Effect of food quality on rate of growth and development of the pelagic copepod *Pseudocalanus elongatus* (Copepoda, Calanoida)

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ABSTRACT: Rates of body growth, development and egg production of *Pseudocalanus elongatus* were measured in the laboratory, in relation to the taxonomy and physiology of algal food. Four types of experiments were performed to measure the copepod's response to (1) 7 algal species of similar size and shape, but different taxonomic groups, (2) inferior food species that were offered with good food as a check of toxicity, (3) nitrogen limitation and the growth rate of food species, and (4) highly unsaturated fatty acids that were supplied with inferior food to test for lack of essential fatty acids. Grazing was measured to check that the offered food was really ingested. The best food species proved to be *Rhodomonas* sp., which induced a fast rate of development, good somatic growth and egg production and low mortality. The development rate was almost equally fast with *Thalassiosira weissflogii*, *Gymnodinium simplex* and *Tetraselmis suecica*, but the rates of somatic growth or egg production were lower and mortality generally higher than with *Rhodomonas* sp. Three algal species, *Dunaliella* sp., *Amphidinium* sp. and *Chrysochromulina polylepis*, were poor food; copepod development was not completed, the rates of somatic growth and egg production were low and mortality was high. Ingestion was equally high with most of the species; only *C. polylepis* was not eaten. No clear toxic effects were found when the 3 poor-food species were offered in mixtures with *Rhodomonas* sp. N-limited *Rhodomonas* sp. did not reduce the rate of copepod development in comparison to a N-replete culture; however, N-limited *T. weissflogii* reduced the development rate to the low level of poor-food species. No effect of different growth rates of *Dunaliella* sp. was found. Lipids rich in highly unsaturated fatty acids supplied with *Dunaliella* sp. did not substantially improve the slow development and low egg production observed with this species. The weight-specific somatic growth rate was always higher than the weight-specific egg production rate, especially with less optimal food, which seems to hamper the estimation of the secondary production of copepods based on egg production alone. It is concluded that large differences in the food quality of different algal species are due to differences in digestibility or in mineral and biochemical composition.

KEY WORDS: Copepod · Food quality · Development · Somatic growth · Egg production · Taxonomy · N-limitation · Growth rate · Fatty acids · Secondary production

INTRODUCTION

Mesozooplankton growth rate and reproduction are determined by temperature, food quantity (Checkley 1980) and food quality (Huntley et al. 1987, Støttrup & Jensen 1990, Jónasdóttir 1994). Egg production of copepods in nature is generally assumed to be food limited, while juvenile growth often seems to be dependent on temperature alone (McLaren 1978, Peterson et al. 1991, Huntley & Lopez 1992). Nevertheless, at times, somatic growth may be food limited as well (Klein Breteler & Schogt 1994 and references therein). Although correlating mesozooplankton production and crude estimates of the food concentration (such as chlorophyll *a* or organic carbon concentration) is often attempted in the field, it is clear that such relations may be obscured by the food quality, which can be connected with either the taxonomy or the physiology of the algae. Factors affecting food quality may include cell size (Berggreen et al. 1988), cell morphol-

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ogy (Infante & Abella 1985, Burns 1987), toxicity (Lampert 1981, 1982, Nizan et al. 1986), mineral composition (Kiørboe 1989, Sterner & Hessen 1994) or content of some biochemical components, such as amino acids (Cowie & Hedges 1996), polyunsaturated fatty acids (Ahlgren et al. 1990, Jónasdóttir 1994, Müller-Navarra 1995), sugars (Cowie & Hedges 1996, Brown et al. 1997) or vitamins (Brown et al. 1997). The present research focuses on the effect of taxonomy of food species, with preliminary experiments on the effect of biochemical composition, nitrogen limitation and growth rate of algae.

Regarding taxonomic groups, blue-green algae have usually proven to be low quality food, or even harmful (Burns 1987, Lampert 1987, Gliwicz & Lampert 1990, review by Bernardi & Giussani 1990, Sellner et al. 1996). Green algae have also often been shown to be nutritionally poor food, although they are generally ingested by copepods (Hart & Santer 1994). The food quality of diatoms and dinoflagellates has been more contradictory. In several studies diatoms have been shown to be less nutritious than dinoflagellates (Hitchcock 1982, Kleppel et al. 1991), or to induce low hatching success and high mortality of copepod eggs (Ianora et al. 1995 & 1996, Miralto et al. 1995, Ban et al. 1997). However, copepod egg production is often correlated with diatom blooms (Kiørboe & Nielsen 1994), and no negative effects of diatoms on hatching success was found by Jónasdóttir & Kiørboe (1996). Dinoflagellates have also been correlated with high egg production of copepods (Kleppel 1992, 1993), although certain species may have harmful or even toxic effects (Carlsson et al. 1995).

An explanation for differences in nutritive value among different algae may be their different biochemical composition. While gross composition of proteins, carbohydrates and lipids in different algal classes is mostly not related to their nutritive value, the content of specific nutrients may be of importance (Brown et al. 1997). The amino acid content of algae has been found to be relatively similar in all groups and the content of vitamins and sugars variable and species specific, but the fatty acid content of microalgae shows systematic differences according to taxonomic groups (Brown et al. 1997). Diatoms, dinoflagellates, prymnesiophyceans and cryptomonads are generally found to be rich in nutritionally important highly unsaturated fatty acids (HUFA); $20:5\omega3$ (eicosapentaenoic acid, EPA) is generally abundant in diatoms, and $22:6\omega3$ (docosahexaenoic acid, DHA) in dinoflagellates, while prasinophyceans seem to contain only moderate and variable amounts of these fatty acids, and green algae contain none at all (Dunstan et al. 1992, Reitan et al. 1994, Zhukova & Aizdaicher 1995, Brown et al. 1997).

In addition to taxon-related differences, physical and chemical factors, such as nutrient availability and light intensity, have an effect on algal physiology, which affects their quality as food for mesozooplankton. It has been shown that nutrient availability may influence the concentration and composition of proteins and carbohydrates (Morris et al. 1983) as well as lipids and fatty acids (Mayzaud et al. 1989) in the phytoplankton cell. For example, nitrogen limitation causes the protein content of cells to decrease and the carbohydrate content to increase (Uriarte et al. 1993). In nutrient-limited algae, the amount of saturated fatty acids and the total lipid content increases (Parrish & Wangersky 1990), while the amount of highly unsaturated fatty acids, especially those of the $\omega3$ group, decreases (Reitan et al. 1994). Further, the age of the phytoplankton culture and the growth rate of algae affect various cell components. A decrease in nitrogen content and increase in C:N ratio are observed in slowly growing diatoms (Kiørboe 1989, Jónasdóttir 1994), as well as an increase in the extracellular carbohydrate production (Myklestad 1977, Malej & Harris 1993). Negative effects of nitrogen limitation and slow growth rate of algae on feeding, somatic growth and reproduction of planktonic crustaceans are reported by Cowles et al. (1988), Butler et al. (1989), Kiørboe (1989), Giani (1991), Mitchell et al. (1992), Sterner (1993) and Jónasdóttir (1994).

Egg production of copepods is sometimes shown to correlate to somatic growth of sub-adult stages, which makes it possible to estimate the secondary production of a whole copepod community based on female egg production alone (Berggreen et al. 1988, Hay 1995, Saiz et al. 1997). However, somatic tissue and eggs have a different chemical composition, which demands a different nutritional composition of the food (Sterner & Hessen 1994). Lipid content of copepod eggs and gonads of females has been found to be higher than in the rest of the copepod body (Gatten et al. 1980), suggesting that lipids would be more critical to egg production, while proteins would be more important to somatic growth (Kiørboe et al. 1985). Also, different fatty acid groups have been shown to correlate more with egg production than with somatic growth (cf. Jónasdóttir 1994 and Müller-Navarra 1995). Thus, the effect of food quality on the growth rate of sub-adult stages and on egg production is not necessarily similar, as was shown for *Calanus pacificus* by Razouls et al. (1991).

Until now only a few studies have examined the development and growth rates of marine copepods in relation to food quality. Most of the studies on the effect of food quality on marine mesozooplankton have concentrated either on feeding activity and ingestion (e.g. Berggreen et al. 1988, Cowles et al. 1988), or egg pro-

duction (Kiørboe 1989, Støttrup & Jensen 1990, Jónas-dóttir 1994), while studies dealing with somatic growth have mostly been performed with freshwater organisms (e.g. Ahlgren et al. 1990, Xu & Burns 1991, Sterner 1993, Hart & Santer 1994, Müller-Navarra 1995, Twombly & Burns 1996). The few marine studies that have dealt with food quality and copepod growth or development rates, have concentrated on cell size (Berggreen et al. 1988, Klein Breteler et al. 1990), measured only naupliar development (Huntley et al. 1987), or studied only a few food species from 1 or 2 taxonomic groups (Mullin & Brooks 1970a, b, Paffenhöfer 1976, Razouls et al. 1991). These studies show the effect of cell size and toxicity of food species on the feeding activity of copepods and, hence, on mesozooplankton production. In the present study we wanted to study food quality effects connected with different algal taxa, irrespective of the effects of size, shape and toxicity of food species. To test the hypothesis of similar dependence of egg production and somatic growth on food quality, we measured both somatic growth and egg production rates of copepods in relation to food quality. The calanoid copepod *Pseudocalanus elongatus* (Boeck) was fed in excess with different algal species from different taxonomic groups, but of similar size and shape, and in a size range (equivalent spherical diameter 7 to 10 μm) that can be efficiently used by most developmental stages of small copepod species (Berggreen et al. 1988). Grazing experiments were conducted to make sure that observed differences among food species were not due to different rates of ingestion. Toxicity of species inducing low growth rate was tested by offering them in a mixture with the good food *Rhodomonas* sp. In addition, preliminary experiments were performed using algae grown at different growth rates and nutrient supply regimes, and production was measured when highly unsaturated fatty acids were supplied with the algae, to test for possible effects of algal physiology and biochemical composition.

