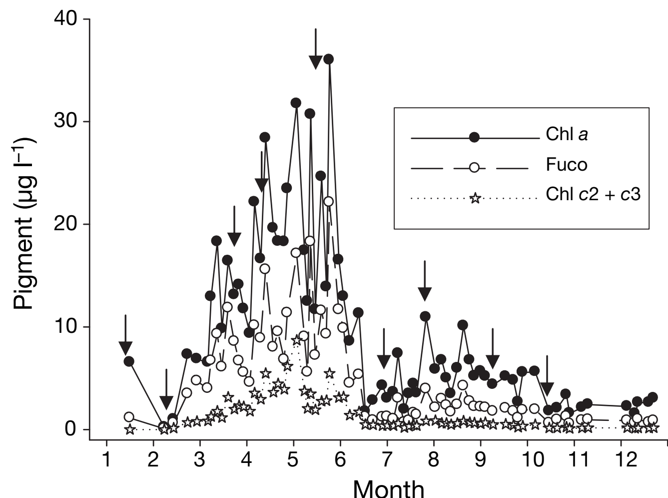

Fig. 1. Annual succession of the dominant phytoplankton pigments at the sampling area in 2001 (µg l^{-1}). Chl *a* and fucoxanthin represent diatoms; chl *c*2 and chl *c*3 represent mainly prymnesiophytes. Arrows indicate the sampling dates of the copepod experiments. fuco: fucoxanthin

year 2002 (Cadée & Hegeman 2002), the peak of the *Phaeocystis* bloom was exceptionally short (1 to 2 wk) with a relatively low biomass (<10 µg chl *c*2 + *c*3 l^{-1}). Our copepod experiments missed this *Phaeocystis* peak, as well as the highest of the short-term diatom peaks, with most of the sampling points during the spring being at the chl *a* level of 10 to 15 µg l^{-1} (Table 1, Fig. 1). Fig. 1 shows the sampling dates relative to the annual phytoplankton succession.

The concentration of POC was high throughout the year, with lowest values during the winter (400 to 500 µg C l^{-1}) and peak values during the spring bloom and autumn (900 to 1400 µg C l^{-1}). Similar changes were observed in PON, although, in proportion to POC, PON increased more during the spring bloom, resulting in the lowest POC:PON ratio during March–May (5.4 to 5.8). However, the POC:PON ratio was always relatively low and never exceeded 8, indicating that the phytoplankton community was not nitrogen-limited during the sampling period (but see Philippart et al. 2000). In contrast, the POC:chl *a* ratio during the winter was extremely high, up to 500 to 600, indicating a high amount of detritus in the water, whereas the POC:chl *a* ratio during the spring bloom was more typical for the coastal areas of the North Sea (70 to 90) (Table 1).

The total concentration of fatty acids and sterols correlated strongly with the seston POC concentration ($r = 0.91$ and 0.92 for fatty acids and sterols, respectively; $p < 0.01$). The concentrations were highest in October and during the spring bloom in March–May (40 to 105 and 5 to 10 µg l^{-1}, respectively). The fatty acid composition of the seston was mostly dominated by saturated and monounsaturated fatty acids, namely C16:0 (21 to 39% of the total fatty acids), C16:1 + C16:2 (12 to 24%), C14:0 (6 to 22%), and C18:1 (6 to 18%). The concentrations and proportions of polyunsaturated fatty acids (PUFAs) correlated with the chl *a* concentration ($r = 0.94$, $p < 0.01$), being highest in May, when they represented ca. 19% of the total fatty acids. Most of the sterols possessed a double bond at the C5-position (Δ5-sterols). Concentrations and proportions of different fatty acids and sterols are presented in detail in Table 3.

Copepod egg production and development

There were significant differences in weight-specific egg production both between the 2 copepod species (2-way ANOVA; $F_{1,71} = 12.7$; $p < 0.001$) and among the different months (2-way ANOVA; $F_{9,71} = 6.3$; $p < 0.001$). The egg production of both species peaked in April (12 ± 2 and 5 ± 0.5 eggs female^{-1} d^{-1} for *Temora longicornis* and *Pseudocalanus elongatus*, respectively), followed by a moderate egg production during late spring and autumn (4 to 7 and 2 to 3 eggs female^{-1} d^{-1}, respectively) and a low egg production during the winter months and June (≤2 eggs female^{-1} d^{-1}). The times of egg production of *T. longicornis* could be approximately divided into months with highest egg production (July, March, April), low to moderate egg production (May, September, October, December, January, and February) and very low egg production (June; Tukey's HSD; $p < 0.05$). In general, *P. elongatus* egg production was less variable among months: egg production in May was significantly higher than in January–February (Tukey's HSD; $p < 0.05$), but otherwise no significant differences between months were detected. For most of the year, egg production of the 2 species responded similarly to the food environment, with a notable exceptions of July, when the egg production of *T. longicornis* was at a maximum but the egg production of *P. elongatus* was low to moderate, and June, when the egg production of *P. elongatus* was moderate but the egg production of *T. longicornis* was very low (Tukey's HSD; $p < 0.05$; Fig. 2A). There were no significant differences in the egg production of copepods feeding on *Rhodomonas* sp. between the months (Kruskal-Wallis ANOVA on ranks; $p > 0.05$), but average egg production of the cultured copepods on the good-quality standard food was 5 ± 1 and 3 ± 0.5 eggs female^{-1} d^{-1} for *T. longicornis* and *P. elongatus*, respectively (data not shown), similar to the previous egg production rates measured in these cultures (Koski et al. 1998, 2006). It should be noted that the typical egg produc-

