Marine Biology (1996) 125: 743–750

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Copepod recruitment and food composition: do diatoms affect hatching success?

Received: 4 December 1995/Accepted: 12 January 1996

Abstract Laboratory experiments were conducted to differentiate between factors controlling the hatching success of copepod eggs. Factors that could affect viability of eggs; *viz* food quality, female condition and external factors were investigated. In a series of experiments the copepod *Acartia tonsa* Dana was fed several different diets while egg production and hatching success were monitored. The diet was analysed for fatty acid content as an indicator of food quality. Both egg production and hatching were found to be affected by the nutritional quality of the food. Hatching was also highly dependent on female fertility. External effects were tested by exposing eggs to diatom extracts. Negative effects were only evident at high extract concentrations, but disappeared when aeration was supplied to the solution. Oxygen measurements showed that failure to hatch was due to hypoxia in the extracts. No inhibitory or toxic effects of diatom cell components on hatching could be found.

Introduction

Egg production rates are frequently measured and used as an indicator of population growth of copepods in nature. However, using egg production rates can cause a significant overestimation of recruitment if the viability of eggs is not 100%. Therefore, an understanding of the factors influencing copepod hatching success is of major importance.

Several studies have reported production of nonviable eggs both in natural and in laboratory populations of copepods in which hatching percentages were found to range from zero to 100%. Including the possibility of resting egg formation, these studies suggest that the reasons for variable hatching success may be dietary based (Ambler 1985; Arnott et al. 1986; Ianora et al. 1992; Ianora and Poulet 1993; Jónasdóttir 1994; Guisande and Harris 1995), due to exposure to low oxygen levels (Uye and Fleminger 1976; Ambler 1985; Roman et al. 1993; Lutz et al. 1994) or environmental contaminants (Buttino 1994), to infertility due to lack of mating/remating (Parrish and Wilson 1978; Ianora et al. 1989), or to inhibition of hatching due to unknown toxic diatom cell components (Poulet et al. 1994; Ianora et al. 1995).

The importance of diatoms as a high-quality food source for copepods has recently been questioned (Kleppel et al. 1991; Poulet et al. 1994; Ianora et al. 1995), and Jónasdóttir (1994) reported that the status of the diatom culture affected the quality of diatoms as food for copepods. The studies of Poulet et al. (1994) and Ianora et al. (1995) suggest particularly that diatoms contain toxins or inhibitors that may suppress normal development of copepod eggs. This is at variance with the classical comprehension that diatoms form the basis of the grazing food chain in the ocean (e.g. Legendre 1990). For this reason the present study focused on diatoms as food for copepods.

One can in general distinguish between three possible controls on egg hatching. Two may be categorized as maternal effects, namely nutritional control, which affects the quality of the eggs, and female condition, such as female age and fertility. The third control is the influence of external factors on eggs (effects of chemicals, oxygen deficiency and/or presence of bacteria). The goal of the experiments presented in this paper has been to differentiate between these potential controls on egg hatching. Experiments were conducted in the laboratory using the copepod *Acartia tonsa* Dana. Females were fed a variety of phytoplankton diets, and egg production and hatching success were observed.

Communicated by L. Hagerman, Helsingør

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Eggs were also exposed to phytoplankton extracts in order to further investigate the potential negative effects of phytoplankton exudates on hatching as reported by Poulet et al. (1994) and Ianora et al. (1995). We chose fatty acids as an indicator of nutritional quality of phytoplankton because of their high concentration in the eggs and ovaries of copepods (Sargent and Falk-Petersen 1988; see also Harrison 1990 for other crustaceans). Additionally, some specific dietary fatty acids have been found to be well correlated with egg production in *Acartia* spp. (Jónasdóttir 1994) and fertility and development of some cladocerans (Ahlgren et al. 1990). However, the importance of other nutritional components (e.g. vitamins and amino acids) for egg production and hatching should not be underestimated even though those components were not chosen to be analysed in this study.

Materials and methods

Culturing and chemical analysis of phytoplankton

Four different diatom species were used as food; *viz Thalassiosira weissflogii* (ca. 14 µm ESD; equivalent spherical diameter), *Thalassiosira rotula* (ca. 20 µm ESD), *Phaeodactylum tricornutum* (ca. 3.5 µm ESD) *Chaetoceros affinis* (ca. 12 µm ESD). The flagellate *Rhodomonas baltica* (ca. 7.5 µm ESD) was used as a high-quality food control (Støttrup and Jensen 1990). Phytoplankton cultures were grown in chemostats in order to control growth rates of the algal cells. Silica was added to the growth media (B1) for the diatom cultures. Cultures were grown under continuous illumination at 15 °C and were monitored and counted daily with a particle counter (ELZONE 180, Particle Data, Inc., fitted with 60, 120 or 240 µm orifice depending on cell size). Every other experimental day, culture aliquots were filtered onto combusted GF/F filters for later fatty acid analyses. After filtration the sample was immediately placed in a micro vial, oxygen flushed out and replaced with argon, and the sample frozen at −80 °C.