METHODS

Cultures. Chemostat cultures of all algae were kept at 15°C on a 16 h light:8 h dark cycle, at a light intensity of ca 150 μE m^{-2} s^{-1}. F/2 medium (Guillard 1975) was used for the cryptophycean *Rhodomonas* sp., the chlorophycean *Dunaliella* sp., the prasinophycean *Tetraselmis suecica*, the prymnesiophycean *Chryso-chromulina polylepis* and the diatom *Thalassiosira weissflogii* cultures. Dinoflagellate medium (Hansen 1989) was used for the dinoflagellate *Gymnodinium simplex* and *Amphidinium* sp. cultures. Dilution rate of most cultures was kept near 0.2 d^{-1}. With *Dunaliella* sp., 2 different dilution rates (0.2 d^{-1} and 0.5 d^{-1}) were

used. Most of the algal cultures were in a steady state during the experiments (more than 10 turnovers of flask content); however, *Amphidinium* sp. was used in the experiments almost immediately after the start of the culture (0 and 6 turnovers in Expts A and B, respectively; see Table 1). *T. suecica* culture sometimes grew strongly on the wall of the container, thus chemostat conditions were not always met.

In nitrogen-limited cultures, nitrogen-poor (200 μM NO$_3$) f/2 medium was used for N-limited *Rhodomonas* sp. and N-limited *Thalassiosira weissflogii*. Since the cell concentration in the N-limited *Rhodomonas* sp. culture was always about 10 times lower than in the N-replete culture, and since nitrate concentration in the culture media of N-limited *T. weissflogii* was seriously depleted and not different from zero, we assume that both cultures were truly nitrogen limited. Dilution rates of these cultures were ca 0.05 d^{-1}. N-limited cultures were also in a steady state during the experiments, only N-limited *T. weissflogii* may not have been in a steady state during C/N analyses (2 turnovers), which may have led to an underestimation of the C:N ratio during the experiments (more than 10 turnovers).

Mean cell volume was measured from the algal cultures, using an ELZONE electronic particle counter (Particle Data Inc.) at the beginning and at the end of each experiment, and the average value was used to calculate the food concentration in the experiments. Since the algal species used are characterised by a simple, mostly round shape without spines, it is assumed that the measured cell volume is representative of the volume available for copepods during grazing. To measure the particulate organic carbon and nitrogen content of algae, a few ml of algal culture was filtered to a combusted GF/C filter, stored at −50°C and analysed using a Carlo Erba CHN analyser. Carbon and nitrogen contents of cells were measured 1 to 3 times during the period of study and corrected for observed differences in cell volume between experiments. Volumes, carbon contents and C:N ratios of cells and dilution rates of cultures are given in Table 1.

The copepod *Pseudocalanus elongatus* was reared in the laboratory in semi-continuous stock cultures at 15°C and in a semi-natural day/night cycle of strongly dimmed light. Cultures were fed with a mixture of autotrophic species, *Rhodomonas* sp. and *Isochrysis galbana*, and a heterotrophic dinoflagellate, *Oxyrrhis marina*, in concentrations >300 μg C l^{-1} (Klein Breteler 1980, Klein Breteler et al. 1982, 1995). Two different *P. elongatus* strains were used in the experiments. The first one was bred in the laboratory for 23 generations before the start of the experiments. After this culture was lost for inexplicable reasons, we isolated new copepods from the Dutch Wadden Sea during winter, as before, and continued the experi-

Table 1. Dilution rate (d^{-1}) of cultures and mean volume (µm^3), carbon content (pg) and C:N ratio of cells fed to 2 different strains (I and II) of *Pseudocalanus elongatus*, number of experiments focusing on development, egg production and grazing (N: nauplii; C: copepodites; Ad: adults) and range of concentrations of food by volume (ppm), carbon (µg C l^{-1}) and nitrogen (µg N l^{-1}) in the experiments. Food level 1: ca 1 to 3 ppm; food level 2: ca 3 to 8 ppm; food level $\frac{1}{2}$ (single species controls in experiments with mixed species): ca 1 to 2 ppm. Rh: *Rhodomonas* sp.; RhN: N-limited *Rhodomonas* sp.; Th: *Thalassiosira weissflogii*; ThN: N-limited *T. weissflogii*; Gy: *Gymnodinium simplex*; Am: *Amphidinium* sp.; Te: *Tetraselmis suecica*; Du: *Dunaliella* sp.; DuL: *Dunaliella* sp., low dilution rate; DuH: *Dunaliella* sp., high dilution rate; Ch: *Chrysochromulina polylepis*. L$_1$, L$_2$ and L$_3$: lipid supplements 1, 2 and 3, respectively. (A) and (B) after species name indicate experiments which were treated separately (cf. Fig. 1, Tables 2–7). (pre) preliminary experiment; (–) value missing; blank spaces: no experiment done

Food species	Algal cultures				No. of experiments			Range of concentration		
	Dilution rate	Cell volume	Cell carbon	C:N ratio	Development	Egg production	Grazing (N/C/Ad)	ppm	µg C l^{-1}	µg N l^{-1}
Strain I										
Food level 1										
Rh	0.16	170	42	5.9	6	2 + pre	12/7/6	2.1–2.3	508–580	86–98
RhN	0.04	195	74	7.7	4		6/3/1	1.9–2.6	710–1010	92–131
ThN(A)	0.06	369	101	9.6	1			3.0	840	88
Gy	0.19	212	30	5.0	3	1	4/6/3	2.1–3.0	290–420	58–84
Te(A)	0.18	227	59	9.4	2	2	6/4/	2.6–3.1	690–780	73–83
DuL	0.17	93	19	9.0	1		4/1/	–	–	–
DuH	0.50	104	13.5	5.0	2	2	8/3/	1.3–1.5	170–200	34–40
Ch(A)	0.25	106	16	8.5	1		1/ /	2.6	400	47
Ch(B)	0.25	123	19	8.5	2	2	/6/	1.9–3.1	290–480	34–56
Food level 2										
Rh	0.16	170	42	5.8	2		2/6/2	4.4	1100	190
RhN	0.05	195	74	7.7	1		/4/1	4.3	1620	210
Th	0.14	623	219	8.1	3	2	4/6/2	4.9–6.2	1710–2200	211–272
ThN(B)	0.04	369	101	9.6	1		2/2/	7.7	2120	221
Am(A+B)	0.19	443	102	4.5	2	2	4/4/	4–7.8	930–1790	207–398
Te(A)	0.20	227	59	9.4	1		/ /1	4.7	1220	130
Te(B)	0.21	227	20	12.5	1		1/ /	5.3	470	38
DuL	0.17	93	19	9.0	1			–	–	–
DuH	0.50	104	13.5	5.0	2			2.8–3.2	360–415	72–83
Ch(A)	0.25	106	16	8.5	1		2/ /	4.9	740	87
Food level $\frac{1}{2}$										
Rh	0.16	170	42	5.9	2			1.4	340	58
Am	0.18	443	102	4.5	1			2.2	510	113
Ch	0.22	123	19	8.5	1			1.1	160	19
Strain II										
Food level 1										
Rh	0.15	160	40	5.9	2	5		1.4–2.0	345–510	58–86
DuH	0.45	93	12.1	5.0	3	5		1.2–1.5	150–195	30–39
DuH + L$_1$	0.45	93	12.1	5.0	3	4		1.2–1.5	150–191	30–38
DuH + L$_2$	0.45	93	12.1	5.0	1	4		1.2–1.5	150–191	30–38
DuH + L$_3$	0.45	93	12.1	5.0	1	4		1.2–1.5	150–191	30–38
Food level $\frac{1}{2}$										
Rh	0.15	160	40	5.9	4			0.8	204–225	35–38
Du	0.45	93	12.1	5.0	4			0.7–0.8	88–100	18–20

ments with this strain after it was cultivated through 8 generations. In the stock cultures with mixed food, no significant differences occurred between the 2 strains for either the development rate or body size. However, since in the present experiments the development time, growth rate, mortality and egg production of the 2 strains when grown on *Rhodomonas* sp. did differ significantly from each other (ANCOVA p < 0.05), the few experiments (with *Dunaliella* sp. and *Rhodomonas* sp. controls) that were conducted with

the second strain, were treated separately (strain II in Tables 1 to 8).

Experiments. Experiments were repeated 1 to 4 times using 7 different algal species grown at various conditions, as described before. The number of different experiments and range of food concentration in experiments (in ppm and in carbon and nitrogen concentration) are given in Table 1.

In all experiments, *Rhodomonas* sp. served as a control to make sure that no additional variability was

introduced due to different copepod generations or strains. Food was provided in excess. Every day 80% of the food medium was renewed. We aimed to achieve a concentration by volume of 2 ppm, which corresponds to ca 400 µg C l^{-1} based on the volume of *Rhodomonas* sp. and has been found to be a sufficient food level for maximum copepod growth with algae of comparable size (Berggreen et al. 1988, Støttrup & Jensen 1990) and large diatoms (Vidal 1980a) and for near maximum phytoplankton concentrations during the spring bloom in the North Sea (Gieskes & Kraay 1977). As a control for food sufficiency, experiments with about double this amount (ca 4 ppm, sometimes up to 8 ppm) were performed with all species except *Gymnodinium simplex*. The actual carbon content in the experiments varied, but was in most cases over ca 300 µg C l^{-1}. Concentrations were established using 2 µm filtered sea water (Whatman Gamma 12-20). Experiments with nitrogen-limited algae were conducted in nitrogen-poor sea water containing 0.00 µM NO$_3$ and 0.03 µM NO$_2$.