Table 3. Concentration (µg l^{-1}) and distribution (% FA) of fatty acids and sterols in Marsdiep at the start of the experiments, as well as the proportion of total fatty acids and sterols from the % POC. See Table 2 for abbreviations; Δ-notation fo sterols indicates double bond position(s). nd: no data

	Dec	Feb	Mar	Apr	May	Sep	Oct
SAFA							
C14:0	4.0 (16)	1.8 (6.4)	17 (22.6)	5.1 (13.8)	13.7 (15.6)	1.0 (10.4)	7.3 (7.0)
C15:0	1.4 (5.6)	2.3 (8.3)	3.6 (4.8)	4.2 (9.8)	4.4 (5.0)	0.7 (7.1)	6.4 (6.1)
C16:0	5.9 (24.1)	10.8 (39.1)	17.7 (23.7)	13.1 (35.4)	18.2 (21.5)	3.4 (35.5)	29.9 (28.5)
C17:0	0.2 (0.8)	0.6 (2.3)	1.3 (1.7)	1.3 (3.0)	1.4 (1.6)	0.3 (2.9)	3.0 (2.8)
C18:0	1.3 (5.3)	3.2 (11.5)	2.9 (3.8)	3.1 (7.2)	3.6 (4.1)	1.3 (13.5)	10.1 (9.6)
C19:0	nd	nd	nd	nd	nd	nd	0.3 (0.3)
C20:0	0.2 (0.6)	0.4 (1.6)	0.2 (0.3)	1.0 (2.3)	0.5 (0.5)	0.1 (0.6)	2.1 (2.0)
C22:0	0.2 (0.6)	0.7 (2.7)	1.1 (1.4)	0.7 (1.7)	0.6 (0.6)	0.1 (1.5)	3.2 (3.0)
C23:0	0.1 (0.1)	0.3 (1.2)	nd	0.2 (0.6)	nd	nd	0.5 (0.5)
C24:0	0.3 (1.2)	0.4 (1.4)	0.06 (0.1)	0.5 (1.10)	0.2 (0.2)	0.1 (0.7)	3.6 (3.4)
Total SAFA	13.4 (54.9)	20.6 (74.5)	43.7 (57.5)	29.0 (68.5)	42.5 (48.4)	7.0 (72.7)	66.5 (63.4)
MUFA							
C16:1	0.6 (2.4)	nd	1.3 (1.7)	0.6 (1.4)	1.8 (2.1)	1.1 (12)	2.5 (2.4)
C16:1 + C16:2	4.9 (20)	3.3 (12.3)	18.4 (24.7)	nd	18.1 (21.4)	nd	15.9 (15.1)
C18:1	2.8 (12)	3.4 (12.0)	4.6 (6.1)	6.3 (15)	9.3 (11.0)	1.3 (14)	19.1 (18.1)
C18:1 + C18:2	nd	nd	2.2 (2.9)	nd	nd	nd	nd
Total MUFA	8.3 (34)	6.7 (24.3)	26.5 (35.5)	6.8 (19)	29.2 (34.5)	2.5 (25.8)	37.5 (35.7)
PUFA							
C:16PUFA	0.3 (1.2)	nd	0.8 (1.1)	nd	nd	nd	nd
C16:2 + C16:3	nd	nd	1.3 (1.8)	5.5 (13)	3.1 (3.5)	nd	nd
C16:3	0.3 (1.2)	nd	nd	nd	nd	nd	nd
C16:4	nd	nd	nd	nd	2.6 (3.0)	nd	nd
C18:2	0.4 (1.5)	0.3 (1.2)	nd	nd	nd	nd	nd
C18:2ω6	0.5 (2.0)	nd	1.0 (1.3)	nd	nd	0.2 (2.0)	nd
C20:4ω6 + C20:5ω3	1.0 (3.9)	nd	2.6 (3.4)	1.0 (2.4)	8.4 (9.6)	nd	1.0 (0.9)
C22:6ω3	0.3 (1.1)	nd	nd	nd	1.9 (2.0)	nd	nd
Total PUFA	2.7 (10.9)	0.3 (1.2)	5.8 (7.7)	6.5 (17.6)	16.1 (19.0)	0.2 (2.0)	1.0 (0.9)
Total FA	24.3	27.7	75.9	42.3	87.7	9.6	105.0
FA (% POC)	4.5	5.6	7.8	4.6	7.9	1.3	7.8
Sterols							
$\Delta^{5,22}$C26:2	0.2	nd	nd	nd	nd	nd	nd
$\Delta^{5,22}$C27:2	0.1	0.1	0.6	0.9	0.8	0.1	1.3
Δ^{5}C27:1	0.7	1.8	1.4	1.8	2.6	0.7	4.9
$\Delta^{5,22}$C28:2	0.4	0.3	0.8	0.7	1.0	0.1	0.7
$\Delta^{5,24(28)}$C28:2	0.5	nd	1.9	0.3	2.0	0.1	0.6
Δ^{5}C28:1	0.1	0.2	nd	0.1	0.4	nd	0.4
$\Delta^{5,22}$C29:2	nd	nd	nd	0.7	nd	nd	nd
Δ^{5}C29:1	0.2	0.5	0.3	0.7	0.5	0.1	1.4
Δ^{7}C28:1	nd	nd	0.2	0.2	nd	0.1	0.4
Δ^{7}C29:1	nd	nd	nd	0.2	nd	nd	nd
Total sterols	2.3	3.0	5.1	5.6	7.3	1.2	9.6
Sterols (% POC)	0.4	0.6	0..5	0.6	0.7	0.2	0.7

