Marine Biology (1994) 121: 67–81

S. H. Jónasdóttir

Effects of food quality on the reproductive success of *Acartia tonsa* and *Acartia hudsonica*: laboratory observations

Received: 22 February 1994 / Accepted: 24 June 1994

Abstract The effect of the chemical composition of food on the reproductive success of the copepods *Acartia tonsa* Dana and *A. hudsonica* Pinhey was studied in the laboratory. Laboratory-reared individuals were fed one of three monoalgal diets at different stages of growth: the diatom *Thalassiosira weissflogii*, the flagellate *Rhodomonas lens* and the dinoflagellate *Prorocentrum minimum*. The diet was analyzed for carbon, nitrogen, protein, carbohydrate and fatty acid content. Reproductive success was measured as eggs female^{-1} day^{-1} (E_r) and as the hatching success of the eggs. The E_r of *Acartia* spp. was correlated with protein and specific fatty acids [16:1ω7 (negative), 20:5ω3, 22:6ω3, and 18:0 (positive)] and, especially, the fatty acid composition of the algae expressed as the ω3:ω6 and 20:22 fatty acid ratios. The youngest diatom cultures and exponentially-growing flagellates displayed the highest E_r; the lowest E_r was recorded for females fed the senescent diatom cultures. The development time of eggs was affected by the age of the phytoplankton culture fed to the female. Hatching success of eggs decreased with the age of the algal culture, but no correlation was found with the measured chemical components of the food.

Introduction

Temporal changes in both the physical and chemical composition of seston in the oceanic environment are well reported (Kattner et al. 1983; Morris et al. 1983; Sakshaug et al. 1983; Fraser et al. 1989; Mayzaud et al. 1989) and take place, for example, as a result of changes in species composition (phytoplankton, microzooplankton, detritus, etc.), physiological state of the phytoplankton, and nutrient availability. Mayzaud et al. examined changes in the chemistry of particles from winter to fall, and found strong seasonality for most chemical and biochemical components (carbon, nitrogen, protein, carbohydrates and lipids). Similarly, Morris et al. reported changes associated with the growth, development and decay of individual phytoplankton blooms. Laboratory and field studies have shown that phytoplankton undergo compositional changes in their lipid classes and specific fatty acids as nutrient availability changes (Kattner et al. 1983; Parrish 1987; Parrish and Wangersky 1987; Mayzaud et al. 1989) as well as with the age of the phytoplankton culture (Ackman et al. 1964; Pugh 1971; Webb and Chu 1982). Protein and carbohydrate content of algal cells also changes when nutrients are depleted (Harrison et al. 1977; Raymont 1980; Morris et al. 1983).

The effects of such compositional changes of phytoplankton on zooplankton growth and reproduction are not well understood. Different food types elicit different reproductive responses in copepods (Marshall and Orr 1952; Nassogne 1970; Checkley 1980; Arnott et al. 1986). However, with the exception of nitrogen in a few studies (Checkley 1980; Cahoon 1981; Kiørboe 1989), the specific compositional constituents in the diet that cause different reproductive responses in copepods have not been identified.

The few previous studies on the nutritional requirements of crustaceans indicate that fertility and development may require specific fatty acids (Cladocera: Conklin and Provasoli 1977; *Acartia tonsa*: Støttrup and Jensen 1990; shrimp: Jones et al. 1979). The lipid content of copepod eggs and gonads of females is higher than that of the whole individual (Giese 1966; Gatten et al. 1980), suggesting the potential importance of lipids in egg production by copepods. Previous studies showed that crustaceans do not or cannot easily biosynthesize the polyunsaturated fatty acids (PUFAs) ω3 and ω6, and that these fatty acids are found in the crustacean in proportion to their availabil-

Communicated by N. H. Marcus, Tallahassee

S. H. Jónasdóttir
Marine Sciences Research Center,
State University of New York at Stony Brook,
Stony Brook, New York 11794-5000, USA

Present address: Danish Institute for Fisheries and Marine Research, Charlottenlund Castle, DK-2920 Charlottenlund, Denmark

ity in the its diet (Lee et al. 1971; Castell 1982; Sargent and Falk-Petersen 1988; Bourdier and Amblard 1989; Fraser et al. 1989). Additionally, the PUFAs 20:5 and 22:6 of the ω3 type are essential for somatic growth and survival of some crustaceans (Kanazawa et al. 1977; Jones et al. 1979; Martin 1980). Both 20:5 and 22:6 PUFAs of dietary origin are enriched in the eggs and nauplii of *Calanus finmarchicus* (Sargent and Falk-Petersen 1988). Kiørboe et al. (1985) also suggested that lipids were important for egg production, while protein was important for somatic growth of nauplii and copepodites.

Numerous egg-production studies of zooplankton have been conducted both in the laboratory and in the field. Egg-production studies usually have not considered either hatching success of the eggs produced (% viability), or survival of the offspring. The first stage after hatching (Naupliar Stage NI) is of particular interest, as it is a non-feeding stage (Landry 1983) in which the nauplii utilize maternally-provided energy reserves. Thus, the hatching success and survival through the initial naupliar stages may be more appropriate measures of the actual recruitment to the population (Ambler 1985; Ianora et al. 1992), and, hence, may be more important parameters in estimating population growth than is the number of eggs produced.

The specific objectives of the present study were to investigate the effects of food of different chemical composition on the reproductive success of copepods in the laboratory and to try to identify some of the chemical components affecting the reproductive success. This paper describes egg-production responses of *Acartia tonsa* Dana and *A. hudsonica* Pinhey in a series of experiments in which the copepods were fed on three monoalgal cultures at different stages of growth: the diatom *Thalassiosira weissflogii*, the flagellate *Rhodomonas lens* and the dinoflagellate *Prorocentrum minimum*. Phytoplankton were analyzed for total protein, total carbohydrate, specific fatty acids and carbon and nitrogen content. Although a detailed study of the special requirements for individual amino acids and vitamins would also be of interest (Conklin and Provasoli 1977; see also Dadd 1982 and Harrison 1990), this study focused on specific fatty acids. This choice was based on the results of the studies mentioned above. Specific attention was given to the 20:5, 22:6, ω3 and ω6 PUFAs in the diet.

Materials and methods

The experiments tested the effects of dissimilar food conditions on egg-production rates of the copepods *Acartia tonsa* Dana and *A. hudsonica* Pinhey. The expected relationship between egg-production rate and food concentration is a curvilinear function, known as the functional-response curve. In order to obtain a functional-response curve, the egg-production experiments were run at four food concentrations, and the egg-production rates were compared to the chemical composition of the food. Before each experiment, the copepods were acclimated for 48 h to their food (algal type and condition) so that measured egg-production rates would reflect ingestion under experimental food conditions.

Phytoplankton cultures

Phytoplankton were grown in batch cultures using *f*/2 media (Guillard 1975) at 16 °C under fluorescent light (10 h light:10 h dark cycle). The silica in the diatom media was neutralized with HCl, which also minimizes precipitation of silica hydroxide (M. J. Perry personal communication). As food for each egg-production experiment, three algal cultures were grown: two to feed the copepods on the two acclimation days and an additional one for the experimental day. The cultures were started at three different initial concentrations to ensure that each culture was at similar growth stage on the day it was used (see Jónasdóttir 1992).

When the experiment was started, an aliquot of the algal culture to be used as food was filtered onto combusted GF/C filters for chemical analyses. Duplicates were filtered for each of the chemical components to be analyzed. Filters were folded and put into 2 ml cryogenic vials and frozen at –90 °C immediately after filtration. Samples for carbon and nitrogen content were filtered through combusted GF/F filters which were folded and wrapped in combusted foil and dried at 60 °C. An aliquot of the batch culture was fixed with Lugol's solution for cell counting. Culture densities were determined using a 1 ml Sedgewick rafter counting cell and 10× magnification on a compound microscope. Between 100 and 300 cells from a known volume were counted to estimate the cell density. Daily growth rates were calculated from these counts.

Three phytoplankton species were used in the different experiments: the diatom *Thalassiosira weissflogii* (cell diam ≃14 μm; volume estimated at 1500 μm^3), the dinoflagellate *Prorocentrum minimum* (cell diam ≃20 μm; estimated volume 3500 μm^3), and the flagellate *Rhodomonas lens* (cell diam ≃9 μm; estimated volume 226 μm^3).

Chemical analysis of food

Proteins were determined using bicinchoninic acid (BCA) protein assay reagent (Pierce) (Smith et al. 1985). Protein was solubilized from the cells on the filters using 1% sodium dodecyl sulfate (lauryl) detergent prior to analysis. The light extinction of the protein solution was quantified on a spectrophotometer at a wavelength of 562 nm. Carbohydrates (CHO) were measured by the 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) assay described by Burney and Sieburth (1977), Johnson and Sieburth (1977), and Johnson et al. (1981). The light extinction of the carbohydrate solution was measured at 635 nm on a spectrophotometer. Carbon and nitrogen content of the samples were measured on a Perkin Elmer 240 B elemental analyzer.

Fatty acids were analyzed by gas chromatography on a capillary column following standardized methods of fatty acid analysis as described by Peltzer et al. (1984) and Wakeham and Canuel (1990). Lipids were extracted by ultrasound sonication into CH_2Cl_2-methanol (2:1, v/v) and the fatty acids trans-methylated with BF_3-methanol to form fatty acid methyl esters (FAME). The FAME fraction was purified and separated from the other lipid compound classes on a short, fully activated silica-gel column (Peltzer et al. 1984). The FAME sample in hexane and with a known amount of C_{19} fatty acid as an internal standard, was injected into a gas chromatograph (Carlo Erba 2150) using hydrogen as a carrier gas at 0.8 kg cm^{-2}. The temperature program was increased from 100 to 230 °C in 3 min, and then remained isothermal at 230 °C for 15 min. Peaks from chromatograms were compared to SUPELCO FAME I and II standards for specific fatty acid identification, and the integrated peaks of each fatty acid compared to the peak area of the C_{19} internal standard.

As phytoplankton cultures were not axenic, the chemical measurements reflect both phytoplankton and bacterial composition. A detailed description of each of the chemical analyses is outlined in Jónasdóttir (1992).