To check for toxicity, *Chrysochromulina polylepis*, *Dunaliella* sp. and *Amphidinium* sp. were mixed with *Rhodomonas* sp. In experiments with mixtures, the concentration of each species was kept at half the nominal level (1 to 2 ppm), and control experiments were performed with both species alone to reveal any effect of the low amount of food.

In addition, experiments were conducted with *Dunaliella* sp. with 3 different lipid supplements. Lipids used to supplement the food were ICES emulsions obtained from P. Coutteau (University of Gent, Belgium). These emulsions are of the Selco(r) type, containing lipids (62% on wet weight basis), vitamins, antioxidants, preservatives, emulgators and water, and are widely used in aquaculture to supplement extra HUFA to, e.g., *Artemia* nauplii (Coutteau & Mourente 1997, Coutteau & Sorgeloos 1997). We used ICES series 2 in which 30% of the lipids consist of the HUFA 22:6ω3 (DHA) and 20:5ω3 (EPA) in a ratio of 0.6, 2 and 4 in lipid supplements 1, 2 and 3, respectively. Control experiments were performed with filtered sea water and lipid supplement 1. The lipids were supplied in an amount equal to about 50% of the dry weight of the algae. When diluted to filtered sea water, lipids formed droplets of ca 5 µm in diameter, which is only slightly smaller than *Dunaliella* sp., thus it was assumed that copepods were able to ingest the lipid droplets.

Development and growth experiments were started with newly hatched nauplii (stages I to III) from a recently (<4 d before) matured generation. Nauplii smaller than 125 µm were caught on a 50 µm sieve, flushed a few times with filtered sea water to remove the previous food particles, and placed into 1180 ml bottles containing the experimental food suspension.

Bottles were put on a roller-apparatus turning at 1 rpm; rolling in these bottles does not affect the development rate of *Pseudocalanus elongatus* (Klein Breteler et al. 1997). Temperature and light conditions remained the same as in the stock culture. The experiments were started with a high concentration of nauplii (ca 1000 ind. l^{-1}), which were harvested 3 to 4 times wk^{-1} to keep the biomass (in dry weight) constant. The medium was replaced in proportion at every harvest. Thus ca 15 adult ind. l^{-1} were left at the end of the experiment. Three times per week, the harvest (200 ml of the content of each experimental bottle) was filtered on a 50 µm sieve and flushed to a petri dish. Individuals were anaesthetised with 20 to 40 µl 5% MS 222 and developmental stages were determined and counted under a binocular microscope. After that, the individuals were flushed 3 times in 9% ammonium formate (HCO$_2$NH$_4$) to remove salts, and pipetted with 40 µl of ammonium formate into small platinum dishes. Dry weight was measured after 24 h drying at 60°C in an oven, using a microbalance (Sartorius micro XM 1000P) with an accuracy of 1 µg. Unfortunately, no copepod weights using *Dunaliella* sp. at a low dilution rate were obtained. Samples with total weight less than 0.03 mg were omitted from the results. Experiments were generally terminated after the first significant appearance of adults; thus the duration of copepodite stage V is largely excluded from the results.

To check for possible crowding in the development experiments, 4 control bottles with a lower concentration of nauplii (ca 40 ind. l^{-1}) were used in parallel in 3 experiments with *Rhodomonas* sp. and in 2 experiments with *Tetraselmis suecica* and *Dunaliella* sp. One control bottle was finished every fourth day, and copepod numbers, developmental stages and mean dry weight were determined as for the experimental bottles.

Development rate was expressed as the increase of the mean stage (calculated from the stage frequency distribution in the samples) over the course of time. Although this is a simple way to approximate development time (Klein Breteler et al. 1994), it allows a less frequent sampling schedule and suffices to detect differences among cultures. Growth rate was estimated from the observed mean dry weight, which was assumed to increase exponentially over the course of time according to

$$W_t = W_0 e^{gt}$$

where W_t is the weight (mg) at time t, W_0 is the weight at time 0, g is the instantaneous growth rate (d^{-1}) and t is the elapsed time (e.g. Paffenhöfer & Harris 1976, Huntley & Lopez 1992).

Mortality appeared to be more or less constant within experiments. Hence, it was described by

$$N_t = N_0 e^{Zt}$$

where N_0 is the number of individuals (l^{-1}) at time 0, N_t is the number of individuals, corrected for mortality due to sampling, at time t and Z is the instantaneous rate of mortality (d^{-1}). N_t was corrected for sampling mortality by multiplying the observed number of animals by

$$[V/(V-v)]^{(n-1)}$$

where V is the volume of the experimental bottle, v is the volume of the sample and n is the rank number of the sample. Slopes g and Z of the linear regressions of $\ln W_t$ and $\ln N_t$, respectively, with t were used to test for differences between experiments, using an analysis of covariance (ANCOVA). When obvious differences were observed among repeated experiments, the rates of development, growth and mortality were estimated separately for each.

Grazing experiments were performed with nauplii, copepodites and adults taken directly from the harvest of the development experiments, which implied an almost full life-time adaptation to the pertinent food. Repeatedly, experiments were performed with all algal species that produced enough survivors for an adequate grazing experiment. Ca 30 individuals of naupliar stages IV to VI, ca 20 individuals of copepodite stages III to IV or ca 10 adult copepods (males and females) were placed in 2 replicate bottles of 225 ml (nauplii and copepodites) or 1180 ml (adults), and left on the roller-apparatus for 24 h at the same temperature, light and food conditions as during their prior development. After 24 h, individuals were collected and counted and developmental stages were determined as described before. The concentration of algae in all bottles was counted at the beginning and at the end of the experiment with the ELZONE particle counter. Clearance rate and ingestion were calculated, using the method of Frost (1972), from the disappearance of particles in bottles containing copepods compared to the average of 2 control bottles without copepods. Dry weights of individuals in the grazing experiments were derived from the mean stage, using the common linear regressions between log dry weight and mean stage, observed for groups of food species in the development experiments (see 'Results').

Egg production experiments were performed at food conditions comparable to those in the development experiments (Table 1), including lipid supplements with *Dunaliella* sp. Preliminary egg production experiments were carried out using females collected from the stock culture less than 2 d after maturation of the major part of the population. From these experiments egg production appeared to continue even after 6 d in filtered sea water. Therefore, we decided to adapt

females from early copepodite stages onwards, before their major build-up of reserve lipids. Copepodite stages II to IV were collected from the stock culture using a 200 µm sieve, flushed to a petri dish, lightly anaesthetised with a small amount of 5% MS 222 (20 µl/3.3 ml filtered water), transferred with a pipette to a petri dish containing the desired food solution and placed in 1180 ml bottles with the same food concentration. Ca 30 copepodites were placed in each bottle to yield >5 females that had successfully moulted into the adult stage after the adaptation period, even in filtered sea water. During adaptation (5 to 7 d), 80% of the food medium was renewed daily. At the end of the adaptation period individuals were filtered to a petri dish, lightly anaesthetised with MS 222, and 3 to 15 adult females without egg sacs were picked out and placed in 1180 ml bottles. After 48 h copepods, eggs and the few nauplii found were collected in a petri dish using a 50 µm sieve; eggs and nauplii were counted and eggs were left to hatch for another 72 h in petri dishes containing 2 µm filtered sea water. Since *Pseudocalanus elongatus* carries egg sacs, and since the development time of eggs at 15°C is longer than 48 h (2.06 d; Klein Breteler et al. 1994), cannibalism was assumed not to affect the results of the egg production experiments. Lipid reserves of the females were visually estimated using a scale of 1 to 4 (Klein Breteler & Gonzalez 1988), after which the females were counted and prepared for dry weight analysis as described before.

The gross growth efficiency of juveniles and adults was estimated from the growth constant (g) of subadult stages and from weight-specific egg production rate of females, respectively, divided by the weight-specific ingestion (both in carbon). Carbon content of copepods was assumed to be 41% of the dry weight (Williams & Robins 1982) and carbon content of eggs 0.14 g C egg^{-1} (Frost 1989).