Fatty acids were analysed by gas chromatography on a capillary column following the procedure described in Whyte (1988). Lipids were extracted for 24 h in CH_2Cl_2/methanol (2:1, v/v) with a known amount of C_{17} fatty acid added to the sample. The fatty acids were transmethylated with BF_3-methanol to form fatty acid methyl esters (FAME). The FAME sample was injected into a gas chromatograph (GC, Hewlett Packard 5890 equipped with split/splitless injection system) using helium as a carrier gas at 1.0 ml min^{-1}. The injection temperature was 250 °C, and the temperature program was 100 °C isothermal for 2 min and increased from 100 to 300 °C at 5 °C min^{-1}. Peaks from chromatograms were compared to Sigma and Larodan FAME standards for specific fatty acid identification, and the integrated peaks were compared to the peak area of the C_{17} standard.

Preparation of extracts

Phytoplankton for preparation of extracts were grown in batch cultures. Extracts were made either from cells in exponential or stationary growth phase (Table 1). An aliquot of known cell concentration of each of the phytoplankton cultures was filtered onto GF/C filters. The cells retained on the filter were re-suspended in 25 ml of filtered seawater (0.25 µm) and sonicated with a probe for 10 min. The content was kept cold during sonication by submerging the beaker containing the sample in an ice bath. The sonicate was pipetted into a test tube and spun down at 4500 rpm for 10 to 20 min, or until the supernatant was clear. The supernatant was then pipetted into a 25 ml plastic bottle and frozen at −80 °C. These stock solutions of algal extracts were thawed and diluted to the desired concentration (cell volume equivalents) prior to use.

Table 1 Extract experiments. Phytoplankton cultures, growth stages and extract concentrations as equivalent cell volume concentrations (10^6 µm^3 ml^{-1}) used in the hatching experiments

Phytoplankton extracts	Extract concentrations (10^6 µm^3 ml^{-1})
Thalassiosira weissflogii, exponential	0, 12.5, 25, 50, 150
Thalassiosira weissflogii, stationary	0, 25, 50, 150
Chaetoceros affinis, exponential	0, 150
Skeletonema costatum, exponential	0, 150
Phaeodactylum tricornutum, exponential	0, 120
Phaeodactylum tricornutum, stationary	0, 150
Thalassiosira rotula, exponential	0, 50, 150

Egg production and hatching experiments

Maternal effects – Effects of diet and female age on egg production and hatching

Effect of food was examined using seven different diets, namely fast growing (1.0 divisions d^{-1}) *Thalassiosira weissflogii, Thalassiosira rotula, Phaeodactylum tricornutum, Chaetoceros affinis* and *Rhodomonas baltica* and slow growing (0.1 divisions d^{-1}) *T. weissflogii* and *P. tricornutum*. In addition 0.5-µm filtered seawater served as a starvation control. Phytoplankton was added at super abundant concentrations (5×10^6 µm^3 ml^{-1}; corresponds to phytoplankton bloom concentration) to ensure maximum ingestion rate.

Female copepods that had matured within a week were obtained from laboratory cultures of *Acartia tonsa* (Støttrup et al. 1986). For each diet, 14 females were placed in each of six 1-litre bottles. The bottles were filled to the rim (total volume 1200 ml) and sealed without a head space, mounted on a slowly rotating wheel (1 rpm) and incubated at 17 ± 1 °C. No males were added to the bottles. Females were transferred daily to a fresh food suspension by carefully pouring the content of the bottle through a submerged 180 µm sieve. The females were counted and pipetted into the fresh food suspension. The eggs were retained on a 43 µm mesh and either fixed with Lugol's solution or allowed to hatch. In the latter case the eggs were washed into 320-ml bottles containing filtered seawater and sealed without a head space. These bottles were placed on a plankton wheel for 48 h, which is sufficient time for all vital eggs to hatch at 17 °C (McLaren et al. 1969; Ambler 1985). The nauplii and remaining eggs were fixed with Lugol's solution for later counting.