tion of our cultured *T. longicornis* was lower than the maximum rates measured in the field, which can be up to 50 eggs female^{-1} d^{-1} (see e.g. Peterson & Bellantoni 1987; see 'Discussion').

The quality and/or quantity of seston affected the development rates of both *Temora longicornis* and *Pseudocalanus elongatus*, with a similar response of both species to the seasonal changes in food (Fig. 2B, Table 4). In March, copepods developed until late copepodite stages, with a relatively high growth rate of 17 to 18% body wt^{-1} d^{-1}, while in July and September only part of the population was able to moult into copepodites, and in winter (December–February) the development was mostly arrested in nauplii stages IV–V, and the growth rate was low (4 to 11% body wt^{-1} d^{-1}) (Fig. 2B, Table 4). The low success of juveniles in July was due to an extremely high mortality of 50 to 60% d^{-1} (Table 4), which only allowed a low development (Fig. 2B) although at a high growth rate (Table 4). Mortality in December, January, and March was 11 to

14 % d^{-1}, while higher mortality was observed in February (21 and 40 % d^{-1} for *T. longicornis* and *P. elongatus*, respectively) and September (26 and 61 % d^{-1}, respectively) (Table 4).

Effect of food quantity and quality

Comparison of functional responses, following the approach of Müller-Navarra & Lampert (1996), suggested a difference in egg production response of *Temora longicornis* and *Pseudocalanus elongatus* on natural seston. While the egg production of *P. elongatus* during most of the months was similar or higher than expected based on the carbon concentration, *T. longicornis* egg production was mostly lower, indicating a limitation by food quality for *T. longicornis* but not for *P. elongatus* (Fig. 3A). Only when the food concentration was expressed as chl *a* was *T. longicornis* egg production mostly on or above the functional response line, although a few months (May and June) with a potential food quality limitation remained. For *P. elongatus* it even seemed that the food quality of seston was sometimes higher than that of *Rhodomonas*

Table 4. *Temora longicornis* and *Pseudocalanus elongates.* Weight-specific growth (µg C [µg C]$^{-1}$ d^{-1}) and daily mortality (d^{-1}) of juveniles in December 2000–September 2001 (slope of the significant regressions between the carbon weight or ln number of individuals and time; mean ± SD) and length of the incubation periods (d). Length of incubation period mainly depended on mortality, since incubation was stopped either when all individuals were dead or when the first adults started to appear (see 'Materials and methods'). Specific growth and mortality with a high concentration of *Rhodomonas* sp. and without food (filtered sea water; FSW) are included for comparison. ns: not significant

Month	*T. longicornis*			*P. elongatus*		
	Growth	Mortality	d	Growth	Mortality	d
Dec	0.08 ± 0.01	−0.14 ± 0.03	12	0.10 ± 0.001	−0.11 ± 0.03	14
Jan	0.10 ± 0.008	−0.21 ± 0.03	9	0.04 ± 0.004	−0.40 ± 0.08	9
Feb	0.08 ± 0.004	−0.14 ± 0.02	9	0.11 ± 0.009	−0.20 ± 0.03	9
Mar	0.17 ± 0.003	−0.13 ± 0.02	15	0.18 ± 0.001	−0.16 ± 0.02	15
Jul	0.18 ± 0.002	−0.48 ± 0.03	9	0.17 ± 0.006	−0.61 ± 0.16	9
Sep	0.09 ± 0.001	−0.26 ± 0.04	11	0.14 ± 0.001	−0.47 ± 0.08	7
Rhodomonas sp.	0.18 ± 0.02	−0.07 ± 0.01	22	0.20 ± 0.01	−0.09 ± 0.01	21
FSW	ns	−0.67 ± 0.11	7	ns	−0.68 ± 0.14	6

