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Effect of heterotrophic versus autotrophic food on feeding and reproduction of the calanoid copepod *Acartia tonsa*: relationship with prey fatty acid composition

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ABSTRACT: We determined the egg production efficiency (EPE: egg production/ingestion) and egg viability of the copepod *Acartia tonsa* Dana under different heterotrophic and autotrophic diets. EPE was estimated in adult females either as the slope of the linear relationship between specific egg production (EPR) and ingestion rates, or as the quotient: EPR/ingestion rate. The diets, offered in monoculture, were the heterotrophic ciliates *Strombidium sulcatum* or *Mesodinium pulex*, the heterotrophic dinoflagellate *Gymnodinium dominans*, the autotrophic cryptophyte *Rhodomonas salina* and the autotrophic dinoflagellate *Gymnodinium sanguineum*. The diets were also analyzed for fatty acid contents and composition, relationships with EPE and reproductive success were determined. Clear differences were found in the fatty acid contents and the composition of the different diets offered, but these differences did not correspond with variability in EPE. However, egg viability was correlated with ingestion of certain prey essential fatty acids; interestingly, our data do not show that ciliates and heterotrophic dinoflagellates are nutritionally superior prey for marine copepods, contrary to general expectations.

KEY WORDS: Copepod · *Acartia tonsa* · Ciliate · Dinoflagellate · Egg production · Food quality · Fatty acids · Egg production efficiency · Hatching success

INTRODUCTION

Marine pelagic systems are characterized by the presence of a wide variety of autotrophic and heterotrophic species, which represent potential food items for omnivorous copepods. Copepod diets have recently been shown to be much more diverse than traditional concepts based on the classical food chain implied. Special interest has been devoted to the role of large heterotrophic protists (ciliates and dinoflagellates) in the diet of copepods, because they have been proposed as an intermediate link between the microbial loop and higher trophic levels (Sherr & Sherr 1988, Verity & Paffenhöfer 1996). In fact, several studies have demonstrated that copepods have high clearance rates on microzooplankton (i.e. ciliates and heterotrophic dinoflagellates) compared to those on phytoplankton, indicating positive selection for microzoo-

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plankton (e.g. Dolan 1991, Fessenden & Cowles 1994, Zeldis et al. 2002).

Although in most environments microzooplankton have been shown to be an important contribution to the carbon and nitrogen food ration of copepods (Turner & Anderson 1983, Stoecker & Sanders 1985, Merrell & Stoecker 1998), very few investigations have examined their actual contribution to copepod reproduction, and evidence is often indirect. For instance, the frequent lack of correlation observed in many field studies between *in situ* growth rates of zooplankton and the concentration of phytoplankton (e.g. Dam et al. 1994, Ohman & Runge 1994, Saiz et al. 1999, Calbet et al. 2002) has been used to argue that ciliates may contribute to zooplankton diet. Some field studies have also found correlations between ciliate concentrations and copepod egg production (Kleppel et al. 1991, White & Roman 1992). Stoecker & Egloff (1987) provided more direct evidence from the laboratory, by observing that egg production rates (EPR) of *Acartia tonsa* Dana were enhanced in the presence of ciliates.

In our opinion, further insight into the role of heterotrophic prey on copepod diet requires consideration of its nutritional value and, consequently, its biochemical composition. *A priori* one suspects that differences in metabolism between autotrophism and heterotrophism may result in different biochemical composition of protists. Unfortunately, knowledge of the biochemical composition of planktonic heterotrophic protists is very scarce. Conventional stoichiometric theory based on carbon and nitrogen limitation would suggest a higher nutritional quality of ciliates compared with autotrophs (Stoecker & Capuzzo 1990).

However, recent studies including other indicators of nutritional food quality (e.g. fatty acid composition) appear to be contradictory. For example, Ederington et al. (1995) observed that the bacterivorous ciliate *Pleuronema* sp. as food resulted in lower copepod EPR than the diatom *Thalassiosira weissflogii*, and related this effect to the ciliate's lack of 20:5ω3 (eicosapentaenoic acid, EPA) and 22:6ω3 (docosahexaenoic acid, DHA) fatty acids. It is unclear, however, how representative the bacterivorous scuticociliate *Pleuronema* might be of truly planktonic ciliates (Vaqué et al. 1997, Pitta & Giannakourou 2000).