Each experiment lasted for 10 d. At the end of each experiment, 20 females were randomly taken from the six bottles and measured for prosome length. Female carbon content was calculated from cephalothorax length according to the regression in Berggreen et al. (1988). Carbon content of eggs (n = 420 randomly measured for size throughout the experiment) was estimated from egg volume assuming 1.4×10^{-6} µg C µm^{-3} (Kiørboe et al. 1985).

Effect of fertility on egg production and hatching

In order to differentiate between female age and female fertility experiments including males were set up. Fast growing *Thalassiosira*

weissflogii was used as food (5×10^6 μm^3 ml^{-1}). Five bottles with 14 females each, and five with 12 females and 3 males each were set up. Females chosen for these experiments were 1 wk older than in the previously described experiments. Otherwise the experiments were as described in the previous subsection. Lost and dead males were replaced daily so that a total number of 3 $bottle^{-1}$ was retained throughout the 10-d experiment.

External effects

Ten females were placed in each of 8 to 12 1-litre bottles containing 5×10^6 μm^3 ml^{-1} suspension of fast growing *Thalassiosira weissflogii* from a chemostat (1.0 divisions d^{-1}). Females were transferred daily into fresh food suspensions. The second and forth batches of eggs were collected for hatching (after 48 and 96 h of feeding). Eggs were put into different concentrations of phytoplankton extracts as shown in Table 1. Four bottles were set up for each concentration of extract. Two of the bottles were sealed without a head space (total of 320 ml) and mounted on a plankton wheel, while the other two were filled to 270 ml and gently aerated (by bubbling air) during the entire egg incubation period. In the *T. weissflogii*-extract experiments only one bottle of each concentration was aerated. Nauplii and any remaining eggs were fixed in Lugol's solution after 48 h of incubation and later counted.

In a separate experiment, a range of concentrations of algal extracts (0, 50, 100 and 150×10^6 μm^3 ml^{-1} cell volume equivalents) in replicates were set up in order to measure oxygen depletion in sealed bottles throughout 48 h of incubation. There were no eggs in these experiments. One or two samples from each extract concentration were terminated after 6, 12, 24 and 48 h of incubation. Oxygen was measured with a Winkler's titration method (Strickland and Parsons 1972).

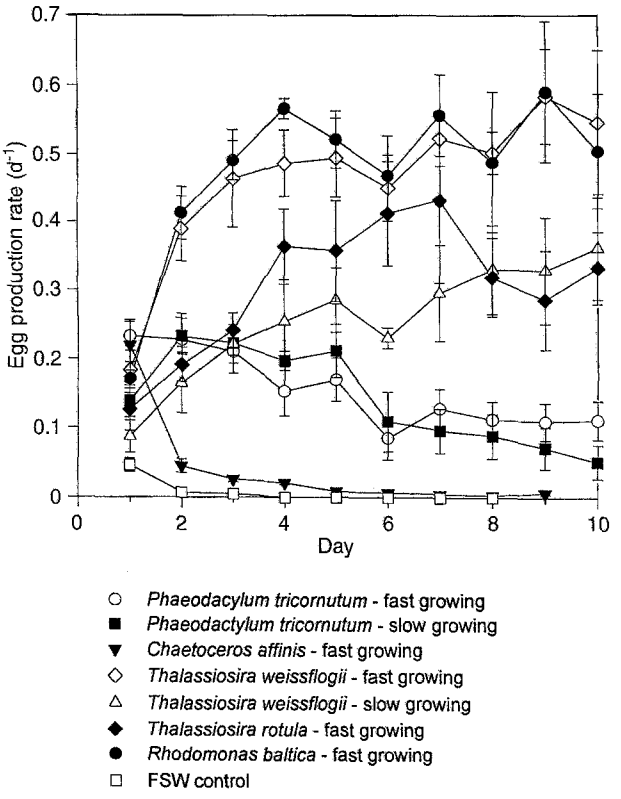

Fig. 1 *Acartia tonsa*. Specific egg production rates (μg C_{egg} μg C_{φ}^{-1} d^{-1} ± 1 SD) of females fed different diets at concentrations of 5×10^6 μm^3 ml^{-1} as a function of time (d from start of experiment)

Results

Maternal effects

Effects of diet and female age on egg production and hatching

The egg production (expressed as μg C_{egg} μg C_{φ}^{-1} d^{-1}) of *Acartia tonsa* differs greatly with food type (Fig. 1). As can be seen with most treatments, the egg production rates on the first day of the experiments are affected by preceding food conditions. Therefore the comparisons of specific egg production rates between treatments are based on measurements after completion of the first 24 h. There are highly significant differences in egg production depending on food types (one-way ANOVA, $F = 333.9$, $p > 0.0001$): a high egg production rate, 0.5 to 0.75 d^{-1}, on a diet of fast growing *Thalassiosira weissflogii* and *Rhodomonas baltica*; medium egg production, 0.25 to 0.40 d^{-1}, on slow growing *T. weissflogii* and fast growing *T. rotula*; low egg production, 0.1 d^{-1}, on fast and slow growing *Phaeodactylum tricornutum*; and almost no egg production while feeding the fast growing *Chaetoceros affinis*.