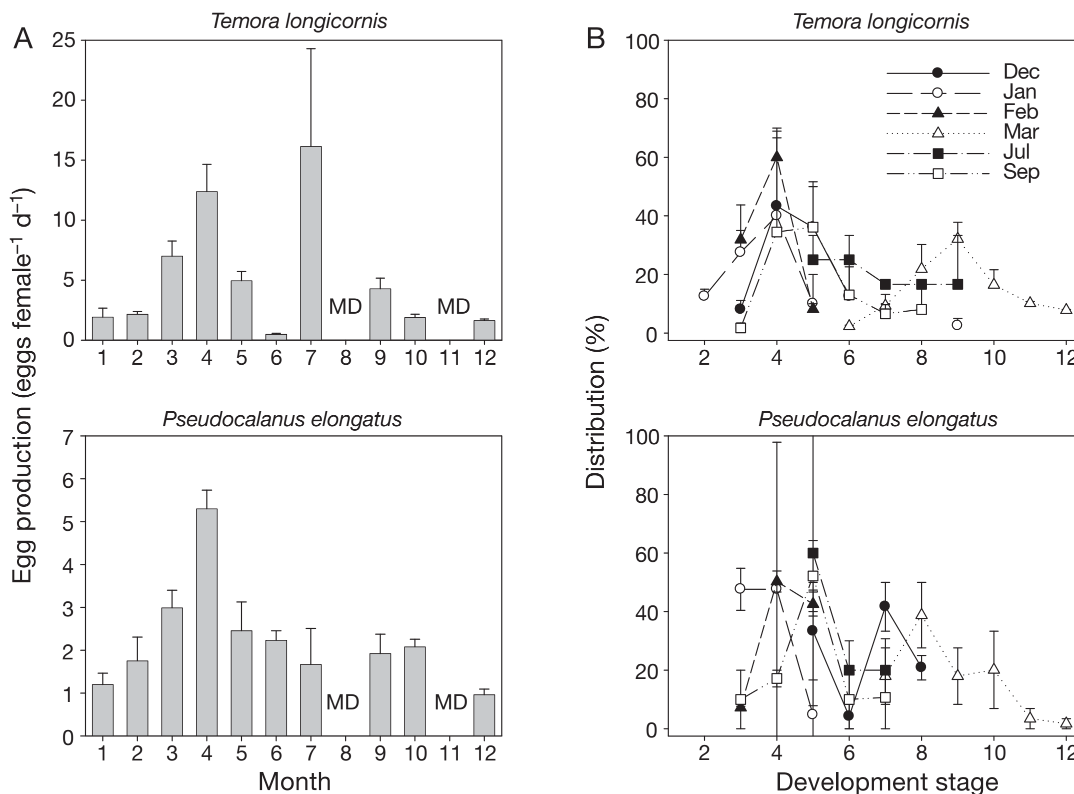

Fig. 2. *Temora longicornis* and *Pseudocalanus elongatus.* (A) Egg production (eggs female^{-1} d^{-1}) and (B) final stage distribution (%) of *T. longicornis* and *P. elongatus* in December 2000–October 2001 (mean ± SE). MD: missing data. Length of the incubation periods is indicated in Table 4

sp.; the month of April appeared to be especially beneficial, when the egg production of both species was ca. 2× higher than in a corresponding concentration of *Rhodomonas* sp., irrespective of whether the food concentration was expressed as POC or chl *a* (Fig. 3A). The approach of Müller-Navarra & Lampert (1996) thus suggests no qualitative food limitation for *P. elongatus*, but a dependency on phytoplankton (chl *a*) combined with a potential food quality limitation for *T. longicornis*.

The juvenile growth of both species appeared potentially limited by food quality, when the food concentration was expressed as POC. However, when the food concentration was expressed as chl *a*, the growth of