Copepod rearing

Copepods for the experiments were obtained in 1991 by towing a 210 μm mesh-size plankton net in Long Island Sound (LIS), New

Table 1 Phytoplankton species and batch-culture conditions used in egg-production experiments with *Acartia tonsa* and *A. hudsonica* (*EE* early exponential; *ME* mid-exponential; *LE* late exponential; *S* senescent; *Tw Thalassiosira weissflogii*; *Rl Rhodomonas lens*; *Pm Prorocentrum minimum*; *h A. hudsonica*; *t A. tonsa*)

Experiment No.	Algal type	Batch culture conc (cells ml^{-1})	Divisions d^{-1}	Culture stage	Code
A. tonsa					
1	*T. weissflogii*	25000	0.58	EE	*Tw*EE-*t*
2	*T. weissflogii*	100000	0.12	ME	*Tw*ME-*t*
3	*T. weissflogii*	180000	0.06	LE	*Tw*LE-*t*
4	*T. weissflogii*	300000	0.07	S	*Tw*S-*t*
5	*R. lens*	40000	0.84	EE	*Rl*EE-*t*
6	*R. lens*	200000	0.55	LE	*Rl*LE-*t*
7	*R. lens*	300000	0.56	S	*Rl*S-*t*
8	*P. minimum*	9000	0.86	EE	*Pm*EE-*t*
A. hudsonica					
9	*P. minimum*	70000	0.33	LE	*Pm*LE-*h*
10	*T. weissflogii*	40000	0.58	EE	*Tw*EE-*h*
11	*T. weissflogii*	300000	0.05	S	*Tw*S-*h*
12	*R. lens*	50000	1.08	EE	*Rl*EE-*h*
13	*R. lens*	400000	0.14	S	*Rl*S-*h*

York (40° 50′N; 73° 07′W). They were reared under controlled conditions in batch culture in the laboratory. The zooplankton species used were the copepods *Acartia tonsa*, found in LIS during the summer and fall, and *A. hudsonica*, which is abundant from early fall through winter and spring. Experiments with *A. tonsa* were run in July through September, and with *A. hudsonica* in April to May with one experiment (No. 9) in September. The copepod cultures were kept at 16 °C in dim light on a 14 h light:10 h dark cycle. Actively swimming individuals (predominately females, but also males and Copepodite Stage V) were picked from fresh field samples. Approximately 40 copepods were placed in each of several 2-liter beakers containing GF/C filtered 27‰ seawater from LIS. The water was lightly aerated. The copepods were fed excess food consisting of a mixture of three exponentially growing algal species (*Thalassiosira weissflogii*, *Rhodomonas lens* and *Prorocentrum minimum*) twice a week. Three quarters of the water was replaced every 7 to 10 d and eggs and nauplii were separated from the copepodites and adults. Removing females for experiments, additionally diluted the cultures and allowed the nauplii to grow without grazing pressure from adults.

Experiments

Egg production

Actively swimming females were chosen from the zooplankton batch cultures for the experiments. Four females were placed in a Plexiglas container which had a 210 µm screen on the lower end and was hung inside a 1-liter beaker filled to a total of 800 ml with filtered seawater and the experimental food. This resulted in ≈200 ml of water inside each container. The 210 µm screen was coarse enough to prevent cannibalism of the eggs spawned by the females by allowing the eggs to fall easily through the screen while keeping the copepods inside the container. Incubations were conducted at 16 °C in dim light on a 14 h light : 10 h dark cycle.

A series of experiments was run to determine the effect of the growth stage of algal monocultures and the species of phytoplankton on the egg production of copepods (Table 1). Experiments 1 to 8 measured egg-production rates of *Acartia tonsa* and Experiments 9 to 13 the rates of *A. hudsonica*. The algal culture used was diluted into four different food concentrations (in triplicate) with the exception of Experiments 12 and 13, in which the cultures were diluted into five algal concentrations (in duplicate). These concentrations ranged from extreme food limitations to excess food in order to examine the egg-production response at both stressed-food and excess conditions. The concentrations were based on algal-cell volume to facilitate comparison among the three algal species. The cell-volume concentrations used were 7.5×10^5, 15×10^5, 30×10^5, and 60×10^5 $\mu m^3 ml^{-1}$ in Experiments 1 to 11 and 4.5×10^5, 9×10^5, 15×10^5, 30×10^5, and 60×10^5 $\mu m^3 ml^{-1}$ in Experiments 12 and 13. (For comparison, 7.5×10^5 $\mu m^3 ml^{-1}$ equals ≈500, 3300, and 215 cells ml^{-1} for *Thalassiosira weissflogii*, *Rhodomonas lens*, and *Prorocentrum minimum*, respectively.)

Copepods were acclimated at the experimental conditions (food type and concentration) for 48 h prior to the experiment. For the first acclimation day, food suspension were made from the first phytoplankton culture in the three-culture series described in the preceding section. The food suspensions on the second acclimation day were made from Culture 2. Females were carefully transferred to the fresh food suspension with the wide end of a Pasteur pipette, and the old food suspension and eggs were discarded. The experiment started on Day 3 with food suspension from Culture 3. Copepods from each beaker were transferred from their containers into small dishes 24 h later, and prosome length was measured. The copepods were dipped into distilled water to remove salt, placed in small tared aluminum cups, and dried at 60 °C for later dry-weight measurements. The contents of each beaker were carefully poured through a 20 µm-mesh screen and the eggs were rinsed into a counting chamber and counted. If mortality over the 3 d of an experiment exceeded one individual in a beaker, the data from that beaker was excluded.

Egg-development time, hatching success and naupliar survival

Concurrent with the egg-production incubations, another set of experiments was set up to measure hatching success of eggs. All incubations were conducted at 16 °C. Twenty-five females were placed in a 2-liter beaker and acclimated for 48 h using the same procedure as described in the preceding section, at an algal concentration of 22.5×10^5 $\mu m^3 ml^{-1}$. This concentration is close to the critical food concentration. After acclimation, females were carefully transferred to the experimental food conditions for an additional 24 h, after which time the eggs were removed and 20 to 30 were placed together in each of several 15 ml wells (tissue-culture plates) filled with filtered seawater. Hatching was checked every 24 h for 3 to 4 d or until no egg had hatched during the last 24 h period. After the eggs had hatched, 18 to 38 nauplii were selected for naupliar survival measurements. Each nauplius was placed in a 2 ml well in a glass culture plate containing filtered seawater, and survival was checked every 24 h for 2 to 3 d.

Due to an overestimation of the development times of eggs for some of the experiments described in the preceding paragraph, the series of development times was incomplete. Therefore, a separate set of experiments to determine the development time of eggs was carried out for *Acartia tonsa* females only; these were fed on *Thalassiosira weissflogii* and *Rhodomonas lens* (Experiments D1 to D6 in Table 2). Three different algal growth stages were used in this series of experiments. Three paired experiments using both food types

Table 2 *Acartia tonsa*. Experiments to determine development times. Culture stages of *Thalassiosira weissflogii* and *Rhodomonas lens* as in Table 1

Experiment No.	Algal type	Batch culture conc (cells ml^{-1})	Divisions d^{-1}	Culture stage
D1	*T. weissflogii*	27000	0.50	EE
D2	*T. weissflogii*	70000	0.45	ME
D3	*T. weissflogii*	160000	0.07	LE
D4	*R. lens*	30000	0.39	EE
D5	*R. lens*	175000	0.30	ME
D6	*R. lens*	440000	0.01	S

were carried out simultaneously (Experiments D1 and D4; D2 and D5; D3 and D6: Table 2). The same 50 females (25 for each food type) were used in all these experiments, which were run for a total of 9 d: after each paired experiment, the females were mixed and split randomly again for the next set. The experiments were carried out in a manner similar to that used for the hatching experiment, and the eggs were incubated at 16 °C. After the 72 h feeding period, the females were carefully removed, all eggs were sieved from the water with a 20 µm-mesh screen, and the females were again placed in the experimental beakers. After 4 h they were removed and any newly spawned eggs were sieved out carefully with a 20 µm screen. Twenty to 30 eggs were placed in each of several 15 ml wells as described in the preceding paragraph. Time zero (T_0) was set at 2 h after the initiation of the experiment, i.e., females were removed at T_2. Eggs were checked for hatching at T_{16} and every 4 h thereafter until no more eggs had hatched for ≥12 h. The average development time was calculated as $\Sigma(h \cdot T)/\Sigma h$, where h is the number of eggs hatched at Time T.

The sizes of eggs and nauplii were measured in Experiments D1 to D6. At T_2, 6 to 10 eggs (0 to 4 h-old) were fixed in formalin for size determinations. At least 10 nauplii per experiment were preserved in formalin 0 to 4 h after hatching (Naupliar Stage NI). Separate experiments on the effect of formalin preservation on egg sizes indicated no significant differences (one-way ANOVA, P=0.82) in sizes of *Acartia tonsa* eggs before and after 1 wk of fixation. Size measurements were made by videotaping the image of the eggs and nauplii through a compound microscope. The outlines of the images on the screen were traced on acetate. A micrometer ruler was videotaped and traced in the same manner and used to calibrate the size measurements. Measurements were made using a digitizing pad (Summagraphics MacTablet), and calculations were performed using the NIH (National Institute of Health) image-analysis software on a Macintosh II computer. Length was measured twice for each nauplius and the mean of the two measurements was used for calculations. Three cross-sections were generally measured for each egg. Egg volume was calculated, assuming a spherical shape, as: $4/3\pi r_1 r_2 r_3$, where r_1, r_2, and r_3 are the radii of the eggs (one-half of each of the three cross-sections measured). When only two cross-sections were measured, the equation used was $4/3\pi r_1 r_2^2$, where r_2 is the shorter radius of the two cross-sections measured (Allan 1984).

Results

Egg production

Egg production of both *Acartia tonsa* and *A. hudsonica* followed the expected functional response pattern (Fig. 1). That is, egg-production rate increased curvilinearly with increasing food concentration up to a maximum level (E_{max}) and stayed constant with further increases in food concentrations. As the copepods do not produce eggs at zero food concentration, a line was drawn from zero to the E_r measured at the lowest food concentration to demonstrate the functional response curve. This assumes no threshold concentrations of food for egg production, but this study did not test E_r at a low enough concentration to confirm that this was actually the case.

A comparison of the egg-production rates was conducted using a Model 1 three-way general linear model (GLM) ANOVA, Type III sums-of-squares (SAS) which permits missing cells in the ANOVA design. The effects of E_r of the phytoplankton species, age of the batch culture and the four experimental food concentrations were determined as well as the interactions among all these variables.

The egg-production rates of both copepod species were observed to be a function of both food concentration ($P<0.001$) and algal type ($P<0.0001$). The E_r increased with increasing food concentration until a critical concentration was reached. The E_r of *Acartia tonsa* females (the test pools the means of all algal stages) was highest when they were fed *Rhodomonas lens*; lower but similar values were recorded for females fed *Thalassiosira weissflogii* and *Prorocentrum minimum*. However, the interaction of algal species and culture age was highly significant for the E_r of *A. tonsa* ($P<0.0003$), while the interaction between algal age and food concentration was non-significant. These results indicate that while the age of the culture may affect E_r, the nature of such age effect is dependent on the algal species used as food. As just one culture age was tested for *P. minimum*, effect of culture age could be tested for *R. lens* and *T. weissflogii* only.

Similarly, E_r was higher for *Acartia hudsonica* fed *Rhodomonas lens*, than when this species was fed *Thalassiosira weissflogii* or *Prorocentrum minimum*. The age of the cultures did not affect egg-production rates of *A. hudsonica* when data from all three algal species were combined. However, a combined effect of culture age and food concentration was significant on the E_r of *A. hudsonica* ($P<0.05$). That is, for this copepod species, the effect of culture age on E_r was greater at high than at low food concentrations.