The age of the females during the 10-d experiment apparently did not affect the egg production rates as the change in egg production with time differs among algal treatments; it stayed constant with the fast growing *Thalassiosira weissflogii*, *Rhodomonas baltica* and *Chaetoceros affinis*, decreased with both stages of *Phaeodoctylum tricornutum* and *T. rotula* and increased with the slow growing *T. weissflogii*.

Female survival after the 10-d experiment (Fig. 2) was higher than 60% and similar for most of the treatments, but lower for slow growing *Thalassiosira weissflogii* and fast growing *Chaetoceros affinis*. Egg production rate and female survival were, thus, not closely related.

The food type and the age of the female (time) both significantly affected hatching success of the eggs (two-way ANOVA, $F = 60.5$, $p = 0.0001$ and $F = 16.6$, $p = 0.0001$ for food type and time, respectively; Fig. 3). The interaction was not significant. Hatching success of eggs was lowest while females were feeding on slow growing *Phaeodactylum tricornutum* but highest while they were feeding on fast growing *Thalassiosira weissflogii* and *Rhodomonas baltica*. The error in the hatching estimate of eggs from females fed *Chaetoceros affinis* is large as it is based on very few eggs (31 eggs on Day 6, 18 eggs on Day 9). Hatching declined with time in all treatments. The slopes of decline are not significantly different among the food treatments (test of equality of slopes, $F = 0.8$, $p = 0.6$, compared from Day 6 and on).

There was no direct relationship between hatching success and egg production rate (Fig. 4). However, at

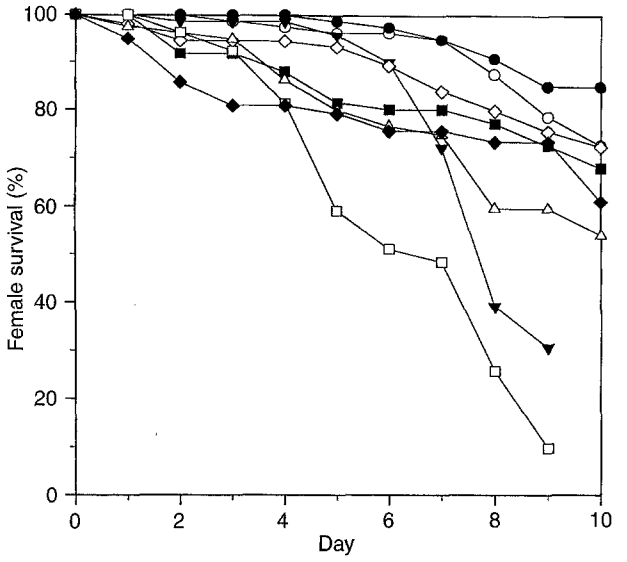

Fig. 2 *Acartia tonsa.* Female survival (%) on different diets as a function of time (d from start of experiment). Symbols as in Fig. 1

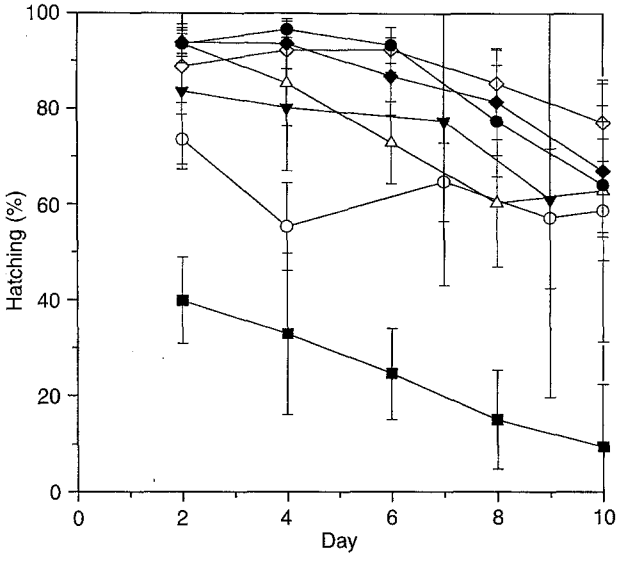

Fig. 3 *Acartia tonsa.* Hatching success of eggs (% hatched ± 1 SD) from females fed different diets at concentrations of 5×10^6 µm^3 ml^{-1} as a function of time (d from start of experiment). Symbols as in Fig. 1

high egg production rates hatching is never lower than 60%, while at low egg production rates hatching ranged from 15 to 100%.