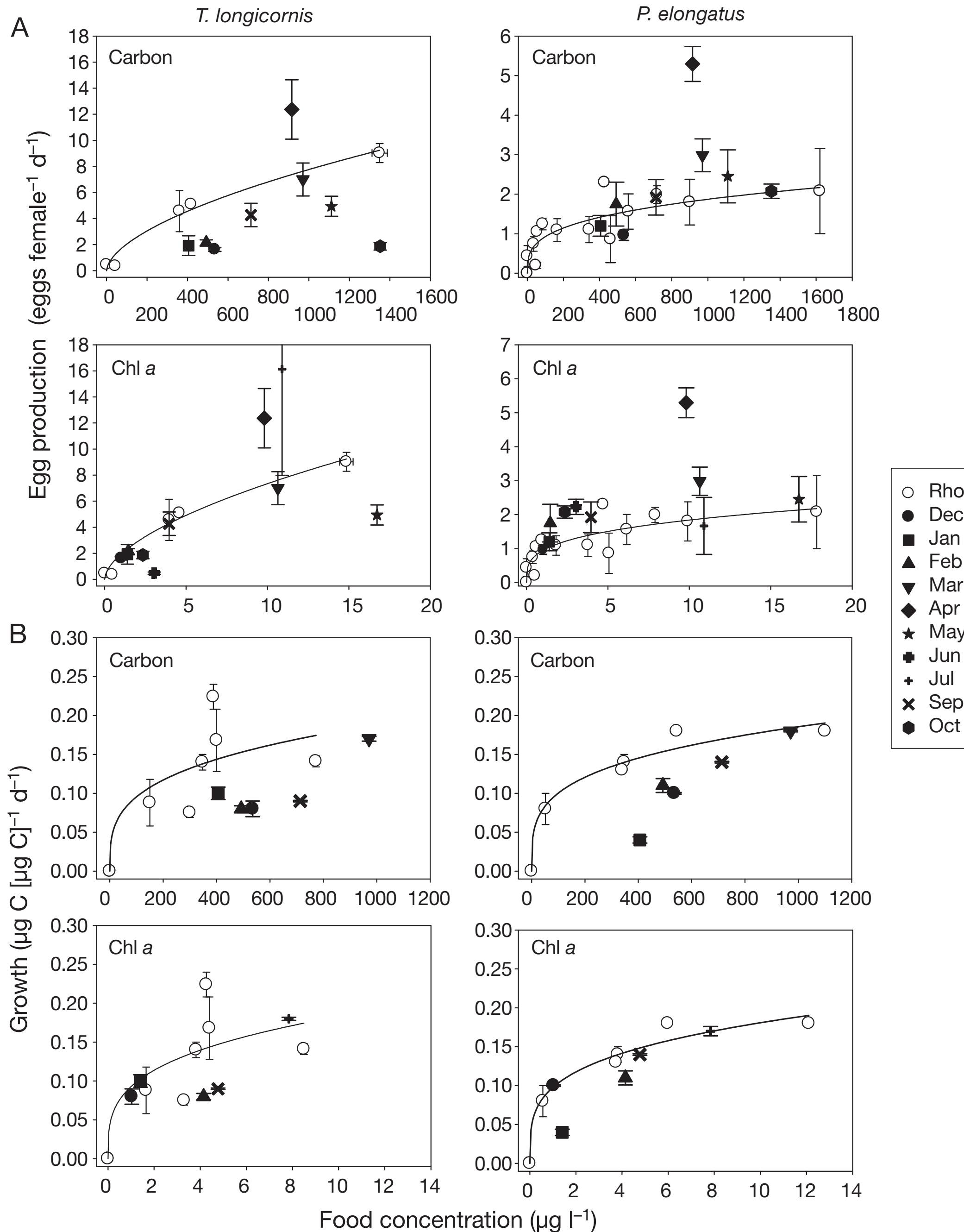

Fig. 3. *Temora longicornis* and *Pseudocalanus elongatus*. (A) Egg production (eggs female^{-1} d^{-1}) and (B) growth (µg C [µg C]$^{-1}$ d^{-1}) of *T. longicornis* and *P. elongatus* fed *Rhodomonas* sp. (○) and in natural seston (filled symbols; mean ± SE) as a function of carbon and chl *a* concentrations (µg l^{-1}). The curved line represents a power function of the functional response of egg production/growth on *Rhodomonas* sp. Symbols below the line indicate limitation by food quality. Rho: *Rhodomonas* sp.

Table 5. *Temora longicornis* and *Pseudocalanus elongatus.* MLR coefficients and their significance for the model (egg production = constant + [C1 PC1] + [C2 PC2] + [C3 PC3] + [C4 PC4] + [C5 PC5]; growth = constant + [C1 PC1] + [C2 PC2] + [C3 PC3]). Values reported are the best fit for a stepwise backward regression. Egg production is expressed in number of eggs female^{-1} d^{-1}, growth as daily specific growth rate (µg C [µg C]$^{-1}$ d^{-1}). See Table 2 for the variables representing different principal components. Coefficients describe the expected increase/decrease in egg production (eggs female^{-1} d^{-1}) or growth (% body wt) per a unit change of the independent variables. Significant coefficient at *p < 0.05, **p < 0.01, and ***p < 0.001

	Coefficient	Value	SE	*F* (-to-remove)	*F* (-to-enter)	p
Egg production						
T. longicornis	Constant	2.754	0.640			
N = 8	C1	0.778	0.101	59.125		0.002**
R^2 = 0.938	C2				0.250	0.643
Adj. R^2 = 0.891	C3	−0.725	0.176	17.043		0.015*
	C4				0.102	0.766
	C5	−4.812	1.458	10.890		0.030*
P. elongatus	Constant	1.844	0.174			
N = 8	C1	0.290	0.027	111.661		<0.001***
R^2 = 0.974	C2				0.935	0.388
Adj. R^2 = 0.940	C3	−0.285	0.048	35.697		0.004**
	C4				0.122	0.745
	C5	−2.375	0.396	36.044		0.004**
Growth						
T. longicornis	Constant	7.002	0.933			
N = 5	C1	1.004	0.201	24.885		0.015*
R^2 = 0.892	C2				0.110	0.762
Adj. R^2 = 0.857	C3				0.738	0.453
P. elongatus	Constant	−1.892	3.110			
N = 5	C1				0.044	0.847
R^2 = 0.869	C2	0.0212	0.005	19.860		0.021*
Adj. R^2 = 0.825	C3				0.539	0.516

Pseudocalanus elongatus closely followed the functional response line, while for *Temora longicornis*, 2 months (February and September) with a potential food quality limitation remained. In contrast to egg production, growth was never higher on seston than on a corresponding concentration of *Rhodomonas* sp. (Fig. 3B).