RESULTS

Growth and development rates

Development was not isochronal; copepodite stages lasted longer than naupliar stages with most food species. For algal species that enabled copepods to complete development, the rate of development tended to be slower in the first copepodite stages than in nauplius stages, and later copepodite stages lasted even longer (Fig. 1, Table 2). This is commonly found in *Pseudocalanus* spp. (McLaren et al. 1989, Kiørboe & Sabatini 1995), but not with a mixture of food (e.g. Klein Breteler et al. 1994, 1995). Although we used food species that were in a narrow size range, it is
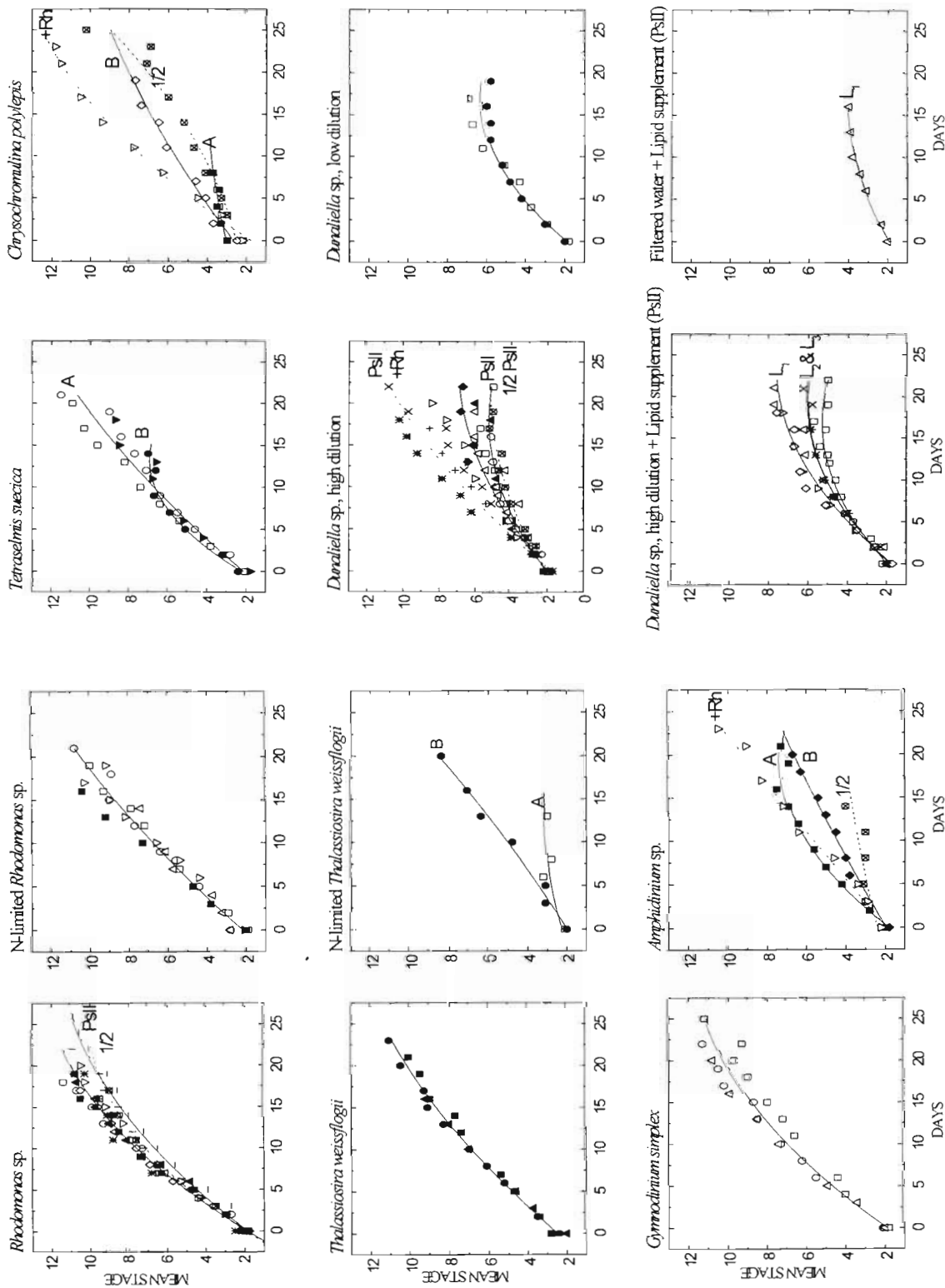
Fig. 1. *Pseudocalanus elongatus*. Mean life stage as a function of time, showing development of individuals fed with different food species and in filtered sea water. Mean stages: 2–6, naupliar stages I to V; 7–11, copepodite stages I to V; 12, adults. A 3rd order polynomial was used to describe development. Different symbols indicate different experiments: (□, O, ◇, △, ▽, +, ×, *, |, –) food level of 1 to 3 ppm; (■, ●, ◆, ▲, ▼) food level of 3 to 8 ppm. Separate curves were fitted when results of experiments were obviously different (A and B), or when the 2nd strain of *P. elongatus* was used (PsII). (+Rh, ----) Experiments with *Rhodomonas* sp. addition, and (1/2, ⊠, ---) the control of this mixture experiment at half the nominal food level. L₁, L₂ and L₃: supplement of lipids of different fatty acid ratios (see 'Methods')

Table 2. *Pseudocalanus elongatus*. Average development time from nauplius stage II to copepodite stage I and from copepodite stage I to copepodite stage V in experiments with 2 different copepod strains. (∞) Development not completed. ($\frac{1}{2}$) single species controls at 1 to 2 ppm used in experiments with mixed species. FW: Filtered sea water. Other abbreviations as in Table 1

Copepod strain	Food species	Development time (d) N II–C I	C I–V	Total
Strain I	Rh	8.9	13.5	22.4
	Rh($\frac{1}{2}$)	10.0	18	28
	RhN	10.1	14.2	24.3
	Th	9.7	15.4	25.1
	ThN(A)	∞	∞	∞
	ThN(B)	17	∞	∞
	Gy	10.3	13.1	23.4
	Te(A)	10.5	14.5	25
	Te(B)	12	∞	∞
	DuL	19.6	∞	∞
	DuH	21.6	∞	∞
	Am(A)	20.1	∞	∞
	Am(B)	26.5	∞	∞
	Am($\frac{1}{2}$)	∞	∞	∞
	Ch(A)	∞	∞	∞
	Ch(B)	16.5	∞	∞
	Ch($\frac{1}{2}$)	22	∞	∞
	Rh + Am	14	14	28
	Rh + Ch	9.2	14.2	23.4
Strain II	Rh	11.0	14.6	25.6
	Rh($\frac{1}{2}$)	12.5	17.4	29.9
	DuH	∞	∞	∞
	DuH($\frac{1}{2}$)	∞	∞	∞
	DuH + L$_1$	16	∞	∞
	DuH + L$_2$	25	∞	∞
	DuH + L$_3$	23	∞	∞
	FW + L$_1$	∞	∞	∞
	Rh + Du	11	12.9	23.9

probable that none of the sizes of algae offered alone was optimal food for all stages of *P. elongatus*. Such a dependency of optimal food size on copepod age was shown by Gruzov (1985), Berggreen et al. (1988) and Klein Breteler et al. (1990).

There was no detectable effect of crowding. Development rates in experimental bottles (starting with high concentration of nauplii) were similar to those in control bottles with low concentrations of individuals (results not shown). Slopes of log mean stage against log time were not significantly different (ANCOVA, p > 0.05).

There was no significant difference among successive copepod generations. The development of *Pseudocalanus elongatus* with *Rhodomonas* sp. was comparably fast in all the experiments conducted with the same strain. However, there was a significant difference in development rates between the 1st and 2nd *P. elongatus* strain (cf. experiments with *Rhodomonas* sp. and *Dunaliella* sp. in Fig. 1 and Table 2).

There was no systematic difference between the single and double food concentrations used. At the double concentration (solid symbols in Fig 1) with one species (N-limited *Rhodomonas* sp.) development was slightly faster, while with other species (*Tetraselmis suecica*, *Dunaliella* sp. and *Chrysochromulina polylepis*) it was sometimes slower. Only with N-limited *Thalassiosira weissflogii* did it seem that there was a considerable difference, but this comprised one experiment only. At the still lower food concentration ($\frac{1}{2}$, dashed line Fig. 1) used in controls of mixture experiments with *Rhodomonas* sp., *C. polylepis* and *Amphidinium* sp., development was generally slower than at single and double concentrations; however, with *Dunaliella* sp. such a reduced development rate did not occur (Fig. 1). Overall, the results seem to indicate that the intended conditions of excessive food were achieved at the single food level in all food species.

Large differences of development rates were obtained with the different species of algae used (Fig. 1). Development was fastest with *Rhodomonas* sp., *Thalassiosira weissflogii* and *Gymnodinium simplex*. With these species, copepods were able to complete their development in 22 to 25 d, which is slightly slower than found in previous studies (cf. Paffenhöfer & Harris 1976, Vidal 1980a, b, Klein Breteler et al. 1982, 1995). In the 2nd *Pseudocalanus elongatus* strain, the development with *Rhodomonas* sp. was completed in 26 d (Table 2). In experiments with *Tetraselmis suecica*, similar rapid development was obtained; however, in one of the experiments (Expt B), development stopped in the first copepodite stages. With the rest of the food species, development was slow and incomplete. There was no difference in the low development rate between low and high dilution rates of *Dunaliella* sp. Nitrogen limitation in *Rhodomonas* sp. had no obvious effect compared to the N-replete culture, but nitrogen limitation in *T. weissflogii* dramatically turned this species to a poor-quality food. When *Rhodomonas* sp. was mixed with poor-food species, the development rate was similar to that with *Rhodomonas* sp. alone at the comparable low (*Amphidinium* sp.) or even the higher 'single' concentration (*Chrysochromulina polylepis* and *Dunaliella* sp.). Lipid supplements to *Dunaliella* sp., particularly supplement 1 (22:6ω3/20:5ω3 ratio of 0.6), somewhat increased the development rate of nauplii, but development was not completed with any of the lipid supplements, nor with lipid supplement 1 in filtered water (Fig. 1, Table 2).