The fatty acid composition of the food affected both egg production rates and hatching success (Figs. 5 and 6, respectively). Egg production was high when both $\omega 3:\omega 6$ and $22:6\omega 3$ versus $20:5\omega 3$ fatty acid (from now on called 22:20 ratio) in the diet were high. However, the egg production rate could also be high at low ratios, but never low at high ratios indicating the importance of other cell constituents in the diet not measured in

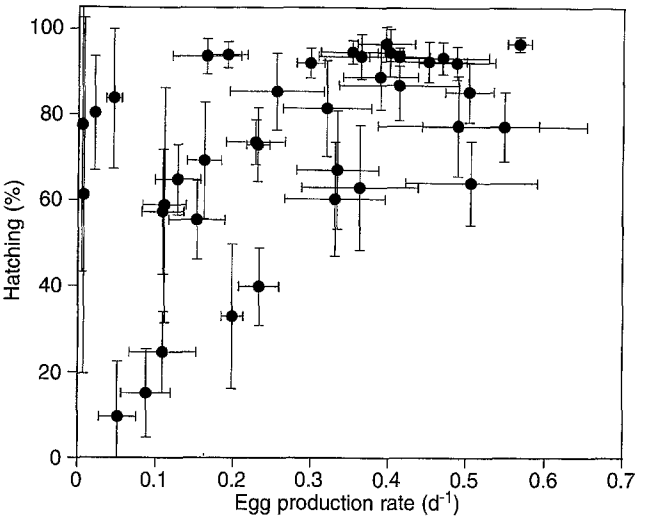

Fig. 4 *Acartia tonsa.* Correlation between hatching success (%) and specific egg production rate (d^{-1}). *Error bars* indicate SD

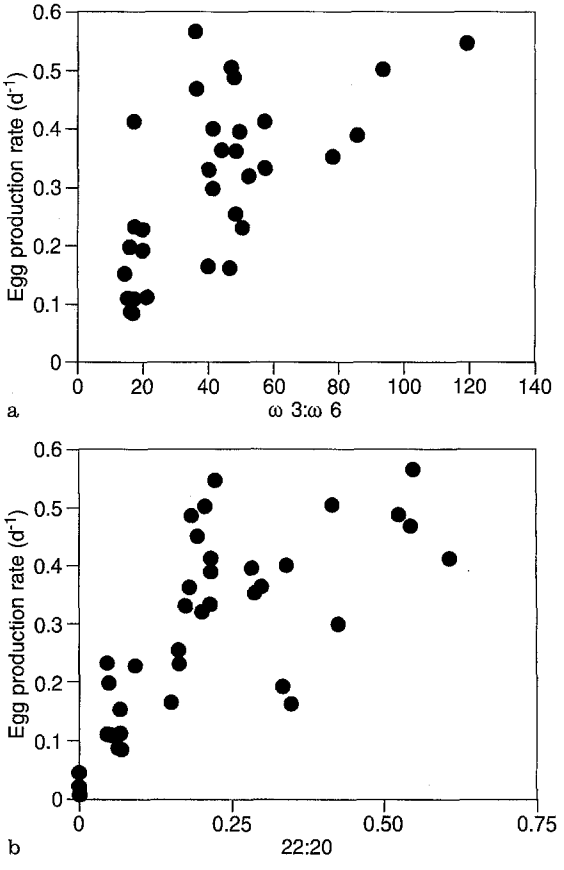

Fig. 5 *Acartia tonsa.* Specific egg production rate (d^{-1}) as a function of **a** $\omega 3:\omega 6$ and **b** 22:20 ($22:6\omega 3$ vs $20:5\omega 3$) fatty acid ratios in the food

this study (Fig. 5). The same is also evident with the hatching success of *Acartia tonsa* eggs. We also found that egg hatching was negatively correlated to the concentrations of both saturated (SAFA) and mono-un-