In order to further test the significance of the observed differences in E_r when *Acartia* spp. was fed different-aged phytoplankton cultures of the same species, a Student's t-test was applied to the maximum egg-production rates measured at the highest food concentration used in the experiments, 60×10^5 µm^3 ml^{-1}. The test showed that for both copepod species fed *Thalassiosira weissflogii* (Fig. 1 a, d), the youngest algal culture yield the highest E_{max}, the E_{max} then decreased with increasing culture age. A significant difference (at $P<0.05$) was found between the mean E_{max} of *A. tonsa* females fed the youngest and the two oldest *T. weissflogii* cultures (*Tw*EE-*t* vs *Tw*LE-*t* and *Tw*S-*t*). The difference between the culture at mid-exponential growth and the oldest culture was also significant (*Tw*ME-*t* vs *Tw*S-*t*). There was no significant differences in the E_{max} of *A. hudsonica* fed the two different culture stages of *T. weissflogii*.

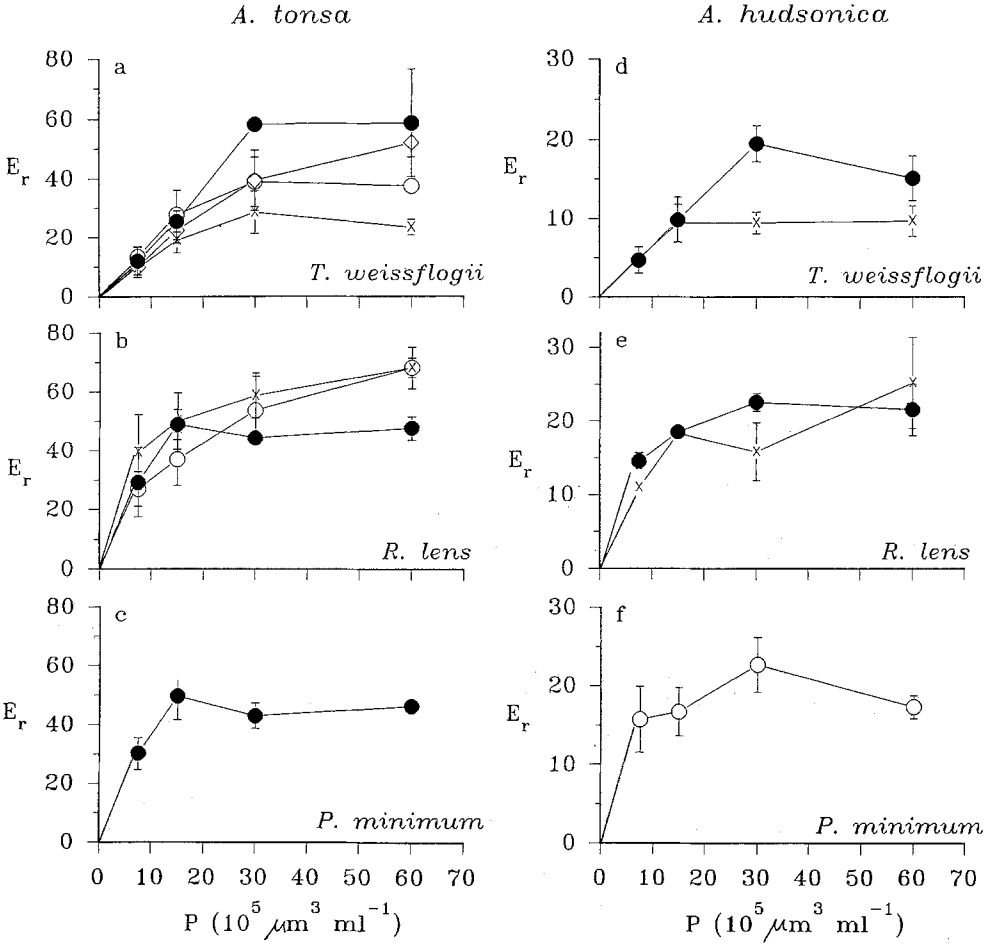

Fig. 1 *Acartia tonsa* and *A. hudsonica*. Egg-production rates (E_r, eggs female^{-1} d^{-1}) expressed as means ±1 SE, as a function of volume-based food concentration (P, µm^3 ml^{-1}) when different-aged cultures of *Thalassiosira weissflogii*, *Rhodomonas lens* and *Prorocentrum minimum* were fed to females (Growth stages of phytoplankton cultures = ● early exponential, EE; ◇ mid-exponential, ME; ○ late exponential, LE; × senescent, S)

Table 3 Chemical composition (pg) of phytoplankton cultures used in experiments, standardized to cell volume of 1000 µm^3 (*C* carbon; *N* nitrogen; *CHO* carbohydrate; experiments numbered and coded as in Table 1). Where values have no standard error, only one sample could be analyzed

Experiment no. and code	C	N	C:N	Protein	CHO
1 *Tw*EE-*t*	66.99±5.51	13.48±0.95	4.97	47.69±9.4	0.82±0.2
2 *Tw*ME-*t*	74.46±0.72	14.11±1.74	5.28	65.80±1.2	24.69±0.5
3 *Tw*LE-*t*	82.66±0.44	13.16±0.64	6.28	54.06±2.1	32.81
4 *Tw*S-*t*	68.56±0.69	11.55±0.77	5.94	48.39±14.6	18.52±1.8
5 *Rl*EE-*t*	224.06±5.60	54.41±6.42	4.12	228.03±0.8	14.28±1.7
6 *Rl*LE-*t*	192.47±0.51	59.21±1.61	3.25	261.53±13.2	16.67±0.0
7 *Rl*S-*t*	231.41±5.02	55.02±6.75	4.20	232.75±9.5	13.31
8 *Pm*EE-*t*	72.48±9.80	12.19±0.70	5.62	372.50	4.88
9 *Pm*LE-*h*	55.04±1.47	9.32±0.23	5.91	42.91±3.4	16.36±0.2
10 *Tw*EE-*h*	58.11±1.32	10.95±0.35	5.29	48.86±7.4	9.97±0.5
11 *Tw*S-*h*	53.84±0.39	7.58±0.11	7.11	19.85±0.8	12.77±0.8
12 *Rl*EE-*h*	203.35±2.58	46.61±0.91	4.37	257.59±12.6	17.45±6.4
13 *Rl*S-*h*	234.40±2.79	54.26±0.38	4.31	233.70±22.7	33.29±2.8

This trend of lower E_{max} with increasing culture age was not observed for the egg production of either copepod species fed the flagellate *Rhodomonas lens* (Fig. 1 b, e). For *Acartia tonsa*, egg-production rates with the early-exponential stage *R. lens* culture were significantly lower than those with the two older cultures (*Rl*EE vs *Rl*LE and *Rl*S). No difference in E_{max} was observed for *A. hudsonica* fed *R. lens* of different ages. Only one experiment was carried out for each *Acartia* species using the alga *Prorocentrum minimum* (Fig. 1 c, f).

Chemical composition of phytoplankton cultures

To facilitate comparison of chemical composition among cells of different sizes, algal cells were normalized to a cell volume of 1000 µm^3 (Table 3). The carbon and nitrogen content of *Rhodomonas lens*, as a function of cell volume, was ≈3 and 4 times higher than that of *Thalassiosira weissflogii* and *Prorocentrum minimum*. Nitrogen concentration decreased slightly with age in cultures of both *T. weissflogii* and *P. minimum*, but not in the *R. lens* cultures. Protein

Table 4 Fatty acid composition (pg) of phytoplankton in Experiments 1 to 13 standardized to cell volume of 1000 μm^3 (*PUFA* polyunsaturated fatty acids; *MUFA* monounsaturated fatty acids; *SAFA* saturated fatty acids; experiments numbered and coded as in Table 1)

Fatty acids	Experiment No.												
	1 *Tw*EE	2 *Tw*ME	3 *Tw*LE	4 *Tw*S	5 *Rl*EE	6 *Rl*LE	7 *Rl*S	8 *Pm*EE	9 *Pm*LE	10 *Tw*EE	11 *Tw*S	12 *Rl*EE	13 *Rl*S
14:0	–	–	–	–	–	–	–	–	–	0.23	0.20	0.90	0.41
15:0	0.03	0.07	0.04	0.08	0.02					0.04	0.03	0.03	
iso 16:0	0.02	0.03	0.02	0.04	0.03	0.04	0.04			0.02		0.05	0.05
16:0	0.43	0.86	0.51	0.85	0.68	0.58	0.55	0.69	0.60	0.49	0.46	2.04	0.87
16:1					0.10	0.05	0.04					0.28	0.10
16:1ω7	0.43	0.98	0.59	1.15	0.12	0.06	0.05	0.07	0.05	0.51	0.47	0.16	0.05
16:1	0.09	0.16	0.07	0.17	0.23	0.22	0.27		0.03	0.08	0.06	0.45	0.54
16:2	0.05	0.07	0.03	0.10						0.05	0.02		
16:3	0.24	0.57	0.26	0.57						0.25	0.20		
16:4	1.03	1.60	0.74	1.94						0.97	0.55		
16:5?	0.02	0.04	0.02	0.03	0.06	0.06	0.06		0.01			0.04	0.08
18:0	0.02		0.01	0.01	0.08	0.08	0.07	0.05	0.03	0.07	0.01	0.22	0.13
18:1ω7	0.02	0.03	0.04	0.05	0.11	0.09	0.06	0.11	0.08	0.10	0.02	0.67	0.05
18:1ω9	0.07	0.04	0.02	0.04	0.61	0.54	0.50	0.09	0.04	0.05	0.02	1.19	0.38
18:2ω6	0.04	0.11	0.05	0.14	0.06	0.07	0.04	0.07	0.07	0.06	0.05	0.23	0.03
18:3ω6	0.02	0.03	0.02	0.04									
unknown		0.02				0.03							
18:3ω3	0.02	0.03	0.03	0.10	1.18	1.51	1.36	0.07	0.07	0.03	0.01	2.80	1.64
18:4ω3	0.08	0.24	0.10	0.12	3.42	3.37	3.86	0.40	0.42	0.17	0.11	6.36	3.76
18:5ω3								1.92	1.93				
20:1								0.08	0.05			0.04	
20:2								0.05	0.04				
20:4ω6	0.02	0.04	0.01	0.04				0.03	0.01				
20:5ω6					0.17	0.25	0.28					0.29	0.65
20:5ω3	0.86	1.78	0.81	1.89	1.38	1.87	1.84	0.08	0.12	1.19	0.78	2.80	2.53
22:1													
22:4					0.05		0.06						
22:4ω6													
22:5					0.02		0.02						
22:6ω6					0.04			0.02				0.04	0.01
22:6ω3	0.24	0.42	0.17	0.48	0.81	1.27	1.01	0.80	0.84	0.29	0.16	1.49	1.35
Total	3.74	7.14	3.53	7.82	9.16	10.07	10.10	4.53	4.41	4.61	3.21	20.08	12.63
PUFA	2.61	4.93	2.22	5.41	7.14	8.36	8.49	3.44	3.51	3.02	1.92	14.02	9.97
MUFA	0.61	1.21	0.71	1.40	1.16	0.96	0.91	0.34	0.26	0.73	0.57	2.79	1.12
SAFA	0.50	0.97	0.58	0.98	0.80	0.70	0.65	0.75	0.63	0.85	0.71	3.24	1.46
ω3	1.21	2.49	1.11	2.58	6.86	8.04	8.10	3.29	3.39	1.68	1.08	13.50	9.30
ω6	0.08	0.18	0.07	0.22	0.23	0.32	0.32	0.10	0.09	0.07	0.07	0.52	0.68
ω3:ω6	15.12	13.83	15.86	11.73	29.82	25.12	25.31	30.90	38.96	24.00	15.43	25.96	13.68
20+18:22	3.55	4.23	4.62	3.95	1.63	1.47	1.81	2.46	2.46	4.08	4.86	1.82	1.86

concentrations were ≈4 times higher in *R. lens* and *P. minimum* (EE) than in *T. weissflogii* cultures, but did not vary greatly with culture age in any of the three species. Carbohydrate concentrations were higher in *T. weissflogii* than in *R. lens* and *P. minimum*, and appeared to be higher in old cultures in all three species.