The MLR model identified dominant phytoplankton pigments (PC1) as the main factor determining the egg production of both copepod species (Table 5). The model coefficients implied an increase in egg production of ca. 0.8 and 0.3 eggs female^{-1} d^{-1} for *Temora longicornis* and *Pseudocalanus elongatus*, respectively, with an increase of 1 µg pigments l^{-1}. Also the different PUFAs C20 + C22 (PC3) and C18 (PC5) had a significant score in the model, although with negative coefficients. These coefficients implied a decrease of 0.7 and 0.3 eggs female^{-1} d^{-1} for *T. longicornis* and *P. elongatus*, respectively, with a 1 µg l^{-1} increase in C20 + C22, and a decrease of 4.8 and 2.4 eggs female^{-1} d^{-1} for *T. longicornis* and *P. elongatus*, respectively, with a 1 µg l^{-1} increase in C18 PUFAs (Table 5).

The multiple regression model for growth identified the dominant pigments (PC1) as the only significant coefficient for *Temora longicornis* growth, implying an increase in specific growth rate by 1.0% body carbon d^{-1} with a 1 µg l^{-1} increase in pigments. In contrast, POC (PC2) scored as the only significant coefficient for *Pseudocalanus elongatus* growth, suggesting a 0.3% increase in specific growth rate d^{-1} with a 1 µg l^{-1} increase in POC concentration (Table 5).

DISCUSSION

Bioassay approach

A bioassay approach has the advantage of separating the effects of temperature, copepod condition, age, and previous feeding history from the effects of food quantity/quality, as well as allowing distinction between the effects of food quantity and food quality. However, inherent in the method is a number of assumptions that, unfortunately, cannot all be evaluated. Among others, the method assumes that (1) seston quality does not change during storage, (2) a difference from the ambient temperature does not affect the experiment, (3) functional responses of cultured copepods are comparable to those of *in situ* animals, and (4) sampling station and sampling frequency adequately represent the study area during the season.

First, the composition and quality of potential food may change during storage due to the different temperature and light conditions. Such effects could include changes in cellular chl *a*, carbon or nitrogen levels, as well as changes in concentration and composition of fatty acids (Verity 1981, Berges et al. 2002, Rousch et al. 2003). In our experiments, the mean monthly temperature in the Marsdiep was close to the experimental temperature (16 ± 3°C) in May–October but much lower in December–April (6 ± 2°C) (van Aken 2003). The impact of such temperature change on the food quality of the seston community is difficult to estimate. The peak egg production rates that we observed in early spring, however, suggest no more than a minor (negative) influence due to storage at lower temperature.

Second, conducting the experiments at a temperature higher than *in situ* is likely to increase the basic metabolism and thus the food demand of the copepods (Ikeda 1985), which could have led to an artificial food limitation in some of our incubations. Using the regression between copepod body weight, temperature, and oxygen consumption from Ikeda (1985) and a respiratory quotient of 0.97 (Harris et al. 2000), an increase in temperature from 6 to 15°C would increase the carbon demand of copepods by ca. 6% (from 0.30 to 0.32 µg C ind.$^{-1}$ d^{-1}), which seems insufficient to induce food limitation in the present experiments. However, the potential effects with changing temperature suggest that this approach should not be used in areas with a large annual temperature range.

Third, there is a possibility that the functional response of cultured copepods deviates from that of their natural congeners (Tiselius et al. 1995). The functional response of natural copepods is difficult to ascertain, due to the variable environment and the unknown feeding history of the copepods that were the main reasons to work with conditioned laboratory animals. Cultured copepods may change their body size after many generations in the laboratory (Klein Breteler & Gonzalez 1982), and they may have a lower egg production than in nature (M. Koski pers. obs.). In general, cultured copepods are likely to be less flexible in their behaviour, lack a diel feeding rhythm, and have higher growth rates and a lower genetic variability (see e.g. Tiselius et al. 1995). Hence, when comparing the functional responses of natural and cultured copepods, a large variability may obscure or bias the true responses in the former, whereas an overreaction in case of a lower flexibility may confound the response of the latter. It remains to be decided if the unknown maternal effects of the *in situ* copepods are a stronger limitation for the interpretation of food quantity/quality studies than the potentially different characteristics of the cultured and *in situ* animals. In our study, the bioassay method allowed us to assess seasonal differences of egg production and development rate in relation to the food environment, which, doubtlessly, are not easily discerned in experiments with natural copepods.