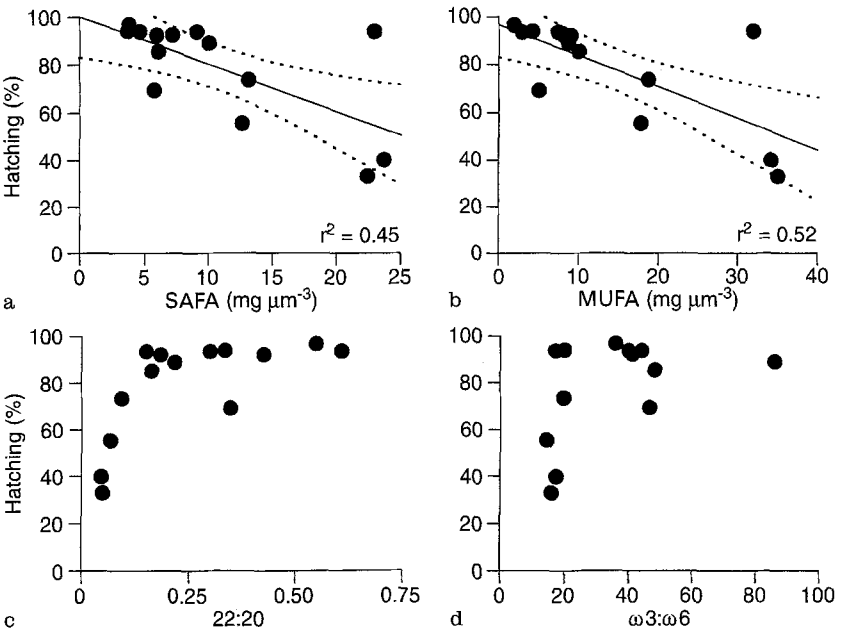

Fig. 6 *Acartia tonsa.* Hatching success as a function of **a** saturated fatty acid (*SAFA*) concentrations (mg μm^{-3}, $\pm$95% confidence limit, significance level of slope: $p = 0.008$); **b** mono-unsaturated fatty acids (*MUFA*) (mg μm^{-3}, $\pm$95% confidence limit, significance level of slope: $p = 0.004$); **c** 22:20 (22:6ω3 vs 20:5ω3) and **d** ω3:ω6 fatty acid ratios in the food

saturated (MUFA) fatty acid content of the food (Fig. 6).

Effect of fertility on egg production and hatching

Egg production rates were identical for females incubated with and without males throughout the 10-d experiment (Fig. 7) and similar to those found in the previous experiments. However, the hatching success of the eggs in the two treatments differed markedly. Hatching of eggs in treatments without males were low (55 to 30%) and showed the same rate of decrease with time as seen in Fig. 3 when testing for the different food types. However, when males were present in the incubation bottles, hatching remained high and close to 100%.

External effects

In the sealed bottles egg hatching was significantly decreased at phytoplankton extract concentrations exceeding 50×10^6 μm^3 ml^{-1} equivalent cell volume concentration (ANOVA, $F = 812.4$, $p < 0.0001$) but close to 100% at lower concentrations (Fig. 8). Extracts from the different phytoplankton species and at different growth phases all resulted in the same effects, and all the phytoplankton species are pooled in the figure. However, there was no effect of phytoplankton extracts on egg hatching in aerated bottles even at the highest concentrations.

Oxygen was depleted in sealed bottles containing extract (Fig. 9). The depletion rate was faster for higher concentrations of extract. This suggests that low hatching at high extract concentrations in sealed bottles is due to low oxygen rather than extract toxicity.

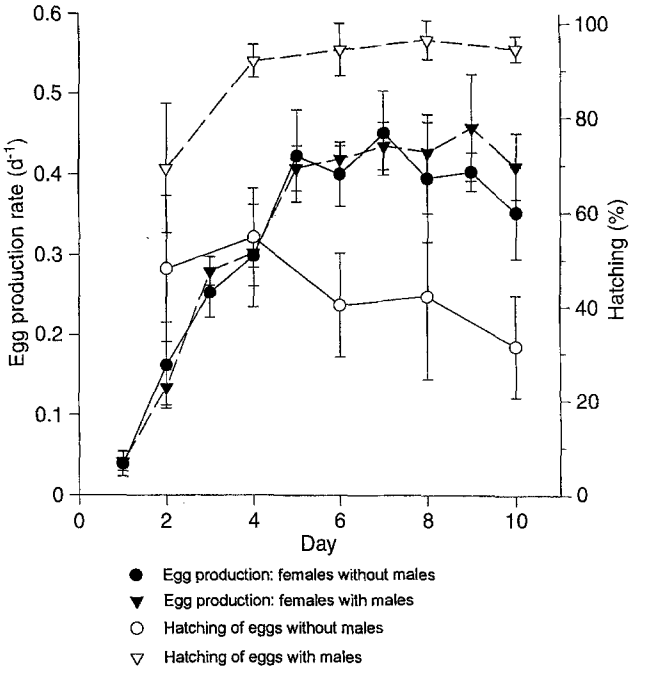

Fig. 7 *Acartia tonsa.* Egg production and hatching from incubations of females alone and females and males feeding on *Thalassiosira weissflogii* at a concentration of 5×10^6 μm^3 ml^{-1}