Based on volume, the total fatty acid concentration of *Thalassiosira weissflogii* and *Prorocentrum minimum* was similar; these species contained about one-third less fatty acids per unit cell volume than did *Rhodomonas lens* (Table 4). The major fatty acid groups (PUFA, monounsaturated fatty acids; MUFA, and saturated fatty acids; SAFA) of the three phytoplankton species differed slightly (Fig. 2). *T. weissflogii* had relatively lower PUFA levels but higher MUFA levels than did either *R. lens* or *P. minimum*. No distinct differences in the amount of SAFA were observed among the phytoplankton species. The three phytoplankton species also differed in specific fatty acid composition. *T. weissflogii* was characterized by the C_{16} series fatty acids and 20:5ω3 (Fig. 3 a, Table 4), whereas the C_{18} series of fatty acids were representative of the flagellates (Fig. 3 b, Table 4). *T. weissflogii* had higher levels of the 20:5ω3 fatty acid than did the flagellates, while the flagellates had greater percentages of the 22:6ω3 fatty acid (Fig. 3 c). *R. lens* and *P. minimum* had a similar fatty acid composition and contained approximately twice the amount of ω3 fatty acids as *T. weissflogii* (Fig. 3 d, Table 4). *P. minimum* had a high content of the 18:5ω3 fatty acid and a low concentration of the 20:5ω3 fatty acid. There were no obvious trends in percentage fatty acid composition as a function of culture age. The most obvious differences in fatty acid concentrations between stages were among the different *T. weissflogii* cultures, but some differences were also evident between the *R. lens* cultures in Experiments 12 and 13 (Table 4).

Effect of phytoplankton chemical composition on egg-production rates

The effects of chemical composition of food on egg-production rates will be described for an algal concentration of 15×10^5 $\mu m^3 ml^{-1}$ only. This concentration represents the critical food concentrations or one that is only slightly limiting for both *Acartia tonsa* and *A. hudsonica* (Fig. 1).

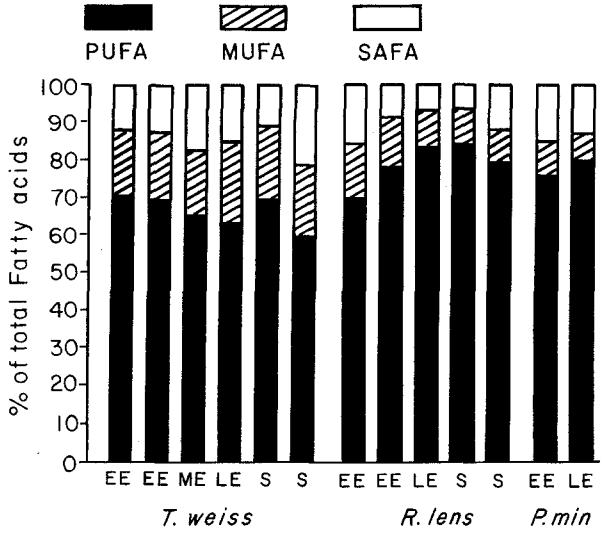

Fig. 2 *Thalassiosira weissflogii*, *Rhodomonas lens* and *Prorocentrum minimum*. Division of fatty acid composition into polyunsaturated (PUFA), monounsaturated (MUFA) and saturated (SAFA) fatty acids. Growth stages of cultures as in Fig. 1

Similar patterns of E_r responses to food composition were obtained at algal concentrations of 7.5 and 60×10^5 $\mu^3 ml^{-1}$ (reported in Jónasdóttir 1992). Results for the intermediate concentration of 30×10^5 $\mu m^3 ml^{-1}$ were subject to large variations and could not be used for comparative purposes. The correlations between the E_r of *Acartia tonsa* and *A. hudsonica* at 15×10^5 $\mu m^3 ml^{-1}$ and volume-based concentrations of each of the measured chemical components of the phytopankton cells (carbon, nitrogen, protein, carbohydrate and total and individual fatty acids) are shown in Table 5, which also gives the correlations between E_r and the C:N, ω3:ω6 and 20:22 fatty acid ratios of the phytoplankton cells.

Carbon, nitrogen and protein content of the cells all exhibited significant correlations with egg production of *Acartia tonsa* (Table 5). No correlation was observed between carbohydrate (CHO) and egg-production rate of *A. tonsa*, while the correlation was significant and positive for *A. hudsonica*.

Not all fatty acids could be considered in testing correlations with E_r, as some occurred in only one of the three phytoplankton species and the concentration range was thus limited to one species only. These fatty acids were 15:0, 16:2, 16:3, 16:4, 18:3ω6 and 20:4ω6 which were present only in *Thalassiosira weissflogii*; 20:5ω6, 22:4 and 22:5 which were only found in *Rhodomonas lens*; and 20:1 and 20:2 which were recorded only in *Prorocentrum minimum*.

Prorocentrum minimum is characterized by a high concentration of 18:5ω3, but not of 20:5ω3. The 18:5 fatty acid does not appear in many microalgae and its nutritional importance is not well known (Harvey et al. 1987; Fraser

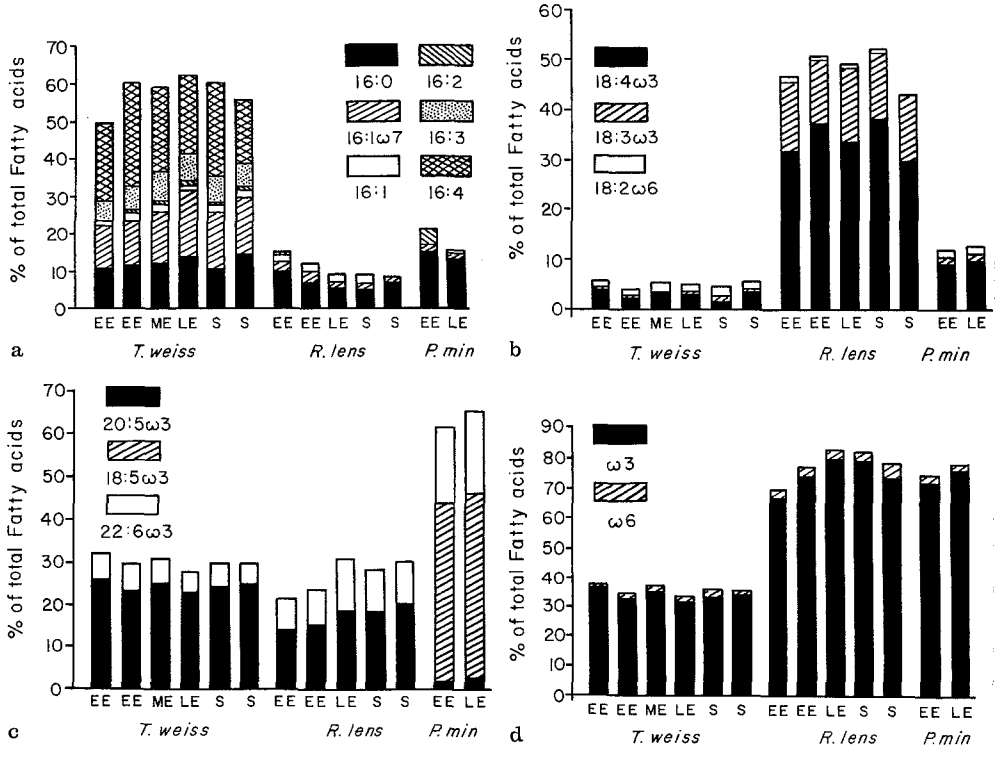

Fig. 3 *Thalassiosira weissflogii*, *Rhodomonas lens* and *Prorocentrum minimum*. Contribution of **a** C_{16}, **b** C_{18}; **c** 20:5ω3, 18:5ω3, 22:6ω3 and **d** ω3 and ω6 fatty acids. Growth stages of cultures as in Fig. 1

Table 5 *Acartia tonsa* and *A. hudsonica*. Summary of correlations of egg-production rates with various chemical components of cells. Null hypothesis tested (H_0; $\beta=0$). Significance levels of the slopes = NS: $P>0.05$; *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$

Component	*Acartia tonsa* (15×10^5 µm^3 ml^{-1}; $df=1,21$)					*Acartia hudsonica* (15×10^5 µm^3 ml^{-1}; $df=1,22$)				
	intercept	slope (±SE)	R^2	F	P	intercept	slope (±SE)	R^2	F	P
Carbon	19.79	0.12 (0.04)	0.291	9.035	**	9.86	0.04 (0.02)	0.313	5.019	*
Nitrogen	24.43	0.36 (0.14)	0.240	6.949	*	10.57	0.15 (0.07)	0.317	5.109	*
Protein	19.50	0.09 (0.02)	0.479	20.203	***	10.76	0.03 (0.01)	0.341	5.686	*
CHO	43.52	-0.53 (0.33)	0.101	2.475	NS	7.39	0.39 (0.17)	0.313	5.011	*
Total FA	23.43	1.66 (1.25)	0.074	1.772	NS	10.20	0.48 (0.22)	0.306	4.853	*
PUFA	22.38	2.40 (1.33)	0.128	3.227	NS	9.96	0.71 (0.29)	0.356	6.084	*
ω3	22.23	3.05 (1.01)	0.293	9.129	**	10.36	0.73 (0.27)	0.402	7.387	*
ω6	26.95	42.82 (34.31)	0.066	1.558	NS	11.08	12.16 (5.28)	0.345	5.793	*
16:0	45.44	-16.85 (23.17)	0.022	0.505	NS	10.04	4.84 (2.51)	0.255	3.758	NS
16:1ω7	46.18	-26.87 (6.25)	0.456	18.450	***	19.02	-18.72 (5.14)	0.547	13.291	**
18:0	20.84	343.87 (82.38)	0.442	17.425	***	11.14	35.67 (19.30)	0.237	3.416	NS
18:1ω7	15.36	301.81 (83.25)	0.374	13.143	**	12.56	8.95 (6.64)	0.141	1.812	NS
18:1ω9	26.81	34.64 (11.58)	0.289	8.949	**	12.32	6.14 (3.36)	0.232	3.334	NS
18:2ω6	48.23	-193.52 (97.04)	0.153	3.977	NS	11.71	27.67 (23.16)	0.116	1.440	NS
18:3ω3	28.67	11.94 (4.61)	0.233	6.714	*	12.02	2.74 (1.24)	0.305	4.820	NS
18:4ω3	27.53	5.22 (1.71)	0.296	9.253	**	11.79	1.27 (0.57)	0.320	5.182	*
20:5+18:5	18.37	10.18 (6.37)	0.104	2.548	NS	6.13	4.14 (1.13)	0.548	13.321	**
22:6	19.51	23.67 (7.71)	0.300	9.478	**	8.42	7.55 (2.03)	0.557	13.825	**
C:N	61.23	-5.23 (3.16)	0.111	2.744	NS	28.88	-2.68 (1.31)	0.276	4.203	NS
ω3:ω6	2.15	1.55 (0.30)	0.555	27.426	***	10.30	0.15 (0.16)	0.071	0.846	NS
(20+18):22	63.30	-9.25 (2.34)	0.415	15.617	***	26.01	-3.66 (0.94)	0.578	15.080	**