Finally, one may question to which degree our sampling area was representative of the coastal North Sea and whether our monthly sampling allowed us to draw conclusions on the seasonal dynamics and food conditions of the copepod populations. The main characteristics of the seston in our samples, namely the overall dominance of large (>5 µm) diatoms, a high concentration of POC during the winter months, several chl *a* peaks of >10 µg l^{-1} during the season, and a relatively low C:N ratio, are typical for the study area (see e.g. Philippart et al. 2000, Cadée & Hegeman 2002). Although these are also regular features of the annual dynamics in the North Sea and nearby areas (see e.g. Kiørboe & Nielsen 1994, Pond et al. 1996, Halsband & Hirche 2001), a more diverse phytoplankton community, with a dominance of *Phaeocystis globosa* in April–May, and a dinoflagellate bloom during late summer, appears more common than a year-round biomass dominance of diatoms (Kiørboe & Nielsen 1994, Pond et al. 1996, Castellani & Altunbaş 2006). Also, the Marsdiep area typically has a *P. globosa* bloom period of 1 to 2 mo, although the bloom intensity has decreased during the last decade (Cadée & Hegeman 2002). Our study year appeared atypical in that respect, since prymnesiophytes, although present, never dominated the phytoplankton biomass, and their peak period was short (Fig. 1, Table 1). Nevertheless, our results are well in agreement with seasonal responses observed by other workers in the North Sea, both with respect to development and egg production. These observations include low egg production during the winter months, peak production during the spring bloom in March–April, intermediate values during most of the summer and early autumn, and a possible secondary peak in late summer (Fransz & Gonzalez 1991, Halsband & Hirche 2001, Halsband-Lenk et al. 2004, Arendt et al. 2005), as well as the generally low biomass increase outside the bloom season (Kiørboe & Nielsen 1994).

Food limitation of egg production

The multiple regression model indicated that the concentration of dominant phytoplankton pigments could largely explain the seasonal variation in egg production of both copepod species (Table 5). However, the approach of Müller-Navarra & Lampert (1996) suggested a clear difference between the 2 copepod species in their response to seston POC versus phyto-

plankton (chl *a*) concentration: while the egg production of *Pseudocalanus elongatus* during most of the months was similar or higher than expected based on carbon concentration, *Temora longicornis* egg production was mostly lower (Fig. 3A). This indication of a food quality limitation of *T. longicornis* was not apparent in the functional response to chl *a*, with the exception of a few months. The difference in the response of the 2 copepod species to POC concentration suggests that *P. elongatus* was using detritus and heterotrophic organisms as a food source, whereas *T. longicornis* was mainly relying on phytoplankton as food.

The approach of Müller-Navarra & Lampert (1996) additionally indicated 2 months with food quality limitation of *Temora longicornis* egg production (May and June) while no quality limitation was evident for *Pseudocalanus elongatus* egg production (Fig. 3A). The results with the sac-spawning and selectively feeding *P. elongatus* correspond with the idea of less food quantity- and less food quality-limited production of sac-spawning (Hirst & Bunker 2003) and selectively feeding copepods, respectively (DeMott 1988, Anderson & Pond 2000). Of the food quality indicators studied, multiple regression analysis identified the long chain polyunsaturated fatty acids C20 + C22 (PC3) and C18 (PC5) as significant factors in explaining the egg production of both species, but with negative coefficients. This is unexpected, since these fatty acids are typically considered essential for copepod nutrition and frequently have a positive correlation with egg production, hatching success, and growth (reviewed by Jónasdóttir et al. 2009). We interpret the negative coefficients to mean that the absolute concentrations of these fatty acids were not limiting copepod egg production in the Marsdiep. The negative coefficients may also be connected to the copepod need to obtain PUFAs in specific ratios. For instance, EPA:DHA (eicosapentaenoic acid, 20:5ω3, and docosahexaenoic acid, 22:6ω3, respectively) and ω3:ω6 ratios have been frequently identified as food quality indicators for copepod reproduction (reviewed by Jónasdóttir et al. 2009). Due to the very low concentrations (below detection) of DHA during large part of the year, we could not include the PUFA ratios in our analyses; thus, reasons for the negative coefficients remain unclear.

There were also several months when the egg production of both species with natural food was higher than expected from the response with a similar chl *a* concentration of *Rhodomonas* sp. (most notably April) (Fig. 3A). Both *Temora longicornis* and *Pseudocalanus* spp. are omnivorous and known to feed on ciliates (Hansen & van Boekel 1991, Fessenden & Cowles 1994). Hansen & van Boekel (1991) suggested that in the Marsdiep area *T. longicornis* feeds on abundant diatoms in March and May but switches to ciliates during a *Phaeocystis* bloom in April. In this area, ciliate concentrations typically peak at the end of the *Phaeocystis* bloom in late April to early May, when ciliate biomass can reach above 200 µg C l^{-1} (van Boekel et al. 1992). A similar post-bloom ciliate peak has been shown to be of both a good-quality and a sufficient quantity to support high egg production of copepods in the North Sea (Dutz & Peters 2008). A contribution due to heterotrophs may thus well explain the peak egg production in April, which was ca. 2× higher than expected based on chl *a* concentration (Fig. 3A).