Growth of *Pseudocalanus elongatus* was exponential and thus constant for all development stages. Hence, ln(dry weight) generally increased linearly over the course of time in most of the food species. With few exceptions, the specific growth rate (*g*, slopes in Table 3) shows an effect of food species similar to that

Table 3. *Pseudocalanus elongatus*. Parameters of linear model of growth relating ln(mean dry weight) (mg ind.$^{-1}$) to time in experiments with 2 different copepod strains. All regressions are significant (*p < 0.05), except the last 4 for strain I, and the last 2 for strain II. Slopes (g) which do not differ significantly (p < 0.05) from each other are grouped by a vertical line (only species with significant regressions included). Abbreviations as in Tables 1 & 2

Copepod strain	Food species	Slope	Intercept	n	R
Strain I	ThN(A)	0.36525	−7.6007	3	1.00 *
	Rh	0.18377	−7.9901	37	0.96 *
	Rh + Ch	0.16623	−7.7223	9	0.99 *
	RhN	0.16245	−7.8147	30	0.94 *
	Gy	0.14724	−7.8666	26	0.91 *
	Ch(B)	0.14436	−8.3321	5	0.96 *
	Rh + Am	0.14371	−8.1690	10	0.96 *
	Te(A)	0.13721	−8.0922	13	0.94 *
	Rh($\frac{1}{2}$)	0.13393	−7.6918	9	0.97 *
	Th	0.12399	−7.5206	23	0.89 *
	DuH	0.09741	−7.9436	24	0.69 *
	Am(B)	0.09284	−8.3179	7	0.82 *
	Am(A)	0.06322	−7.9768	10	0.78 *
	Ch($\frac{1}{2}$)	0.05625	−7.7783	8	0.86 *
	ThN(B)	0.14249	−7.6324	5	0.87
	Te(B)	0.03269	−7.0125	3	0.55
	Am($\frac{1}{2}$)	0.02493	−7.8490	3	0.10
	Ch(A)	−0.03761	−8.4745	8	−0.10
Strain II	Rh + Du	0.15175	−7.6371	26	0.88 *
	Rh	0.10997	−7.3573	19	0.83 *
	Rh($\frac{1}{2}$)	0.09927	−7.4663	19	0.84 *
	Du($\frac{1}{2}$)	0.00510	−7.5892	8	0.04
	Du	−0.25174	−6.5141	3	−0.97

Table 4. *Pseudocalanus elongatus* (Strain I). Parameters of linear regressions relating log mean dry weight (mg ind.$^{-1}$) and mean stage of copepods in experiments with different food species. All correlations are highly significant (p < 0.001), except that of N-limited *Thalassiosira weissflogii*. Common regression indicated per group of regressions that do not differ significantly (p < 0.05) in slope. Abbreviations as in Tables 1 & 2

Group	Food species	Intercept	Slope	n	R
Group 1	Rh	−3.9284	0.1758	43	0.97
	RhN	−3.7262	0.1544	34	0.94
	Th	−3.6567	0.1436	25	0.89
	ThN(A+B)	−3.2880	0.1491	8	0.55
	Gy	−3.8394	0.1631	27	0.95
	DuH	−3.8724	0.1719	25	0.84
	Ch(A+B+$\frac{1}{2}$)	−4.2270	0.2139	23	0.81
	Average	−3.7888	0.1600	184	0.90
Group 2	Te(A)	−3.6747	0.1165	16	0.89
	Am(A+B)	−3.7875	0.1242	17	0.77
	Average	−3.7417	0.1217	33	0.84

Table 3). The growth rate with *T. weissflogii* was intermediate and significantly different from both groups.

The relation between log dry weight and mean stage, used to calculate the mean dry weight of copepods in grazing experiments, was linear with all food species (Table 4). With the exception of *Tetraselmis suecica* and *Amphidinium* sp. (Group 2 in Table 4), the slope of this relation was similar for all food species (Group 1 in Table 4), indicating that the effect of food species was not obviously reflected in the weight of the individual stages. Thus, the growth rate seemed to be less affected by food quality than did the development rate, which is in contrast to short-term responses to food shortage (Miller et al. 1984). Since both prosome length and length-specific weight of *Pseudocalanus elongatus* are positively correlated with food abundance (Klein Breteler & Gonzales 1988), an effect of food quality on weight of stages would also be expected. The small effect of food on weight may possibly be explained by the low survival rate with less optimal food species, which led to missing observations of weight at the premature end of such experiments.

Mortality was relatively constant throughout development in the experiments with all algal species. Mortality was lowest (3 to 6 % d^{-1}) in experiments with *Rhodomonas* sp., N-limited *Rhodomonas* sp. and *Thalassiosira weissflogii* (Table 5). With most other species, as well as with mixtures and lipid supplements, mortality was between 6 and 17 % d^{-1}, which is significantly different from the experiments with the former 3 foods. However, very high mortality was recorded in some experiments with *Tetraselmis suecica* (Expt B), N-limited *T. weissflogii* (Expt A), *Chrysochromulina polylepis*

shown by development rate; good growth of 13 to 18 % body weight d^{-1} was obtained with *Rhodomonas* sp., N-limited *Rhodomonas* sp. and *Gymnodinium simplex*; however, the growth rate with *Thalassiosira weissflogii* was significantly (p < 0.05) lower. Comparably good growth, with the exception of 1 experiment, was obtained with *Tetraselmis suecica*. Slow or negligible growth (<10 % body weight d^{-1}) was found with *Dunaliella* sp. and *Amphidinium* sp. The growth rate with the remaining species was variable (*Chrysochromulina polylepis*) or unreliable (N-limited *T. weissflogii*), and generally not different from zero, due to high mortality (cf. Table 5) and consequently a low number of observations. The growth rate of the 2nd *P. elongatus* strain was much lower than that of the 1st strain; even with *Rhodomonas* sp. the growth rate was only 10 to 11 % body weight d^{-1}. Addition of *Rhodomonas* sp. to the poor- or variable-food species dramatically increased the growth rate to a level of about 15 % body weight d^{-1}, irrespective of the copepod strain. Using ANCOVA, we distinguished 2 groups (1st *P. elongatus* strain) within which there was no significant difference in growth rate (p < 0.05,

Table 5. *Pseudocalanus elongatus*. Parameters of linear model of mortality, relating ln(number of individuals) to time in experiments with 2 different copepod strains. All regressions are significant (p < 0.05), except that of N-limited *Thalassiosira weissflogii* (Expt A) (p > 0.05). Slopes which do not differ significantly (p < 0.05) from each other are indicated with a vertical line. Abbreviations as in Tables 1 & 2

Copepod strain	Food species	Slope	SD	Intercept	n	R
Strain I	Th	0.0389	0.013	6.7378	25	0.53
	Rh(½)	0.0400	0.011	6.7308	7	0.85
	RhN	0.0509	0.020	6.4168	38	0.39
	Rh	0.0561	0.008	6.9020	66	0.68
	ChB	0.0758	0.016	6.7170	8	0.89
	Rh + Am	0.0880	0.017	6.1708	10	0.88
	Ch(½)	0.0970	0.009	6.9384	9	0.97
	Rh + Ch	0.0970	0.009	6.9384	9	0.97
	Am(B)	0.1128	0.015	7.1901	8	0.95
	Am(A)	0.1143	0.009	7.0685	11	0.97
	Gy	0.1260	0.013	7.1970	26	0.89
	Te(A)	0.1291	0.030	7.0214	34	0.60
	DuL	0.1349	0.027	7.1520	17	0.80
	DuH	0.1540	0.016	6.9237	34	0.86
	ThN(B)	0.1599	0.015	7.1737	7	0.98
	Te(B)	0.2929	0.561	7.2189	7	0.89
	Ch(A)	0.4358	0.087	7.9600	14	0.82
	ThN(A)	0.4813	0.171	7.2124	5	0.85
	Am(½)	0.4772	0.057	7.2626	6	0.97
Strain II	Rh(½)	0.0267	0.009	6.7615	21	0.56
	Rh	0.0285	0.010	6.7312	21	0.54
	Rh + DuH	0.0641	0.015	6.8694	32	0.61
	DuH + L₂	0.1316	0.040	7.3721	9	0.78
	DuH + L₃	0.1442	0.041	7.5309	7	0.84
	DuH + Lₜ	0.1483	0.017	7.1764	27	0.86
	DuH(½)	0.1650	0.072	7.2763	13	0.57
	DuH	0.1746	0.037	7.2992	23	0.72
	FW + L₁	0.2143	0.084	7.8302	7	0.75

(Expt A), *Amphidinium* sp. (experiment with food level ½) and filtered water with lipid supplement, in which all the specimens died during the first week of the experiment.

Egg production

Egg production was measured in separate experiments in which individuals were adapted to the pertinent food for 5 to 7 d. Egg production was highest in the 1st strain of *Pseudocalanus elongatus* with *Rhodomonas* sp. and *Thalassiosira weissflogii*, ca 5 eggs female^{-1} d^{-1} (Fig 2), which is generally comparable to literature values (cf. Corkett & Zillioux 1975, Paffenhöfer & Harris 1976, Thompson 1982, Landry 1983, Frost 1985). Somewhat lower egg production was observed with *Tetraselmis suecica* and *Gymnodinium simplex* (2.4 eggs female^{-1} d^{-1}), and in the 2nd copepod strain with *Rhodomonas* sp. Still lower egg production

occurred with *Dunaliella* sp. and *Amphidinium* sp. (<1 egg female^{-1} d^{-1}). No eggs were produced in any of the *Chrysochromulina polylepis* experiments or in experiments with filtered sea water with or without lipid supplement. None of the lipids supplementing the *Dunaliella* sp. diet significantly improved the low egg production with this alga alone (Fig. 2).

Egg production in the 1st *Pseudocalanus elongatus* strain correlated with female lipid content (Table 6), but not in the 2nd strain, for which lipid content remained invariably high, while egg production again was strongly reduced with *Dunaliella* sp., similar to that in filtered sea water. No explanation could be found for this obvious difference between the 2 strains. Apparently, the presence of storage lipid is not sufficient and an adequate food source is necessary for egg production in *P. elongatus*. A similar dependence of reproduction on spring phytoplankton development in addition to lipid stores has been observed in *Calanus finmarchicus* (Diel & Tande 1992).

Hatching success was high (>70%) with *Rhodomonas* sp., *Thalassiosira weissflogii* and *Gymnodinium simplex*. Thus, our experiments did not indicate any obvious inhibitory effect of diatoms on the hatching success of copepod eggs, such as was documented by Ianora et al. (1996). Hatching success with *Dunaliella* sp. and *Tetraselmis suecica* was low (23 to 42%). A similar or lower proportion of eggs hatched in the experiments with *Dunaliella* sp. with lipid supplements (Table 6).