Table 6 *Acartia tonsa* and *A. hudsonica*. Development times and hatching success of eggs, naupliar (*NI*) survival, egg sizes and naupliar size. Values are means ±SE (n number of measurements; – not measured). Experiments numbered and coded as in Tables 1 and 2

Experiment	Development time (h)	Hatching success (%)	NI survival (%)	Egg size (10^5 µm^3)	Naupliar size (µm)
1 *Tw*EE-*t*	–	96.97 ($n=165$)	77.78 ($n=18$)	–	–
2 *Tw*ME-*t*	–	73.72 ($n=137$)	100 ($n=15$)	–	–
3 *Tw*LE-*t*	–	49.69 ($n=161$)	100 ($n=18$)	–	–
4 *Tw*S-*t*	–	39.85 ($n=138$)	100 ($n=18$)	–	–
5 *Rl*EE-*t*	–	94.84 ($n=175$)	66.67 ($n=18$)	–	–
6 *Rl*LE-*t*	–	50.86 ($n=125$)	100 ($n=18$)	–	–
7 *RlS-t*	–	100.0 ($n=204$)	100 ($n=36$)	–	–
8 *Pm*EE-*t*	–	94.72 ($n=284$)	89.47 ($n=19$)	–	–
9 *Pm*LE-*h*	–	89.17 ($n=120$)	100 ($n=20$)	–	–
10 *Tw*EE-*h*	–	6.82 ($n=132$)	71.43 ($n=7$)	–	–
11 *TwS-h*	–	14.81 ($n=81$)	66.67 ($n=12$)	–	–
12 *Rl*EE-*h*	–	34.00 ($n=50$)	82.33 ($n=17$)	–	–
13 *RlS-h*	–	21.05 ($n=114$)	66.67 ($n=18$)	–	–
D1 *Tw*EE-*t*	30.75±0.63 ($n=16$)	93.69 ($n=111$)	–	2.98±1.21	107.74±0.958
D2 *Tw*ME-*t*	32.38±0.55 ($n=21$)	62.61 ($n=115$)	–	–	107.34±0.958
D3 *Tw*LE-*t*	34.80±0.69 ($n=13$)	49.04 ($n=104$)	–	3.07±0.09	111.60±1.118
D4 *Rl*EE-*t*	31.43±0.42 ($n=35$)	74.74 ($n=95$)	–	2.89±0.12	108.64±0.958
D5 *Rl*ME-*t*	31.72±0.46 ($n=29$)	60.00 ($n=110$)	–	2.74±0.07	106.47±0.991
D6 *RlS-t*	29.20±0.79 ($n=10$)	69.15 ($n=94$)	–	3.15±0.10	109.55±1.514

et al. 1989; Bradshaw et al. 1990). These investigators demonstrated that when the fatty acid 18:5 is present in the crustacean diet, it is assimilated but does not appear as 18:5 in the crustacean itself. This indicates an ability in crustaceans to elongate the 18:5 fatty acid to 20:5. Elongation of the fatty acid 18:3ω3 to 20:5ω3 by crustaceans has been reported by several authors (Jones et al. 1979; see also Castell 1982). Therefore, the present study has pooled the 18:5 fatty acid with the 20:5 fatty acid in the case of *P. minimum*. Neither *Thalassiosira weissflogii* nor *Rhodomonas lens* contained the 18:5 fatty acid.

Concentrations of the fatty acids 16:1ω7 and 18:0 were correlated ($P<0.001$) with the E_r of *Acartia tonsa* (16:1ω7 was negatively correlated) as were 18:1ω9, 18:1ω7, 18:4ω3, 22:6ω3 and ω3 ($P<0.01$; Table 5). The egg-production rates of *A. hudsonica* were significantly correlated ($P<0.01$) with 16:1ω7 (negatively), and with 20:5ω3 and 22:6ω3 (positively; Table 5).

The C:N ratio and the fatty acid ratios 20:22 and ω3:ω6 have been shown to be of potential importance for reproduction (Castell 1982; Harrison 1990; Støttrup and Jensen 1990; Ahlgren et al. 1992). In neither species did egg-pro-

duction rates correlate with the C:N ratio of the diet. The ω3:ω6 ratio yielded a highly positive correlation (nonsignificant for *Acartia hudsonica*), and the 20:22 ratio a highly significant negative correlation for both species.

Egg development time, hatching success and naupliar survival

Differences in the development times and hatching success of the eggs were apparent among the different experiments (Table 6). A Model I, two-way nested ANOVA showed that microalgal species (*Thalassiosira weissflogii* and *Rhodomonas lens*: pooled culture-ages) did not significantly affect the development times of *Acartia tonsa* eggs. However, there were highly significant differences between the development times of eggs of copepod fed algae of different age ($F=5.02$, $df=5,141$, $P<0.001$). An a posteriori Tukey honest significant-difference (HSD) test revealed that eggs from females fed the youngest (early exponential, EE) *T. weissflogii* cells developed significantly faster than eggs from females fed older (late-exponential, LE) cells. However, eggs of females fed the LE *T. weissflogii* culture developed significantly slower than eggs from females fed *R. lens* cultures of any age. There were no significant differences between development times of eggs of females fed any of the *R. lens* culture stages.

A *G*-test for analysis of frequencies (Sokal and Rohlf 1981) was made using the LOGLIN statistical program (BIOM Statistical Program, SUNY, Stony Brook, New York) to test the effects of phytoplankton species and age on the hatching success of *Acartia tonsa* eggs. Due to the nature of the analysis, only data from the *Thalassiosira weissflogii* and *Rhodomonas lens* experiments (1 to 7) were used, since the *Prorocentrum minimum* experiment was carried out for only one age group. Results from Experiment D5 (*R*ME) were used to fill in the missing age group in the *R. lens* series. The analysis-of-frequency revealed a highly significant three-factor interaction between phytoplankton species, algal stage (age) and hatching success ($G=154.8$, $df=3$). In other words, the degree of association between algal species and hatching success differed as a function of algal stage. The hatching success of eggs from *A. tonsa* females fed *T. weissflogii* decreased with increasing age of the culture, but the age of *R. lens* cultures did not affect the hatching success.

The hatching success of *Acartia hudsonica* eggs was very low in most experiments (6 to 34% for Experiments 10 to 13), but was high (89%) in Experiment 9. Hatching success of *A. tonsa* eggs appeared to be a function of growth rate of the phytoplankton cultures used to feed the females (Fig. 4); it increased with increasing growth rate ($R^2=0.494$, $P=0.005$). However, when the hatching success of the eggs was correlated with the different chemical components of the cell, no significant correlations were evident for either *A. tonsa* or *A. hudsonica*.

Egg and naupliar sizes were similar in the six treatments (Experiments D1 to D6) for which they were measured (Table 6). The only significant difference in egg size was found between eggs spawned by females fed *Rhodomonas lens* at exponential growth stage (D5, which produced the smallest eggs) and senescent *R. lens* cells (D6, which produced the largest eggs) (Tukey HSD test, $\alpha=0.05$). There were no significant differences between the sizes of the nauplii in these experiments (Tukey HSD test, $\alpha=0.05$). There was a significant relationship between egg size and naupliar size (Fig. 5a; $R^2=0.60$) but no correlation was found between the development times and either the egg or naupliar size for these experiments (Fig. 5b, c).

Survival of NI nauplii to NII stage was always >65%. No correlation was observed between hatching success and naupliar survival or the chemical contents of food and naupliar survival.

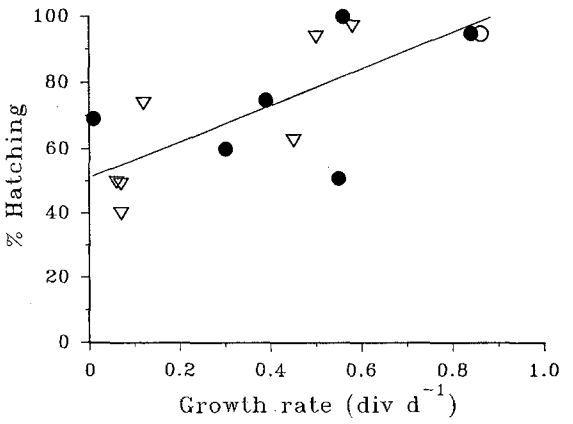

Fig. 4 *Acartia tonsa*. Hatching success of eggs as a function of growth of batch cultures used as food for females broadcasting eggs; $R^2=0.494$ (▽ *Thalassiosira weissflogii*; ● *Rhodomonas lens*; ○ *Prorocentrum minimum*)

Condition factors

The length and weight of the females, measured at the end of the experiments, were used to calculate the condition factor (which is an index of fitness, μg mm^{-3}; Durbin et al. 1983). Table 7 gives the means of the replicates in each experiment, while Fig. 6 plots all the replicates. Egg production and condition of females after the experiments were positively correlated for *Acartia tonsa* ($R^2=0.30$, $P<0.01$), but not for *A. hudsonica* (Fig. 6). No correlation was found with female length (unrelated to weight) and egg-production rate of either species (one-way ANOVA, *A. tonsa*: $df=1,22$, $F=2.62$, $P=0.119$; *A. hudsonica*: $df=1,11$, $F=0.00$, $P=0.99$).