It should also be noted that although *Rhodomonas* sp. is of a good nutritional quality and often used as a standard food in laboratory studies (Kiørboe et al. 1985, among others), it may not be of optimal size for adult copepods (Berggreen et al. 1988). The slope of the functional response of egg production on *Rhodomonas* sp. may thus be less steep and saturate at a higher concentration than on a larger good-quality food (see e.g. Berggreen et al. 1988, Kiørboe et al. 1985). Thus, if the seston contains a high concentration of large ciliates as might have occurred in April, the egg production would be expected to be higher than on the corresponding concentration of *Rhodomonas* sp. However, since the seston concentrations in the present study were typically high, the shape of the functional response curve on *Rhodomonas* sp. should not have large consequences for the interpretation of the results.

Food limitation of juvenile growth

In contrast to the mainly temperature-dependent growth of juvenile copepods (Huntley & Lopez 1992), we observed clear differences in development and growth rates due to changes in food conditions. In fact, only the cohort initiated in spring (March) survived until adult stages, while in the experiments performed during the summer (July), autumn (September), and winter (December–February), development failed before or soon after metamorphosis (Fig. 2B, Table 4). Based on MLR, the growth rate of both copepod species was mainly linked to food quantity, viz. phytoplankton in the case of *Temora longicornis* and POC in the case of *Pseudocalanus elongatus* (Table 5). For neither of the species was there any apparent limitation by the biochemical compounds studied. The bioassay showed a similar connection to food quantity; while POC appeared to be of limiting quality for both copepods, the functional response of growth closely followed the chl *a* concentration (Fig. 3B). There were, however, 3 exceptions: the growth rate of *T. longicornis* in February and September was lower than expected based on chl *a* concentration (Fig. 3B), and the extremely high mortality of both species in July

was clearly out of range considering the high chl *a* concentration (see Tables 1 & 4). There thus appeared to be additional unknown factors or combinations of factors that influenced the growth rate and mortality of both copepod species. In July this may include some deleterious compounds, as indicated by the high mortality rate combined with a high growth rate indicative of a high feeding rate.

Similar to our findings on egg production, we could not identify a clear variable inducing food-quality limitation of *Temora longicornis* growth during February and September. The food quality factors that could potentially limit growth and were not measured here include ratios of PUFAs, absolute or proportional concentration of phosphorus, food size spectrum, low digestibility or assimilation efficiency of food, and specific vitamins. Any of these, or a combination of these with the measured food quality indicators, could limit growth; in a dynamic seasonal environment as the Marsdiep, different limitations could also change in a short time-scale (days to months). The present study was not designed to identify such short-term specific limitations, which would demand a much higher sampling frequency and a variety of food quality variables.

Although many studies have shown maximum growth rates of diverse copepod species in the field (Peterson et al. 1991), food-limited growth has also been observed (Bakker & van Rijswijk 1987, Peterson & Kimmerer 1994). Peterson & Kimmerer (1994) observed an extremely high early mortality of *Temora longicornis* in Long Island Sound, with only spring and early summer cohorts reaching adulthood. Also in Kattegat (Denmark) the secondary egg production peak of *T. longicornis* in late summer did not result in an increase of biomass, which only peaked during early summer (Kiørboe & Nielsen 1994). The failure of *T. longicornis* cohorts to develop has been attributed to high egg and early nauplii mortality due to cannibalism, disease, viruses, parasitism, predation, maternal nutrition, inhibitory compounds, and water quality (Peterson & Kimmerer 1994, Carotenuto et al. 2002, 2006). Our study adds low food quantity and/or quality to the list, suggesting that mortality during juvenile copepod stages can also be too high and growth too slow for a successful development during a major part of the year.

In conclusion, irrespective of its limitations, our study shows that, on an annual scale, both egg production and growth of the dominant copepod species in our coastal area can be relatively well predicted from the food concentration, either phytoplankton (*Temora longicornis*) or a combination of phytoplankton, detritus and/or heterotrophic food items (*Pseudocalanus elongatus*). Taking into account the eutrophic nature of the Marsdiep area, this is somewhat surprising, but it can be explained by the variable nature of the seston. A large part of the POC consists of detritus, which is not likely to be easily accessible food for copepods. In addition, seasonal blooms of inedible algae may occur, such as *Phaeocystis* spp., which generally are not considered as a high-quality food for copepods (reviewed by Nejstgaard et al. 2007). In this respect, the year of our study, 2001, was exceptional due to the absence of *Phaeocystis* spp. The dominance of detritus and the short-lived blooms of various algal species, followed by blooms of heterotrops, all stress the importance of food-quality limitation, which in our study only appeared during a few separate months. Apparently, these events are too short to be observed with a monthly sampling interval. Nevertheless, the present study indicates that besides temperature or body size (Halsband & Hirche 2001), specific, qualitative, and quantitative food conditions are important in regulating the seasonal dynamics of copepods in the coastal North Sea.

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