Discussion

The goal of this study was to investigate the relative importance of maternal and external effects on the

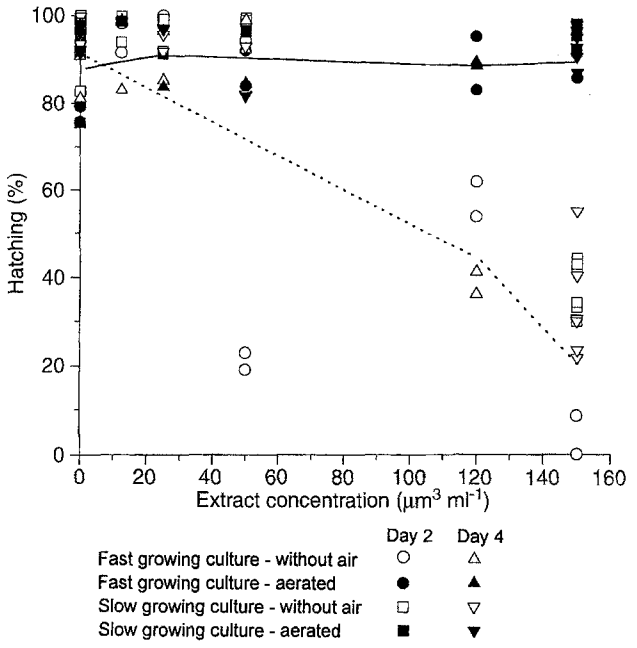

Fig. 8 *Acartia tonsa.* Hatching success (%) of eggs incubated at different concentrations of phytoplankton extracts (as cell volume equivalent; 10^6 µm³ ml^{-1})

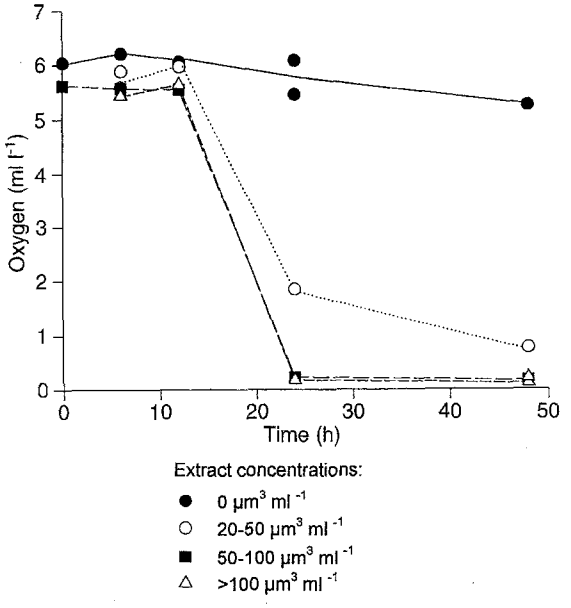

Fig. 9 Oxygen concentration in sealed bottles containing different concentrations of extracts

reproductive success of the copepod *Acartia tonsa.* Quality of food, female age and fertility were examined, and the potential effects of external phytoplankton inhibitors (cf. Poulet et al. 1994; Ianora et al. 1995) on hatching were studied.

Egg production was found to be highly correlated with food type (Fig. 1). An important component of this correlation appears to be food quality as measured by fatty acid composition (Fig. 5). This is in agreement with several previous studies on copepod egg production (phytoplankton type: e.g. Marshall and Orr 1952; Nassogne 1970; Arnott et al. 1986; phytoplankton fatty acids: Støttrup and Jensen 1990; Jónasdóttir 1994). Further, the survival of the females in these experiments differed depending on diet. However, the importance of the different phytoplankton species for survival rank in a different order than that observed for the egg production rates (Fig. 2). This indicates that the nutrient requirement for egg production and the basic subsistence of females differ slightly.

Hatching of *Acartia tonsa* eggs is closely correlated with food type. Correlation with fatty acid composition of the diet (Fig. 6) further show that hatching is to a large extent controlled by maternal nutrition. The significant effect of female age (time) was fully explained by decreasing female fertility (Fig. 7). Parrish and Wilson (1978) have shown that the female has to be re-fertilized in order for the eggs to hatch. In all experiments in the present study the decrease in hatching success occurred at a similar rate after Day 6 of incubation independent of food type (Fig. 3). This indicates that *Acartia tonsa* females require insemination at least 6 d after last mating in order to continue producing fertile eggs.