Discussion and conclusions

The goal of the experiments was to determine the effect of chemical composition of food on the reproductive success of copepods and to identify some of the major chemical

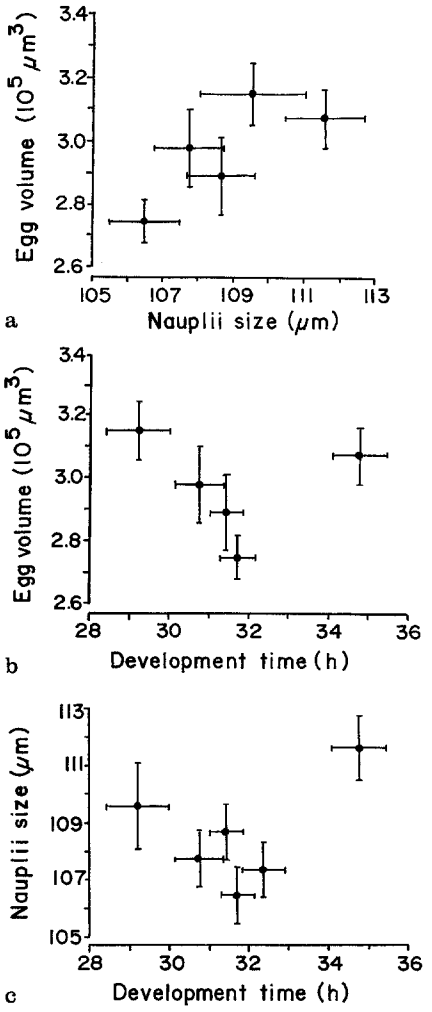

Fig. 5 *Acartia tonsa*. **a** Naupliar size as a function of egg volume (Experiments D1 to D6; $R^2=0.602$); **b** egg volume as a function of development time of eggs ($R^2=0.010$); **c** naupliar size as a function of development time of eggs ($R^2=0.158$). Bars represent ±1 SD of the mean in all cases

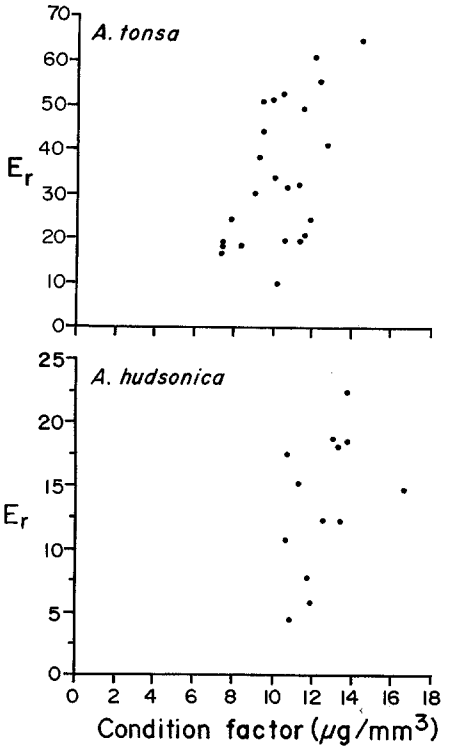

Fig. 6 *Acartia tonsa* and *A. hudsonica*. Egg-production rates (eggs female^{-1} d^{-1}) at algal concentration of 15×10^5 $\mu m^3 ml^{-1}$ as a function of condition of females (μg mm^{-3}). $R^2=0.300$ (**) and 0.184 (NS) for *A. tonsa* and *A. hudsonica*, respectively

Table 7 *Acartia tonsa* and *A. hudsonica*. Mean (±SD) prosome length, female dry weight and condition factor of females at end of experiments. Experiments numbered and coded as in Table 1

Experiment	Prosome length (mm)	Mean weight (μg)	Condition factor ($\mu g\,mm^{-3}$)
1 *Tw*EE-*t*	0.946±0.010	7.35±1.72	8.64±1.78
2 *Tw*ME-*t*	0.929±0.018	6.60±0.81	8.24±0.87
3 *Tw*LE-*t*	0.915±0.030	7.10±0.78	9.39±1.94
4 *Tw*S-*t*	0.896±0.044	8.07±1.02	11.22±0.88
5 *Rl*EE-*t*	0.931±0.029	8.46±2.27	10.34±1.72
6 *Rl*LE-*t*	0.926±0.020	9.25±0.87	11.65±1.03
7 *Rl*S-*t*	0.992±0.028	11.69±2.80	11.92±2.28
8 *Pm*EE-*t*	0.929±0.017	8.72±0.39	10.91±1.06
9 *Pm*LE-*h*	0.719±0.022	5.39±0.12	14.56±1.74
10 *Tw*EE-*h*	0.777±0.013	5.45±0.13	11.62±0.33
11 *Tw*S-*h*	0.810±0.039	5.99±0.61	11.31±1.05
12 *Rl*EE-*h*	0.816±0.026	7.34±0.86	13.48±0.29
13 *Rl*ES-*h*	0.858±0.009	7.48±1.25	11.83±1.59

components of the food which affect the reproductive success of two species of *Acartia*.

Egg production

Acartia tonsa is an opportunistic copepod that does not build up energy reserves but rather invests its entire metabolic production in egg production as soon as food conditions become favorable (Kiørboe et al. 1985). *A. tonsa* has been reported to spawn eggs just 9.5 h after ingestion of food (Tester and Turner 1990), and *A. hudsonica* to respond in less than 24 h to changes in food availability (at 4 °C, Durbin et al. 1992). These species rapidly decrease egg-production rates when food conditions deteriorate (Kiørboe et al. 1985; Durbin et al. 1992).

Production of eggs is energetically expensive. The female has to synthesize genetic material and oocytes and provide nutritionally adequate egg yolk. The embryo must obtain all its nutrition from the egg yolk within the egg. Sufficient nutrition is essential for the female to meet the increased energy expenditure of egg production.

The present results show that the concentration of individual chemical components (volume-based) in their food can greatly affect the egg-producing ability of *Acartia* species. The concentrations of the chemical components measured in the algae used as food in the present study lay within those recorded for the same or similar-sized diatoms and flagellates (protein: Hitchcock 1982; fatty acids:

Ackman et al. 1964, 1968; Volkman 1989; Thompson et al. 1990). However, the absolute concentration of CHO per cell recorded in the present study must be viewed with caution, since it was about two times lower than reported for similar-sized algae (Hitchock 1982; Houde and Roman 1987). As the MBTH method gave a good linearity with increasing concentration of standards, the relative difference in CHO content among algal cells can be used in correlation with egg-production rates.

Maximum egg-production rates differed significantly among most of the treatments (Fig. 1). Factors other than food quality which could have produced differences in egg-production rates (temperature, previous feeding, food concentration) were controlled or kept constant. Body length was measured and was found not to influence egg-production rates.

Uye (1981) observed that adaptation to different temperatures affected egg-production rates in *Acartia clausi*. In the present study this factor could only have affected Experiment 9, in which *A. hudsonica* was fed *Prorocentrum minimum*. The copepods for this experiment were collected in September in waters of ≈15 °C, whereas the other experiments with *A. hudsonica* used the winter population collected in April from waters of 9 to 10 °C. However, the E_r for this particular experiment was similar to that in the other *A. hudsonica* experiments. The temperatures chosen for the experiments were slightly high for *A. hudsonica* and slightly low for *A. tonsa*, based on the in situ temperatures in Long Island Sound. However, several preliminary experiments at 20 °C had proved unsuccessful due to high mortality of females, whereas survival was always close to 100% at 16 °C; thus all experiments were conducted at this latter temperature. The egg-production rates recorded for both species were comparable to the maximum rates determined at similar temperatures in Narragansett Bay (Sullivan and McManus 1986; Durbin et al. 1992) and with rates measured for both *A. tonsa* in Long Island Sound (Beckman and Peterson 1986; Bellantoni and Peterson 1987) and *A. hudsonica* in Long Island embayments during conditions of enriched food (Londsdale et al. 1994). *A. hudsonica* exhibited lower egg-production rates than did *A. tonsa* (Table 5). A similar pattern for these species at the same temperature was observed by Sullivan and McManus (1986).

Although carbon was significantly correlated with E_r in these laboratory experiments (Table 5), it is not a good parameter for estimating in situ egg-production rates (Jónasdóttir 1992). In nature, particulate organic carbon consists of both living and dead particles, whereas in the laboratory experiments only living carbon in the form of phytoplankton cells was present. The similar E_rs measured for *Acartia* spp. fed on particles of 226 μm^3 (*Rhodomonas lens*) and 3650 μm^3 (*Prorocentrum minimum*) excludes the importance of cell size as an indicator of food quality. This is consistent with the findings of Ahlgren et al. (1990) and Støttrup and Jensen (1990), but contrasts with the assumptions of other investigators (Nassogne 1970; Gaudy 1974; Ambler 1986; Verity and Smayda 1989). Thus, the positive correlation of E_r with carbon but not cell size recorded in the present study indicates that for these three phytoplankton species, E_r depends more on the "compactness" of the cell than on cell size alone.

The nitrogen and protein content of the food also correlated well with E_r. Most of the nitrogen is bound in the protein fraction of the food. This positive correlation is understandable, since protein and nitrogenous non-protein compounds (such as free amino acids) are extremely important in the nutrition of female copepods, being essential for the production of DNA, nucleotides, enzymes, hormones and egg yolk proteins (Harrison 1990). Although the protein content of the diet clearly affected the rate of egg production in *Acartia* spp., the chemical constituents providing the best correlation with egg-production rates were in the lipid fraction of the phytoplankton. The specific fatty acid requirements appeared to be different for the two *Acartia* species. Egg production of *A. tonsa* was most highly correlated (positively) with the ω3:ω6 ratio of the cell, and a highly significant negative correlation was recorded for the 20:5 to 22:6 ratio. No correlation with the ω3:ω6 ratio was found for *A. hudsonica*, in which the parameters that accounted for most of the E_r variability were the 20:5 to 22:6 ratio (negative) and the individual 20:5 and 22:6 fatty acids (positive). This result is in agreement with the data of Støttrup and Jensen (1990), who found a similar negative trend between egg production of *A. tonsa* and the 20:22 ratio of its food; they did not measure the ω3 and ω6 fatty acids. The highest E_r for *A. tonsa* reported by Støttrup and Jensen was at a 20:22 ratio of 0.034 in the flagellate *Isochrysis galbana*, and the lowest at a 20:22 ratio of 8 in *Ditylum brightwelli*. Their ratios of 4.5 for *Thalassiosira weissflogii* and 1.5 for *Rhodomonas baltica* are comparable with the ratios of 3.5 to 5 for *T. weissflogii* and 1.5 to 2.0 for *R. lens* in the present study.

The ω3:ω6 ratio has been suggested as an important indicator in metabolic growth and reproduction processes in crustaceans (Castell 1982; Ahlgren et al. 1990; Harrison 1990). Webb and Chu (1982) and Enright et al. (1986) reported a ω3:ω6 ratio of ≥3 to be a good indicator of a nutritious diet for bivalve larval growth. It is likely that copepods have somewhat different requirements than bivalves, but no study on copepod feeding has investigated the effects of this dietary ratio. The present study recorded highest egg-production rates at ω3:ω6 ratios of 25, while rates at a ratio of 3 were very low. Ahlgren et al. (1990) obtained the highest growth response for three cladoceran species on a diet of flagellates containing ω3:ω6 ratios of both 10 and 18. The role of these fatty acids is not well known. The ω3 fatty acids are thought to be important in the maintenance of membrane fluidity in cold environments (Benson and Lee 1975), while there is some evidence that ω6 acids might be precursors for some prostaglandins (Stryer 1988; Harrison 1990). Castell (1982) states that the requirements for ω3 and ω6 fatty acids depend on temperature, salinity, genetic variation and other environmental and biotic factors. The uncertainty of the roles of the ω3 and ω6 fatty acids, as well as those of the 20:5 and 22:6 fatty acids, makes it difficult to explain the reasons for the different nutritional requirements

observed for egg production of *Acartia tonsa* and *A. hudsonica*.