There was a significant difference in mean female dry weight between the 2 different *Pseudocalanus elongatus* strains (Table 6). In the 2nd strain the females were unusually heavy, even compared to former observations at
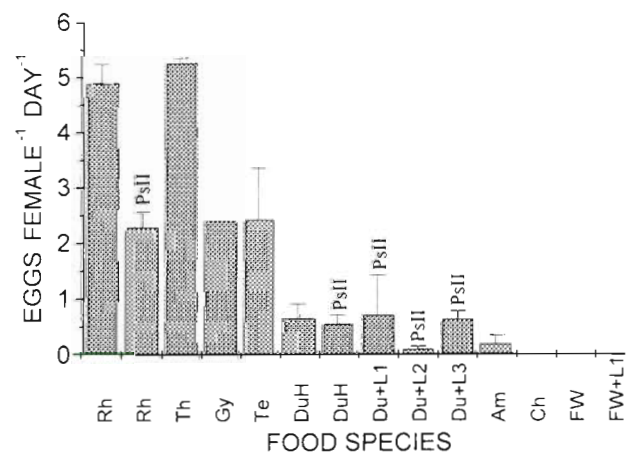

Fig. 2. *Pseudocalanus elongatus*. Egg production of individuals fed with different food species for 5 to 7 d prior to maturity. Abbreviations as in Tables 1 & 2. PsII: Experiments performed with the 2nd *P. elongatus* strain Standard errors are indicated

Table 6. *Pseudocalanus elongatus*. Results of egg production experiments showing number of experiments, number of females in each experiment, mean dry weight and lipid reserves (cf. 'Methods') of females, egg production, weight-specific egg production and hatching success of eggs. Hatching percent in parentheses if not reliable due to low number of eggs. Standard error indicated if more than one measurement was done. Mean dry weights which do not differ significantly indicated with line. Abbreviations as in Tables 1 & 2

Copepod strain	Food species	No. of experiments	No. of females	Mean dry weight (μg ind.$^{-1}$)	Lipid reserve (1–4)	Egg production Eggs ind.$^{-1}$ d^{-1}	% body carbon d^{-1}	Egg hatching No. incubated	% hatched
Strain I	Rh	2	10–11	18	2.7 ± 0.1	4.9 ± 0.4	11 ± 0.7	205	94 ± 0.4
	Th	2	4–12	8.0	3 ± 0	5.3 ± 0.1	11 ± 0.2	170	82 ± 7.1
	Gy	1	8	27	1.5 ± 0.3	2.4	5.1	39	100
	Te	2	9	22	1.4 ± 0.2	2.4 ± 0.9	5.1 ± 2.0	86	23 ± 16
	DuH	2	9	17 ± 0	1.4 ± 0.2	0.7 ± 0.2	1.4 ± 0.5	24	42 ± 29
	Am	2	6–8	22 ± 7.1	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.3	6	(100)
	Ch(B)	2	5–7	13 ± 0.4	1.2 ± 0.3	0 ± 0	0 ± 0	0	
	FW	2	4–6	9.3 ± 1.2	0.2 ± 0.2	0	0 ± 0	0	
Strain II	Rh	6	5–14	42 ± 6	2.8 ± 0.1	2.3 ± 0.3	3.0 ± 0.4	258	74 ± 10
	DuH	5	7–15	26 ± 4	2.5 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	58	31 ± 15
	Du + L$_1$	3	6–15	21 ± 4	2.7 ± 0.1	0.7 ± 0.7	1.4 ± 1.0	51	0
	Du + L$_2$	3	3–13	16 ± 2	2.4 ± 0.2	0.1 ± 0.1	0.2 ± 0.0	7	(0)
	Du + L$_3$	3	3–15	19 ± 3	2.5 ± 0.2	0.6 ± 0.1	0.8 ± 0.2	31	34 ± 4
	FW + L$_1$	4	3–12	16 ± 5	1.7 ± 0.1	0 ± 0	0 ± 0.0	0	

15°C, when in good food conditions the females weighed ca 20 µg rather than 42 µg (Klein Breteler & Gonzalez 1988). This difference seems to be too large to be explained by differences in ripeness of the females, although the heavy weight of females producing low numbers of eggs might suggest that these females contained eggs that were about to be laid. Since the true reason for the difference in female body weight between the 2 strains is not known, we used the average dry weight of the 2 strains separately to calculate the weight-specific egg production. The weight-specific egg production was high with *Rhodomonas* sp. and *Thalassiosira weissflogii* (1st strain, 11 % body carbon d^{-1}), but only intermediate (5 % body carbon d^{-1}) with the other good-food species, *Gymnodinium simplex* and *Tetraselmis suecica*. With the poor-food species, weight-specific egg production was always low. With *Rhodomonas* sp. and the 2nd *P. elongatus* strain, the weight-specific egg production was significantly lower than that with the 1st strain (Table 6). Weight-specific egg production was lower than somatic growth with all food species. With *T. weissflogii* and with *Rhodomonas* sp. (strain I), this difference was small or less than 2-fold, but with *Rhodomonas* sp. (strain II), *T. suecica* and *G. simplex* it was ca 3-fold, and with all the other species and treatments it was more than 5-fold (cf. *g* in Table 3 and % weightspecific egg production in Table 6).

Clearance rate and ingestion

Generally, the clearance rate of nauplii was 0.2 to 0.8 ml ind.$^{-1}$ d^{-1}, that of copepodites 1.5 to 4.5 ml ind.$^{-1}$ d^{-1} and that of adults 3 to 11 ml ind.$^{-1}$ d^{-1} (Fig. 3A). Exceptionally high clearance rates were sometimes obtained in nauplii and adults with *Tetraselmis suecica* (only 1 experiment each) at a high food level. Exceptionally low clearance rates were obtained in most experiments with *Chrysochromulina polylepis*, which is in agreement with previous findings (Nielsen et al. 1990).

The clearance rate was in most of the comparable cases higher at the low than at the high food concentration, indicating regulation of feeding activity with the surplus of food offered (Fig. 3A). This led to more or less similar ingestion rates irrespective of the food level, e.g. with *Rhodomonas* sp. (copepodites and adults, Fig. 3B). Also, large differences in grazing activity of copepodites, e.g. between *Gymnodinium simplex* and *Amphidinium* sp. (Fig. 3A), were mainly due to differences in food concentration, since ingestion rates on these food species were almost the same (Fig. 3B). There was no effect of either N-limitation or dilution rate on grazing of *Pseudocalanus elongatus*; both the algae of N-limited cultures and the cultures grown at a low dilution rate were ingested in amounts similar to those of other cultures. However, large differences in ingestion rates remained due to differences among a few food species. Consistently high ingestion rates were obtained with *Thalassiosira weissflogii* with and without N limitation, and mainly low ingestion rates were obtained with *Chrysochromulina polylepis*. The weight-specific ingestion rates of the different life stages were rather similar, mostly varying between 0.3 and 1.3 µg C (µg C)$^{-1}$ d^{-1} in nauplii, between 0.5 and 1.6 µg C (g C)$^{-1}$ d^{-1} in copepodites, and between
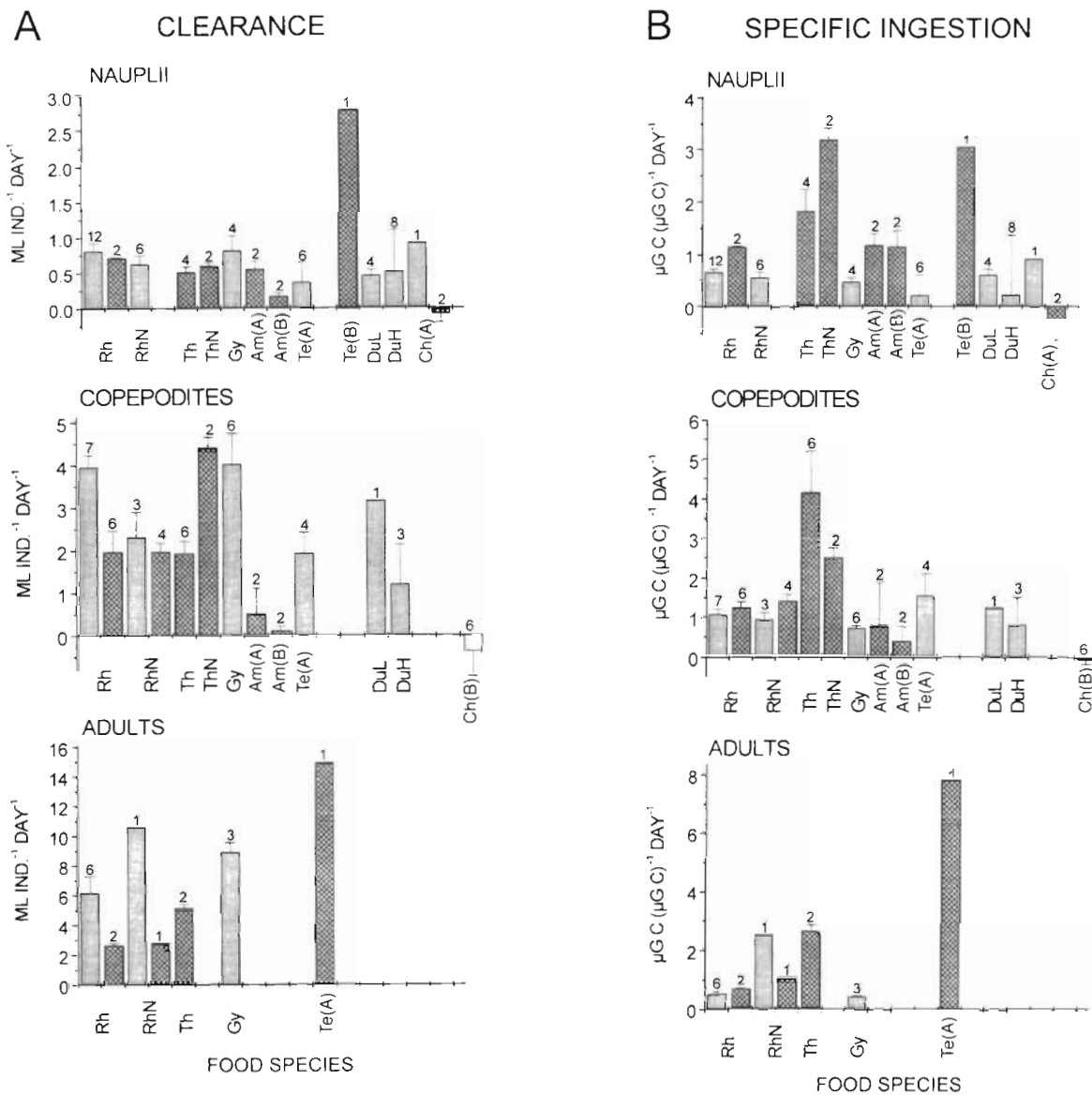
A   CLEARANCE