The main goal of the present study was to determine the nutritional value of heterotrophic prey (ciliates and dinoflagellates) for copepod reproduction. We compared the egg production efficiency (EPE; egg production/ingestion) and egg viability of the copepod *Acartia tonsa* under heterotrophic and autotrophic diets, and analyzed the role of fatty acid composition to explain any differences observed. Furthermore, feeding the heterotrophic cells with different prey, we also explored whether or not the fatty acid composition of heterotrophic organisms depends on the fatty acid composition of their own food and how their ability (or inability) to synthesize copepod essential fatty acids (EFAs) affects their nutritional value as food for copepods.

MATERIALS AND METHODS

Prey cultures. Batch cultures of the autotrophic flagellate *Marsupiomonas pelliculata* and dinoflagellates *Heterocapsa rotundata*, *Gymnodinium sanguineum*, the cryptophyte *Rhodomonas salina* and the diatom *Thalassiosira pseudonana* were grown on either L/1 or F/2 medium (Guillard & Hargraves 1993) under a 12:12 h light:dark cycle.

Three heterotrophic prey were tested: the ciliates *Strombidium sulcatum* and *Mesodinium pulex* (Clone CPH-0006), and the dinoflagellate *Gymnodinium dominans*. Batch cultures of these heterotrophs were grown under different diets in 750 ml flat culture bottles. *S. sulcatum* was grown either on *Marsupiomonas pelliculata* or on natural bacteria developing on wheat-grain; *M. pulex* was grown on *Heterocapsa rotundata*, while *G. dominans* was fed either *Thalassiosira pseudonana* or *Rhodomonas salina*. Cultures of herbivorous heterotrophs were kept under similar light conditions as algae, and were diluted and fed daily. *S. sulcatum* grown on bacteria were kept in darkness and diluted when necessary.

Determination of volume and carbon and nitrogen content of prey. For each of the experiments conducted (see below), samples were taken for size determination of prey. Prey volume was converted to cell carbon and nitrogen contents using factors available in the literature or estimated by us.

Heterotrophs: Volume of heterotrophic prey was calculated by direct microscopic measurement (200 to 400×, n = 50) of acid Lugol's iodine preserved samples (2% final concentration) and assuming simple geometric formulae (ellipsoid for *Strombidium sulcatum* and *Gymnodinium dominans*; sphere for *Mesodinium pulex*). For the estimation of the elemental composition of *G. dominans*, we used the equations pg C cell^{-1} = 0.760 × vol$^{0.819}$ and pg N cell^{-1} = 0.118 × vol$^{0.849}$ provided by Menden-Deuer & Lessard (2000), where the volume is expressed in µm^3. Carbon and nitrogen conversion factors used for ciliates were respectively 0.19 pg C µm^3 and 0.04 pg N µm^3 (corrected for cells preserved in 2% acid Lugol's iodine) according to Putt & Stoecker (1989). Cell losses of both ciliate species due to fixation in 2% acid Lugol's iodine were also estimated to correct ciliate abundance during the experiments by comparing live counts under the stereomicroscope of 1 ml aliquots (n = 20) of water containing few

cells (14 to 20 ciliates ml^{-1}) with 2% acid Lugol's iodine preserved samples processed using our standard counting protocol (see below). In our test, the effect of the fixative reduced the abundance of *S. sulcatum* by 25% (*t*-test, $p < 0.05$) while no effect was observed for *M. pulex*. Accordingly, only *S. sulcatum* abundance in the experiments was corrected for fixation losses.

Autotrophs: Volume of *Rhodomonas salina* was estimated on live cells using an electronic particle counter (Multisizer Coulter Counter). For *Gymnodinium sanguineum*, direct microscopic measurement (200 to 400×, n = 50) of relevant lengths in acid Lugol's iodine preserved cells (2% Lugol's final concentration) were taken and volume estimated assuming an ellipsoid shape.