Other fatty acids not previously reported to be important for copepod or crustacean reproduction are the 18:3ω3, 18:4ω3 and 18:1ω9 fatty acids. The role of these fatty acids is not well understood. The 18:3ω3 and 18:4ω3 fatty acids are thought to be important precursors for prostaglandins (Castell 1982; Harrison 1990). The 18:1ω9 fatty acid is thought to be elongated to 20:1ω9 which can, for example, be used to generate wax esters (Sarget and Falk-Petersen 1988).

Development time of eggs

Factors affecting egg development times are egg size, with larger eggs requiring longer development times than smaller eggs of the same copepod species (McLaren 1965; Corkett 1972; Lonsdale and Levinton 1985) and temperature (McLaren 1965). However, in the present study, no correlation was found between development time and egg size (Fig. 5b); the temperature was kept the same prior to and during all experiments. The parental acclimation temperature (Tester 1985) was also constant. Although the same females were used in all six experiments concerning development time, there was still a significant difference in development times among several of the experiments. The different development times of the eggs cannot, therefore, be due to any genetic differences.

There has been no previous report of the development times of eggs being affected by the nutritional value of the food available to the female copepod, and the present study did not clearly identify the causes for the time differences recorded. A more thorough study is necessary to test if the chemical composition of the food could affect development times of eggs.

Hatching success

During observations and experiments on copepod egg-production, many researchers have reported non-viable eggs (Uye and Fleminger 1976; Parrish and Wilson 1978; Cowgill et al. 1984; Ambler 1985; Arnott et al. 1986; Ianora et al. 1989, 1992). A number of explanations for the non-viability or differences in hatching success have been suggested. In the present study, all the experiments were set up in the same manner, with filtered, oxygen-saturated seawater, so different oxygen levels (Ambler 1985) could not have caused the observed variations in hatching success. The low hatching success of *Acartia hudsonica* eggs in Experiments 10 to 13 could have been due to formation of diapause eggs. The individuals in these four experiments originated from 9 to 10 °C waters in the field and were reared in the laboratory at 16 °C. This increased temperature could have induced the formation of resting eggs (Marcus 1989). The experiments with *A. tonsa* were run at temperatures optimal for this species in the field, so it is not probable that the differential egg hatching observed in *A. tonsa* arose from the production of resting eggs.

Parrish and Wilson (1978) reported that egg viability was related to time since last mating, and associated non-viability with non-fertilized eggs. Ianora et al. (1989) suggested that low hatching success in *Temora stylifera* eggs was due to poor fertilization during broadcast-spawning. In the present study, males were present in all batch cultures and throughout the acclimation period in Experiments D1 to D6. Therefore, it is reasonable to assume that all eggs were fertilized. Moreover, non-fertilized eggs usually disintegrate fairly soon after being spawned and are easily distinguished from fertilized eggs. Non-fertilized eggs were not noted in any of the experiments. Therefore, the observed differences in hatching success probably resulted from differences in the chemical composition of the food fed to the females and/or the condition of the females while producing the eggs.

The copepod embryo is dependent on nutrition from the egg yolk for growth and survival and for sufficient energy to sustain it through one or two nonfeeding naupliar stages. Few studies have concentrated on the chemistry of copepod eggs. Sargent and Falk-Petersen (1988) observed 16:0, 20:5ω3, 22:6ω3 and 24:1ω9 fatty acids of dietary origin in the eggs of *Calanus finmarchicus*. In some crustaceans these fatty acids have been found in 2 to ≤5-fold higher amounts in eggs and ovaries compared to the rest of the female (Hayashi 1976; Sargent and Falk-Petersen 1988), indicating that these fatty acids may be important for embryonic survival and growth. However, in this study, none of the correlations between hatching success and the individual chemical components measured were significant.

No pattern could be detected between diet and naupliar survival: generally, once an egg hat hatched, the nauplius had a good chance of surviving through the non-feeding stages.

Conclusions

This study focused on the specific fatty acids of algal cells in the diet of *Acartia* spp. but did not separate protein or carbohydrates into their specific compounds. However, the importance of other specific components of the phytoplankton cells (such as amino acids and sugars) should not be ignored.

The algal components that appear to be best correlated with *Acartia* spp. egg production are protein and specific fatty acids (*A. tonsa* = 18:0 and 16:1ω7; *A. hudsonica* = 20:5ω3, 22:6ω3 and 16:1ω7) and the ω3:ω6 and 20:22 fatty acid ratios. Females appear to utilize energy from lipids to fuel biosynthesis of eggs, and to direct certain specific dietary fatty acids (probably 20:5, 22:6, ω3 and ω6) directly into vitellogenesis.

These experiments have demonstrated that more care must be taken when selecting food for experimental copepods. The diatom *Thalassiosira weissflogii* has been widely used in feeding and egg-production experiments on various copepods in the laboratory, and as food enrichment in some field studies (Runge 1984; Ambler 1985; Frost

1985; Houde and Roman 1987; Cowles et al. 1988; Jónasdóttir 1989; Durbin et al. 1990). However, the present results indicte that, with the exception of the youngest culture, the nutritional composition of *T. weissflogii* does not favor successful egg production in *Acartia* spp. (in contrast to the flagellate and dinoflagellate food items used). Use of *T. weissflogii* as a food for *Acartia* spp. would probably result in a marked underestimation of their maximum egg-production capacity.

The effect of chemical changes associated with aging of phytoplankton populations on the biology of copepods are well demonstrated in the present study by the egg-production responses (Fig. 1) and the reduction in egg-hatching success (Table 6) of both *Acartia* species fed *T. weissflogii* of different culture stages. This study was not able to clearly determine the chemical component(s) in the aging *T. weissflogii* cultures that caused the different reproduction responses in the copepods. However, based on the results of the present study, there is an indication that the compositional changes of particulate organic matter observed in nature may significantly affect copepod reproduction and growth. If the chemical composition of a diatom bloom changes in a manner similar to that observed in batch culture in the present study, then copepod egg production in situ could justifiably be expected to respond in a similar way to that observed in my experiments. Highest egg production would thus occur during the early development phases of the diatom bloom and decrease gradually as the bloom progressed.

Kleppel et al. (1991) pointed out the potential importance of dinoflagellates in the diet of copepods. The present study, as well as that of Støttrup and Jensen (1990), also suggested that compared to older stages of the diatom *Thalassiosira weissflogii*, some flagellates (*Rhodomonas lens* and *R. baltica*) may constitute a very important food item for *Acartia* spp. The nutritional value of the *T. weissflogii* culture appears to deteriorate with age while the composition of aging flagellates seems more stable. Therefore, the frequent use of diatoms such as *T. weissflogii* should be carefully reconsidered when rearing copepods in the laboratory and/or when conducting experiments in which food quality could be a limiting factor.

Acknowledgements This study was supported by research grants from NATO and Sigma-Xi grant-in-aid of research awarded to the author and by Hudson River Foundation, Grant HRF #012/90A/032 awarded to D. J. Lonsdale and E. M. Cosper. I thank D. J. Lonsdale for her support and advice throughout this study, M. de Angelis for help with the figures and editing of the manuscript and S. Wakeham for use of his laboratory and help with the fatty acid analyses. An earlier version of this study benefited greatly from the comments of E. M. Cosper, C. Lee, W. T. Peterson and M. R. Roman. I also acknowledge N. H. Marcus and two anonymous reviewers for useful comments. This is Contribution No. 969 of Marine Sciences Research Center, SUNY, Stony Brook, New Nork.

References

Ackman RG, Jangaard PM, Hoyle RJ, Brockerhoff H (1964) Origin of marine fatty acids. I. Analyses of the fatty acids produced by the diatom *Skeletonema costatum*. J Fish Res Bd Can 21:747–756

Ackman RG, Tocher CS, McLachlan J (1968) Marine phytoplankter fatty acids. J Fish Res Bd Can 25:1603–1620

Ahlgren G, Gustafsson I-B, Boberg M (1992) Fatty acid content and chemical composition of freshwater microalgae. J Phycol 28:37–50

Ahlgren G, Lundstedt L, Brett M, Forsberg C (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. J Plankton Res 12:809–818

Allan JD (1984) Life history variation in a freshwater copepod: evidence from population crosses. Evolution 38:208–291

Ambler JW (1985) Seasonal factors affecting egg production and viability of eggs of *Acartia tonsa* Dana from East lagoon, Galveston, Texas. Estuar, cstl Shelf Sci 20:743–760

Ambler JW (1986) Effect of food quantity and quality on egg production of *Acartia tonsa* Dana from East lagoon, Galveston, Texas. Estuar, cstl Shelf Sci 23:183–196

Arnott GH, Brand GW, Kos LC (1986) Effects of food quality and quantity on the survival, development, and egg production of *Gladioferens pectinatus* (Brady) (Copepoda: Calanoida). Aust J mar Freshwat Res 37:467–473

Beckman BR, Peterson WT (1986) Egg production by *Acartia tonsa* in Long Island Sound. J Plankton Res 8:917–925

Bellantoni DC, Peterson WT (1987) Temporal variability in egg production rates of *Acartia tonsa* Dana in Long Island Sound. J exp mar Biol Ecol 107:199–208

Benson AA, Lee RF (1975) The role of wax in oceanic food chains. Scient Am 232:77–86

Bourdier GG, Amblard CA (1989) Lipids in *Acanthodiaptomus denticornis* during starvation and fed on three different algae. J Plankton Res 11:1201–1212

Bradshaw SA, O'Hara SCM, Corner EDS, Eglinton G (1990) Changes in lipids during simulated herbivorous feeding by the marine crustacean *Neomysis integer*. J mar biol Ass UK 70:225–243

Burney CM, Sieburth JMCN (1977) Dissolved carbohydrates in seawater. II. A spectrophotometric procedure for total carbohydrate analysis and polysaccharide estimation. Mar Chem 5:15–28

Cahoon LB (1981) Reproductive response of *Acartia tonsa* to variations in food ration and qualitiy. Deep-Sea Res 28A:1215–1221

Castell JD (1982) Fatty acid metabolism in crustaceans. In: Pruder GD, Langdon C, Conklin D (eds) Proceedings of the second international conference on aquaculture nutrition: biochemical and physiological approaches to shellfish nutrition. Louisiana State University, Baton Rouge, pp 124–145

Checkley DM Jr (1980). The egg production of a marine planktonic copepod in relation to its food supply: laboratory studies. Limnol Oceanogr 25:430–446

Conklin DE, Provasoli L (1977) Nutritional requirements of the water flea *Moina macrocopa*. Biol Bull mar biol Lab, Woods Hole 152:337–350

Corkett CJ (1972) Development rate of copepod eggs of the genus *Calanus*. J exp mar Biol Ecol 10:171–175

Cowgill UM, Williams DM, Esquivel JB (1984) Effects of maternal nutrition on fat content and longevity of neonates of *Daphnia magna*. J Crustacean Biol 4:173–190

Cowles TJ, Olson RJ, Chisholm SW (1988) Food selection by copepods: discrimination on the basis of food quality. Mar Biol 100:41–49

Dadd RH (1982) Insect nutrition: relevance to marine invertebrates. In: Pruder GD, Langdon C, Conklin D (eds) Proceedings of the second international conference on aquaculture nutrition: biochemical and physiological approaches to shellfish nutrition. Louisiana State University, Baton Rouge, pp 33–55