Egg production and hatching success were not directly correlated (Fig. 4). However, some relationship is evident between hatching and egg production as high egg production rates tend to result in high quality eggs that hatch, while low egg production can have either high or low hatching success. High food quality therefore sustains high production rates of viable eggs. Apparently, at lower egg production rates, hatching is high when the balance of the required nutrients is favourable, but the egg production is low and the hatching is low when some essential nutrients are missing. This is in agreement with Guisande and Harris (1995) who concluded that hatching success of *Calanus helgolandicus* eggs is dependent on egg size (total organic content of egg), which in turn is correlated to food availability. We suggest furthermore that food quality is of importance in determining egg viability.

The present study shows that both suitable food and the presence of males are essential for production of viable eggs. The few field studies that measure hatching of eggs do not measure these parameters simultaneously with the egg hatching. However, Ianora et al. (1992) showed in a 2-yr seasonal study that the three events with the lowest hatching success of eggs (40 to 60%) coincided with high egg production and low population densities (for *Centropages typicus* in the Gulf of Naples). Our results only allow us to speculate on plausible reasons for low hatching success of eggs in nature when food is sufficient and when population density is low (e.g. during the growth of a spring bloom). We hypothesize that the small population size

may cause failure by females to encounter males, and low hatching is due to nonfertilized eggs.

The present study cannot support the finding of Poulet et al. (1994) and Ianora et al. (1995) that diatom extracts inhibit egg hatching, at least not in the case of *Acartia tonsa* eggs. We found that the low hatching of eggs incubated in high concentrations of phytoplankton extracts is simply due to depletion of oxygen in the incubation water. This is probably due to bacterial decomposition of the substrate. It is clear that copepod eggs will never be exposed in nature to the same high levels of phytoplankton exudates used in the present experiments, not even during peaks of phytoplankton blooms. Therefore, in oxygenated waters external effects of natural diatom populations should not inhibit hatching of *Acartia tonsa* eggs. Exposure to low oxygen levels in the lower water column and sea bed of many coastal systems has been shown do delay embryonic development in copepod eggs (Ambler 1985; Roman et al. 1993), but does not always affect the viability (Lutz et al. 1994). Lutz et al. (1994) showed that newly spawned eggs of *Acartia tonsa* and *Labidocera aestiva* are more sensitive to low oxygen concentrations than older eggs. They also demonstrated that a high percentage of the older eggs exposed to low oxygen concentrations can hatch when they are returned to oxygenated conditions. In our study we used a mixture of old and new eggs (0 to 24 h). We did not test the hatching success of nonhatched eggs after exposure to extracts, but based on Lutz's results we can expect that the eggs in the high extract concentrations in our experiment were either dead (young eggs) or the development was slowed down (older eggs) due to lack of oxygen. Poulet et al. (1994) and Ianora et al. (1995) use newly spawned eggs which are, according to Lutz et al. (1994), very susceptible to low oxygen conditions. Under the high extract concentrations ($>600 \times 10^6$ $\mu m^3 ml^{-1}$ and presumably low oxygen) used in their study the egg development would be expected to halt or the eggs even die.

We conclude that under normoxic conditions hatching of non-diapause, fertilized eggs depends on the nutritional quality of the food. The age of the female does not affect hatching, at least not during the first three weeks after maturation (Fig. 7). We have found no evidence that diatoms have detrimental effects on eggs. Our study shows that diatoms have different nutritional quality, depending on species and physiological status, which in turn controls the quality of eggs. Feeding on the "best" diatom, i.e. fast growing *Thalassiosira weissflogii* cells, results in a high reproductive rate, expressed both as a high egg production rate and a high success in hatching, which is identical to that observed on a non-diatom diet (*Rhodomonas baltica*).

Acknowledgements This research was funded by research grants from NATO (No. 01/B/92/IC), the Icelandic Science Foundation (No. 94-N-099) and NORFA to SHJ and a grant from the Danish Natural Science Research Council (No. 11-0420-1) to TK. The authors greatly acknowledge Dr. S. Poulet for providing an inoculum of the diatom *Thalassiosira rotula* and M. St. John for making the lipid analysis possible. M. Carlsen, S. Klem, J. Melby and C. Mikkelsen provided invaluable assistance in the laboratory, and A. Pii in analysing the fatty acids.

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