Elemental composition (C, N) of *Rhodomonas salina* and *Gymnodinium sanguineum* was estimated by filtering a certain volume of the algal cultures on glass fiber filters (Whatman GF/A), which were kept dried until they were processed with a Carlo-Erba CHN analyzer.

The carbon content of *Acartia tonsa* eggs was estimated by filtering about 5000 eggs on glass fiber filters (Whatman GF/A, n = 3), which were kept dried until analysis with a Carlo-Erba CHN analyzer. In parallel, 3 aliquots of the same volume of eggs were fixed in acid Lugol's iodine and counted. A content of 47.6 ng C egg^{-1} and 11.3 ng N egg^{-1} was obtained, comparable with previous estimates for the same species (Kiørboe et al. 1985).

Lipid analysis. Samples for lipid analyses were collected when cultures were in exponential growth. In the case of *Heterocapsa rotundata*, *Rhodomonas salina*, *Marsupiomonas pelliculata*, *Gymnodinium sanguineum* and *G. dominans*, cells were concentrated by filtering known volumes onto pre-combusted GF/F filters, which were processed as described below.

Ciliates were cultured for weeks with the same diet and abundant supply of food. The day prior to sampling for lipid analysis, the ciliate cultures were fed with a very small amount of food to reduce any interference in the analysis due to the presence of the prey in the water. Ciliates were deliberately not starved before lipid analysis as this could have represented an artifact. As ciliates are very fragile and might not resist a low pressure filtration, they were concentrated (up to 4000 to 6000 cell ml^{-1}) either by their phototactic (*Strombidium sulcatum*) or rheotactic (*Mesodinium pulex*) response and washed twice with filtered seawater. Aliquots of all the concentrated samples were preserved in 2% acid Lugol's iodine and counted under a microscope to estimate cell abundance.

The sample for lipid analyses (3 ml) was placed in a test tube and lipids extracted at –20°C for at least 24 h in CH_2Cl_2/methanol under an argon atmosphere, where the final ratio of the extraction mixture was 8:4:3 (vol:vol:vol) CH_2Cl_2/methanol/water according to Christie (1989). The extraction procedure of Folch et al. (1957) was followed. A known amount of C_{23} fatty acid was added to the sample. Fatty acids were transmethylated with BF_3 in methanol (14%) to form fatty acid methyl esters (FAME). The FAME sample was injected into a gas chromatograph (Hewlett-Packard 5809A, with a Omegawax 320 column and equipped with split/splitless injection system) using helium as a carrier gas at 1.8 ml min^{-1}. The injection temperature was 200°C with an initial column temperature of 80°C. The temperature program was an increase of 40°C min^{-1} to 160°C where it was held isothermal for 1 min. Subsequently, the temperature was increased at a rate of 3°C min^{-1} to 220°C where it was kept isothermal for 17 min. For specific fatty acid identification, peaks from chromatographs were compared to Sigma FAME standards, which contained a few extra known fatty acids, while the integrated peaks were compared to the peak area of the C_{23} standard.

Feeding and fecundity experiments. The calanoid copepod *Acartia tonsa* was obtained from cultures kept at the Danish Institute for Fisheries Research (Charlottenlund, Denmark). Batches of eggs were placed in filtered seawater and after hatching the copepods were grown on the cryptophyte *Rhodomonas salina* until adults. Only young adult females from the same egg batch were used for the experiments. Incubations were conducted with a single prey type per bottle. Copepods (females and some males to ensure fertilization) were conditioned for 48 h to prey type and concentration, as well as to other experimental conditions of light and temperature. Prey suspensions were prepared in 0.2 µm filtered seawater (amended with 5 ml l^{-1} of f/2 medium to compensate for copepod excretion). After the conditioning period, three or four 612 ml Pyrex control bottles (without copepods) and four 612 ml Pyrex experimental bottles (with 6 to 10 adult females of *A. tonsa* added to each) were filled with the desired prey suspension, sealed with plastic film and capped with special care to avoid the presence of bubbles. In general, food concentrations were chosen near satiation levels and scaling the relative abundance of each group to natural occurrences. In the case of *R. salina*, however, we set a larger concentration gradient to show a wider functional response.