Durbin EG, Durbin AG, Campbell RG (1992) Body size and egg production in the marine copepod *Acartia hudsonica* during a winter–spring diatom bloom in Narragansett Bay. Limnol Oceanogr 37:342–360

Durbin EG, Durbin AG, Smayda TJ, Verity PG (1983) Food limitation of production by adult *Acartia tonsa* in Narragansett Bay, Rhode Island. Limnol Oceanogr 28:1199–1213

Durbin AG, Durbin EG, Wlodarczyk E (1990) Diel feeding behavior in the marine copepod *Acartia tonsa* in relation to food availability. Mar Ecol Prog Ser 68:23–45

Enright CT, Newkirk GF, Craigie JS, Castell JD (1986) Growth of juvenile *Ostrea edulis* L. fed *Chaetoceros gracilis* Schütt of varied chemical composition. J exp mar Biol Ecol 96:15–26

Fraser AJ, Sargent JR, Gamble JC, Seaton DD (1989) Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. Mar Chem 27:1–18

Frost BW (1985) Food limitation of the planktonic marine copepods *Calanus pacificus* and *Pseudocalanus* sp. in a temperate fjord. Arch Hydrobiol 21:1–13

Gatten RR, Sargent JR, Forsberg TEV, O'Hara SCM, Corner EDS (1980) On the nutrition and metabolism of zooplankton. XIV. Utilization of lipid by *Calanus helgolandicus* during maturation and reproduction. J mar biol Ass UK 60:391–399

Gaudy R (1974) Feeding four species of pelagic copepods under experimental conditions. Mar Biol 25:125–141

Giese AC (1966) Lipids in the economy of marine invertebrates. Physiol Rev 46:244–291

Guillard R (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum Press, New York, pp 29–60

Harrison KE (1990) The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. J Shellfish Res 9:1–28

Harrison PJ, Conway HL, Holmes RW, Davis CO (1977) Marine diatoms grown in chemostats under silicate or ammonium limitation. III. Cellular chemical composition and morphology of *Chaetoceros debilis*, *Skeletonema costatum* and *Thalassiosira gravida*. Mar Biol 43:19–31

Harvey HR, Eglinton G, O'Hara SCM, Corner DS (1987) Biotransformation and assimilation of dietary lipids by *Calanus* feeding on a dinoflagellate. Geochim cosmochim Acta 51:3031–3040

Hayashi K (1976) The lipids of marine animals from various habitat depths. VI. On the characteristics of the fatty acid composition of neutral lipids from decapods. Bull Fac Fish Hokkaido Univ 27:21–29

Hitchcock GL (1982) A comparative study of the size-dependent organic composition of marine diatoms and dinoflagellates. J Plankton Res 4:363–377

Houde SEL, Roman MR (1987) Effects of food quality on the functional ingestion response of the copepod *Acartia tonsa*. Mar Ecol Prog Ser 40:69–77

Ianora A, Mazzocchi MG, Grottoli R (1992) Seasonal fluctuations in fecundity and hatching success in the planktonic copepod *Centropages typicus*. J Plankton Res 14:1483–1494

Ianora A, Scotto di Carlo B, Mascellaro P (1989) Reproductive biology of the planktonic copepod *Temora stylifera*. Mar Biol 101:187–194

Johnson KM, Burney CM, Sieburth J McN (1981) Doubling the production and precision of the MBTH spectrophotometric assay for dissolved carbohydrates in seawater. Mar Chem 10:467–473

Johnson KM, Sieburth J McN (1977) Dissolved carbohydrates in seawater. I. A precise spectrophotometric analysis for monosaccharides. Mar Chem 5:1–13

Jónasdóttir SH (1989) Effects of food concentration on egg-production rates of two species of *Pseudocalanus*: laboratory observations. J exp mar Biol Ecol 130:33–43

Jónasdóttir SH (1992) Chemical composition of food and the reproductive succes of the copepods *Acartia tonsa*, *Acartia hudsonica* and *Temora longicornis*. Ph.D. dissertation. State University of New York, Stony Brook

Jones DA, Kanazawa A, Ono K (1979) Studies on the nutritional requirements of the larval stages of *Penaeus japonicus* using microencapsulated diets. Mar Biol 54:261–267

Kanazawa A, Teshima S-i, Tokiwa S (1977) Nutritional requirements of prawn. VII. Effect of dietary lipids on growth. Bull Jap Soc scient Fish 43:849–856

Kattner G, Gercken G, Eberlein K (1983) Development of lipids during a spring plankton bloom in the northern North Sea. I. Particulate fatty acids. Mar Chem 14:149–162

Kiørboe T (1989) Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. Mar Ecol Prog Ser 55:229–234

Kiørboe T, Møhlenberg F, Hamburger K (1985) Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. Mar Ecol Prog Ser 26:85–97

Kleppel GS, Holliday DV, Pieper RE (1991) Trophic interactions between copepods and microplankton: a question about the role of diatoms. Limnol Oceanogr 36:172–178

Landry MR (1983) The development of marine calanoid copepods with comments on the isochronal rule. Limnol Oceanogr 28:614–624

Lee RF, Nevenzel JC, Paffenhöfer G-A (1971) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9:99–108

Lonsdale DJ, Cosper EM, Kim W-S, Doall M, Divideenam A, Jónasdóttir SH (1994) Microzooplankton: an important trophic link in Long Island Bays (in preparation)

Lonsdale DJ, Levinton JS (1985) Latitudinal differentiation in embryonic duration, egg size, and newborn survival in a harpacticoid copepod. Biol Bull mar biol Lab, Woods Hole 168:419–431

Marcus NH (1989) Abundance in bottom sediments and hatching requirements of eggs of *Centropages hamatus* (Copepoda: Calanoida) from the Alligator Harbor region, Florida. Biol Bull mar biol Lab, Woods Hole 176:142–146

Marshall SM, Orr AP (1952) On the biology of *Calanus finmarchicus*. VII. Factors affecting egg production. J mar biol Ass UK 30:527–547

Martin BJ (1980) Croissance et acides gras de la crevette *Palaemon serratus* (Crustacea, Decapoda) nourrie avec des aliments composés contenant différentes proportions d'acide linoleique et linolénique. Aquaculture, Amsterdam 19:325–337

Mayzaud P, Chanut JP, Ackman RG (1989) Seasonal changes of the biochemical composition of marine particulate matter with special reference to fatty acids and sterols. Mar Ecol Prog Ser 56:189–204

McLaren IA (1965) Some relationships between temperature and egg size, body size, development rate, and fecundity, of the copepod *Pseudocalanus*. Limnol Oceanogr 10:528–538

Morris RJ, McCartney MJ, Robinson GA (1983) Studies of a spring phytoplankton bloom in an enclosed experimental ecosystem. I. Biochemical changes in relation to the nutrient chemistry of water. J exp mar Biol Ecol 70:249–262

Nassogne A (1970) Influence of food organisms on the development and culture of pelagic copepods. Helgoländer wiss Meeresunters 20:333–345

Parrish CC (1987) Time series of particulate and dissolved lipid classes during spring phytoplankton blooms in Bedford Basin, a marine inlet. Mar Ecol Prog Ser 35:129–139

Parrish CC, Wangersky PJ (1987) Particulate and dissolved lipid classes in cultures of *Phaeodactylum tricornutum* gron in cage culture turbidostats with a range of nitrogen supply rates. Mar Ecol Prog Ser 35:119–128

Parrish KK, Wilson DF (1978) Fecundity studies on *Acartia tonsa* (Copepoda: Calanoida) in standardized culture. Mar Biol 46:65–81

Peltzer ET, Alford JB, Gagosian RB (1984) Methodology for sampling and analysis of lipids in aerosols from the remote marine atmosphere. Tech Rep Woods Hole oceanogr Instn WHOI-84-9:1–104

Pugh PR (1971) Changes in the fatty acid composition of *Coscinodiscus eccentricus* with culture-age and salinity. Mar Biol 11:118–124

Raymont JEG (1980) Plankton and productivity in the oceans. 2nd edn Vol. 1. Phytoplankton. Pergamon Press, Oxford

Runge JA (1984) Egg production of the marine, planktonic copepod, *Calanus pacificus* Brodsky: laboratory observations. J exp mar Biol Ecol 74:53–66

Sakshaug E, Andersen K, Myklestad S, Olsen Y (1983) Nutrient status of phytoplankton cummunities in Norwegian waters (marine, brackish, and fresh) as revealed by their chemical composition. J Plankton Res 5:175–196

Sargent JR, Falk-Petersen S (1988) The lipid biochemistry of calanoid copepods. Hydrobiologia 167/168:101–114

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985). Measurement of protein using bicinchoninic acid. Analyt Biochem 150:76–85

Sokal RR, Rohlf FJ (1981) Biometry. The principles and practice of statistics in biological research. 2nd ed. W.H. Freeman & Co., New York

Stryer L (1988) Biochemistry. 3rd edn W.H. Freeman & Co. New York

Støttrup JG, Jensen J (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. J exp mar Biol Ecol 141:87–105

Sullivan BK, McManus LT (1986) Factors controlling seasonal succession of the copepods *Acartia hudsonica* and *A.tonsa* in Narragansett Bay, Rhode Island: temperature and resting egg production. Mar Ecol Prog Ser 28:121–128

Tester PA (1985) Effects of parental acclimation temperature and egg-incubation temperature on egg-hatching time in *Acartia tonsa* (Copepoda: Calanoida). Mar Biol 89:45–53

Tester PA, Turner JT (1990) How long does it take copepods to make eggs? J exp mar Biol Ecol 141:169–182

Thompson PA, Harrison PJ, Whyte JNC (1990) Influence of irradiance on the fatty acid composition of phytoplankton. J Phycol 26:278–288

Uye S-I (1981) Fecundity studies of neritic calanoid copepods *Acartia clausi* Giesbrecht and *A. steueri* Smirnov: a simple empirical model of daily egg production. J exp mar Biol Ecol 50:255–271

Uye S, Fleminger A (1976) Effects of various environmental factors on egg development of several species of *Acartia* in Southern California. Mar Biol 38:253–262

Verity PG, Smayda TJ (1989) Nutritional value of *Phaeocystis pouchetii* (Prymnesiophyceae) and other phytoplankton for *Acartia* spp. (Copepoda): ingestion, egg production, and growth of nauplii. Mar Biol 100:161–171

Volkman JK (1989) Fatty acids of microalgae used as feed stocks in aquaculture. In : Cambie RC (ed) Fats for the future. Ellis Horwood, Chichester, pp 263–283

Wakeham SG, Canuel EA (1990) Fatty acids and sterols of particulate matter in a brackish and seasonally anoxic coastal salt pond. Org Geochem 16:703–713

Webb KL, Chu F-L E (1982) Phytoplankton as a food source for bivalve larvae. In: Pruder GD, Langdon C, Conklin D (eds) Proceedings of the second international conference on aquaculture nutrition: biochemical and physiological approaches to shellfish nutrition. Louisiana State University, Baton Rouge, pp 272–291