B   SPECIFIC INGESTION

Fig. 3. *Pseudocalanus elongatus* (strain I). (A) Average clearance rate (ml ind.$^{-1}$ d^{-1}) and (B) average weight-specific ingestion rate [µg C (µg C)$^{-1}$ d^{-1}] of N IV–VI, C III–IV and adults. Abbreviations as in Table 1. Light gray bars: food level of 1 to 3 ppm; hatched bars: food level of 3 to 8 ppm. Missing values mainly due to low survival in development experiments. Standard error and number of experiments are indicated for each bar

0.5 and 2.8 µg C (µg C)$^{-1}$ d^{-1} in adults (Fig. 3B), which is comparable to those found in earlier studies with *P. elongatus* (cf. Paffenhöfer & Harris 1976) and *Acartia* spp. (Kiørboe et al. 1985, Støttrup & Jensen 1990, Durbin & Durbin 1992). Focusing on food species that led to poor development, we conclude that the high ingestion observed in nauplii with *Tetraselmis suecica* (Expt B) and in nauplii and copepodites with N-limited *T. weissflogii*, as well as the medium ingestion in nauplii and copepodites with *Amphidinium* sp. (Expts A and B) and *Dunaliella* sp., all indicate that low ingestion was not the reason for the failing or delayed devel-

opment in these experiments. Only in *C. polylepis* was there a clear link between low food consumption and poor development or growth (Figs. 1 & 3B, Table 3).

Gross growth efficiency

Gross growth efficiency of copepods varies according to developmental stage, food concentration and food quality. In our study, growth efficiency of nauplii was higher than that of copepodites and adults (Table 7), which is in contrast to the studies of Paffen-

höfer (1976) and Harris & Paffenhöfer (1976), who found highest gross growth efficiency in early copepodite stages. However, this might have been due to the smaller cell size of algae used in our study. Gross growth efficiency in the double food concentration was lower than in the single concentration, similar to the study of Mullin & Brooks (1970b) and in accordance with a dominant effect of food availability (Straile 1997). Highest growth efficiency of somatic growth, ca 30% for nauplii and ca 20% for copepodites, was obtained with *Rhodomonas* sp., N-limited *Rhodomonas* sp. and *Gymnodinium simplex*, and highest growth efficiency in adults was also obtained with *Rhodomonas* sp. (21%). Irrespective of a negative effect of food level, the growth efficiency with *Thalassiosira weissflogii* and *Amphidinium* sp. was low, <10% for all life stages measured (Table 7), similar to results obtained with N-limited *T. weissflogii* and in one experiment with *Tetraselmis suecica* (Expt B) (gross growth efficiency not shown due to nonsignificant *g*). The growth efficiency of adults was moderate with *G. simplex* (12%), and low with *Tetraselmis suecica* (0.7%). The low growth efficiency of *Pseudocalanus elongatus* feeding on *T. weissflogii* compared to *G. simplex* is in agreement with Conover (1966) and Cahoon (1981), who showed low assimilation efficiency on diatoms compared to dinoflagellates. However, intermediate and high gross growth efficiencies of copepods on some diatom species have also been observed (Mullin & Brooks 1970a, Paffenhöfer 1976, Støttrup & Jensen 1990).

Table 7. *Pseudocalanus elongatus* (Strain I). Gross growth efficiency (%) ± SE of N IV–VI, C III–IV and adults in terms of carbon for algal species that gave rise to significant (p < 0.05) growth (Table 3). Parentheses indicate unreliable data, SE of ingestion exceeding 30 % of the mean value. (–) Value missing; blank space: no experiments done. Lack of data for adults due to low survival in development experiments. Abbreviations as in Tables 1 & 2

Food species	Gross growth efficiency (%)		
	Nauplii	Copepodites	Adults
Food level 1			
Rh	28 ± 1.4	17 ± 1.3	21 ± 2.9
RhN	29 ± 3.9	17 ± 2.4	
Gy	32 ± 2.9	20 ± 0.5	12 ± 1.1
Te(A)	(65 ± 134)	(9 ± 3.4)	0.7 ± 2.6
DuH	(42 ± 50)	(12 ± 62)	–
Ch(B)		(–2.2 ± 690)	–
Food level 2			
Rh	16 ± 0.6	15 ± 1.5	
RhN		12 ± 0.8	
Th	7 ± 1.0	3.0 ± 0.6	4 ± 0.3
Am(A)	5 ± 0.6	(8 ± 23)	–
Am(B)	8 ± 0.5	(24 ± 2400)	

DISCUSSION

We sought to study the effect of food quality on the rates of development, somatic growth and reproduction of the marine copepod *Pseudocalanus elongatus*, concentrating on the effect of taxonomy, irrespective of size, shape and toxicity, of food species, as well as food concentration and feeding activity of copepods. Also, we wanted to test the hypothesis of similar dependence of somatic growth and egg production on food quality.

We found clear differences in all measured variables (development and growth rates, mortality, egg production, female lipid reserves, hatching success and gross growth efficiency) of *Pseudocalanus elongatus* when it was fed with different algal species (cf. summary in Table 8). Our results are in agreement with previous studies showing good food quality of the cryptophycean *Rhodomonas* sp. (Berggreen et al. 1988, Klein Breteler et al. 1995 in mixtures of food) and the diatom *Thalassiosira weissflogii* (Kiørboe 1989), except for the lower somatic growth rate and growth efficiency of the latter species in our study. Also, our results support Hart & Santer's (1994) report of the poor food quality of green algae, in particular that of *Dunaliella* sp. (Støttrup & Jensen 1990, Jónasdóttir 1994). The dinoflagellate *Gymnodinium simplex* and the prasinophycean *Tetraselmis suecica* induced fast development and somatic growth, although survival, egg production, lipid content, hatching success or gross growth efficiency were reduced. However, the second dinoflagellate used in our study, *Amphidinium* sp., together with the chlorophyte *Dunaliella* sp. and the prymnesiophyte *Chrysochromulina polylepis*, proved to be poor food. The contrast between *G. simplex* and *Amphidinium* sp. indicates a low taxonomic conformity of the nutritional characteristics within dinoflagellates, which is in accordance with the studies of Huntley et al. (1987) and Razouls et al. (1991).

Feeding activity of copepods could not explain the differences in the rates of development, growth or mortality observed with different food species. Only with *Chrysochromulina polylepis* was a low growth rate connected to low ingestion, while all the other algae were ingested in sufficient amounts to sustain good growth and reproduction. Also, no clear evidence for toxicity of *C. polylepis*, *Dunaliella* sp. or *Amphidinium* sp. was found. The development of *Pseudocalanus elongatus* in mixture experiments was complete and equally fast (*Amphidinium* sp.) or slightly faster (*C. polylepis* and *Dunaliella* sp.) than in the control experiment with a similarly low concentration of *Rhodomonas* sp. alone (1 to 2 ppm) (cf. Table 2). This excludes the possibility of direct effects through toxic exudates on *P. elongatus*. On the other hand, the

Table 8. *Pseudocalanus elongatus*. Summary of the average effects of different food species on development, growth, mortality, egg production, hatching success, female lipid reserves, ingestion and gross growth efficiency. (+) High response; (0) 'intermediate' response; (–) low response. (++) Ingestion higher than that on *Rhodomonas* sp. Blank spaces: no experiments or unreliable results. N: Nauplii; C: copepodites; Ad: adults. Abbreviations as in Tables 1 & 2

Copepod strain	Food species	Development	Growth	Survival	Egg production	Hatching	Lipid reserves	Ingestion	Gross growth efficiency N	C	Ad
Strain I	Rh	+	+	+	+	+	+	+	+	+	+
	RhN	+	+	+				+	+	+	
	Th	+	0	+	+	+	+	++	–	–	–
	ThN	–		–				++			
	Gy	+	+	0	0	+	0	+	+	+	0
	Te	+	+	0	0	–	0	++			–
	Am	–	–	0	–		–	+	–		
	DuL	–	0					+			
	DuH	–	–	0	–	0	0	+			
	Ch	–	–	–	–		0	–			
	FW				–		–				
	Rh + Am	0	+	0							
	Rh + Ch	+	+	0							
Strain II	Rh	+	0	+	0	+	+				
	DuH	–	–	0	–	0	+				
	DuH + L$_1$	–		0	–	–	+				
	DuH +L$_2$	–		0	–		+				
	DuH + L$_3$	–		0	–	0	+				
	FW + L$_1$	–		–	–		0				
	Rh + Du	+	+	0							

slightly positive contribution by *C. polylepis* and *Dunaliella* sp. with the mixture experiments suggests that at least some of these cells were eaten, which also seems to disprove the possibility of toxic cell components.