Two samples were taken to determine the initial prey concentration (50 ml aliquots preserved in 2% acid Lugol's iodine solution for ciliates and dinoflagellates or 3 Multisizer Coulter Counter measurements for *Rhodomonas salina*). Experimental and control bottles were incubated on a rotating plankton wheel

(speed: 0.2 rpm) at room temperature (17 or 20 ± 1°C) under a 12:12 h light:dark cycle. After ca. 24 h, the contents of the bottles were gently filtered through a submerged sieve (180 µm) to collect the copepods. Once aliquots for assessment of prey abundance were taken, the remaining screened water was filtered through a 20 µm mesh and copepod eggs collected. Eggs were then transferred to 320 ml Pyrex bottles filled with filtered seawater and incubated on a rotating wheel at similar conditions of light and temperature as described above. After 24 h (20°C experiments) or 48 h (17°C experiments), the contents of the bottles were filtered through a 20 µm sieve and the unhatched eggs, empty shells and nauplii collected and counted.

Lugol's iodine solution samples for estimation of ciliate and large dinoflagellate concentrations were settled on 10 ml Utermöhl chambers or alternatively filtered onto 0.45 µm cellulose filters, and the number of cells in the whole chamber or filter enumerated under a light microscope (200×). A total of 300 to 600 cells were counted per sample. Eggs that incidentally appeared in these samples (as they were collected previous to the 20 µm screening) were also counted and included in the calculation of egg production.

Average prey concentration and copepod clearance and ingestion rates were computed according to Frost (1972). EPR was estimated as the number of eggs laid per female and per day. EEPE of *Acartia tonsa* was calculated as the quotient between EPR and ingestion rate, or as the slope of the ingestion rate versus egg production rate linear relationship, after conversion to carbon units using the conversion factors specified above.

RESULTS

Nutritional quality of the prey

Size, carbon and nitrogen contents, and C:N ratios of the prey species are given in Table 1. C:N ratios varied from 3.6 to 4.6 among the studied species, with *Gymnodinium dominans* and *Rhodomonas salina* showing the lowest and the highest values, respectively. C:N ratios of heterotrophic prey were not much lower than those of autotrophs, except for *G. dominans*. The major fatty acid groups of saturated (SAFA), monounsaturated (MUFA), polyunsaturated (PUFA) and highly unsaturated (HUFA; carbon chain length >20) fatty acids were diversely represented in the studied species (Table 2). Except for the ciliate *Strombidium sulcatum*, all other species, either autotrophic or heterotrophic, were rich in PUFAs (38 to 123 fg μm^{-3}). Highest HUFAs concentrations were found in the ciliate *Mesodinium pulex* and the dinoflagellates *G. dominans* and *G. sanguineum*.

Although the specific fatty acid profiles differed among prey, clear differences between autotrophic and heterotrophic prey were not evident (Table 2). High concentrations of the fatty acid 22:6ω3 were observed in the ciliate *Mesodinium pulex* and the dinoflagellates *Gymnodinium dominans* and *G. sanguineum*, with both dinoflagellates being in agreement with a characteristic trend in this phylum (Volkman 1989). The 20:5ω3 fatty acid was well represented in *Rhodomonas salina*, *G. dominans* and *G. sanguineum*, while the 18:3ω3 was found in high concentrations only in *R. salina*. The scarcity of fatty acids in the ciliate *Strombidium sulcatum* (57 to 82 total fg fatty acid μm^{-3}) was mostly due to low PUFA content (less than 25% of total fatty acids). Moreover, the high contribution of the fatty acids 16:1ω7 and 18:1ω7 could be reflections of a bacterial diet (Zurkova & Kharlamenko 1999, Véra et al. 2001).

Together with the cell content of a specific fatty acid in the diet, the ratios between certain EFAs (i.e. ω3:