Since most of the food species were well ingested by *Pseudocalanus elongatus*, and since no clear evidence for toxicity was found, the reason for poor growth success with some of the food species must have been due either to their indigestibility or to an inadequate mineral or biochemical composition. In our excessive feeding conditions, copepods had the chance to compensate for such nutritional inadequacies by increasing food intake. Only in the case of *Thalassiosira weissflogii* did we find evidence for such compensatory consumption. The fast development though moderate somatic growth rate of copepods with N-replete *T. weissflogii* indicates that this food contains all the essential elements required, though in lower proportion, probably in connection with a high inorganic content of the diatom frustule. This was also indicated by the low gross growth efficiency with this species, which is in accordance with studies of Corner et al. (1976) and Støttrup & Jensen (1990). In contrast, increased ingestion of N-limited *T. weissflogii* did not effectively compensate for a possible lower assimilation. With the other algae we did not observe elevated ingestion rates. Therefore, the low somatic growth rates observed with N-limited *T. weissflogii*, *Dunaliella*

sp., and *Amphidinium* sp., and in 1 experiment with *Tetraselmis suecica* (Expt B), as well as the somewhat reduced egg production with *T. suecica* and *Gymnodinium simplex* and low hatching success with *T. suecica*, may be connected to a deficiency of essential food elements.

Two different methods were used to manipulate the food quality of algae: the use of a N-deficient culture medium (*Rhodomonas* sp. and *Thalassiosira weissflogii*), and manipulation of the dilution rate of the culture (*Dunaliella* sp.). However, since dilution rates of cultures grown under N-limitation were also low, the effect of these factors could not be separated in our experiments. Nitrogen limitation and/or low dilution rate of the culture dramatically affected *Pseudocalanus elongatus* rates of growth, development and survival with *T. weissflogii*, but not with *Rhodomonas* sp. or *Dunaliella* sp. Although the C:N ratio in N-limited *T. weissflogii* was not highly altered, though perhaps underestimated (cf. 'Methods'), compared to the N-replete culture, the ratio of 9.6 was the second highest of all the cultures used. The highest C:N ratio occurred in the one unsuccessful experiment with *Tetraselmis suecica* (Expt B) which was connected with heavy wall growth on the algal culture bottle. These unsuccessful cultures of the food algae at high C:N ratios point to a possible N-limited copepod growth in these experiments. However, it would be expected that the shortage of basic elements, such as nitrogen, would

lead to reduced growth and development rates in proportion to the supply rate of the limiting element, as for the egg production response in the study of Kiørboe (1989). Therefore, the abrupt halt in development that we observed even at a small change of the C:N ratio of the food suggests that also other factors apart from N limitation were involved.

Both for growth and egg production, copepods require ω3 group polyunsaturated fatty acids (Støttrup & Jensen 1990), which they cannot synthesize de novo (Fraser et al. 1989). Especially strong correlations have been found between mesozooplankton growth rate and egg production and the abundance of 20:5ω3 (EPA) and 22:6ω3 (DHA) HUFAs in their diet (Jónasdóttir 1994, Müller-Navarra 1995). These essential fatty acids are absent in chlorophyceans (Dunstan et al. 1992, Brown et al. 1997), which is in accordance with measurements in our strain of *Dunaliella* sp. (W. C. M. Klein Breteler, S. Schouten & M Baas unpubl. obs.). Hence, the lack of the HUFAs EPA and DHA may be the reason for inferior growth and development rates of *Pseudocalanus elongatus* when feeding on *Dunaliella* sp. Also, lack of essential HUFAs may explain the reduced egg production and hatching rate of *Pseudocalanus elongatus*, in contrast to good growth and development rates, when feeding on the prasinophyte *Tetraselmis suecica*. According to Ackman et al. (1968), Reitan et al. (1994) and Zhukova & Aizdaicher (1995), *Tetraselmis* sp. may contain considerable quantities of EPA, but not DHA. Hence, if lipids are more important for egg production than for somatic growth, as suggested by Kiørboe et al. (1985), the lack of DHA may be the reason for reduced reproduction of *P. elongatus* feeding on *T. suecica*.

In the diatom *Thalassiosira weissflogii* and the dinoflagellates *Amphidinium* sp. and *Gymnodinium simplex* no clear relation between the copepod's performance and content of EPA or DHA was apparent. In the diatom, the fatty acid composition of the N-limited culture was similar to that of the N-replete culture: both were rich in EPA, with a moderate content of DHA. The dinoflagellate species were both rich in DHA, while only *Amphidinium* sp. contained a moderate amount of EPA (Klein Breteler et al. unpubl. obs.), which is in accordance with published records of fatty acid composition in algae (Ackman et al. 1968, Zhukova & Aizdaicher 1995). Obviously, in the N-limited diatom and in *Amphidinium* sp. essential nutritional deficiencies other than EPA and DHA seem to be involved. With the other dinoflagellate, *G. simplex*, which lacked EPA, reduced egg production similar to that with *Tetraselmis suecica* was observed; however, hatching success with this species was still high, suggesting that the reproduction was less affected than with *T. suecica*. Even if the lack of EPA with this spe-

cies may have caused the reduced egg production, the fast copepod development and growth suggest that *Pseudocalanus elongatus* may synthesize this fatty acid by chain elongation from linolenic acid (18:ω33) (cf. Norsker & Støttrup 1994), which would contradict the fact that EPA is an essential nutritional element in this copepod species.

Direct experimental evidence on the crucial effect of HUFA's on mesozooplankton growth and egg production has until now only been given by Demott & Müller-Navarra (1997) and Weers & Gulati (1997). These authors found improved somatic growth and reproduction of *Daphnia* spp. when fish oil emulsions rich in HUFAs were provided together with the cyanobacterium *Synechococcus elongatus* and the chlorophycean *Scenedesmus acutus*, which alone contained almost no EPA or DHA. We aimed to gather this kind of evidence with marine species by supplying *Dunaliella* sp. with extra HUFAs to copepods. However, our experiments failed to show any significant increase in development rate and egg production of *Pseudocalanus elongatus*. Only with lipid supplement 1 (DHA/EPA ratio of 0.6) was a slightly faster development found than with *Dunaliella* sp. alone, but copepods were still not able to complete their development. Thus, it seems that either copepods are not able to take up lipid droplets from the solution or that the addition of HUFAs does not increase the quality of *Dunaliella* sp. as copepod food. The latter explanation would imply that long chain HUFAs are not the (only) missing factor which causes *Dunaliella* sp. to be poor food for copepods. In general, the low nutritional food value of Chlorophyceae and the correlated absence of HUFA (Brown et al. 1997) may need critical analysis before a true causal relation is found.

In the sea, food quality is determined by chemical composition of seston, which in turn is affected by species composition (phytoplankton, microzooplankton and detritus), physiological state and nutrient availability of algae (Jónasdóttir 1994, Jónasdóttir et al. 1995). Of the algae groups involved in our study, diatoms and dinoflagellates are the most prominent ones in the seasonal development of the phytoplankton community in the sea. While controversy surrounds questions of the nutritional quality of diatoms (cf. Ban et al. 1997), dinoflagellates (excluding species which are toxic or rejected by copepods; cf. Huntley et al. 1986) are generally found to be a good quality food and the preferred food items of copepods in the sea (Kleppel 1992, 1993, Ianora & Poulet 1993). However, the species used in our study did not show such a superior food quality of dinoflagellates over diatoms. Although the nutritious dinoflagellate *Gymnodinium simplex* was good food for juvenile growth, it did not sustain the maximum egg production observed with the

diatom *Thalassiosira weissflogii*. Thus, in contrast to the study of Ban et al. (1997), we did not observe any harmful effects of diatoms on copepod reproduction, but only somewhat reduced juvenile growth. However, N-limited slowly growing *T. weissflogii* in our study was inferior food, which is in agreement with studies of Jónasdóttir (1994) and Kiørboe (1989), who showed the poor food quality of senescent diatoms and cells with a high C:N ratio. It seems possible that only fast growing and nutrient-replete diatom cells are good food for copepods, which in turn would imply that copepod reproduction and somatic growth would be enhanced only in the early phase of diatom blooms, when nutrients are still abundant (cf. Kiørboe et al. 1988).

We did not exhaustively explore the effect of chemical or physical factors on the quality of algae as food for copepods. When nitrogen was limiting, *Rhodomonas* sp. continued to be of good quality. Therefore, it might be reasoned that under natural conditions there will always be some algal species that furnishes the copepods with the appropriate kind of food. Heterotrophic flagellates and ciliates, exploiting different food sources, might also assist in funneling essential food elements towards higher trophic levels. If this were all sufficient, copepod production would only be dependent on temperature and bulk availability of food, which is not true (Kiørboe et al. 1988, Jónasdóttir et al. 1995). Future studies on the role of limitation of algal growth by nutrients and light may bring more clarity to the complex biochemistry of algae, which determines their value as food for zooplankton.

Our results further stress the fact that estimating the secondary production based on egg production underestimates the total production. With all the food species used in our study somatic growth was higher than egg production, the difference being larger with less optimal food species. This differential dependency on food quality is in accordance with the study of Peterson et al. (1991), implying that secondary productivity cannot be estimated from field data on egg production alone.

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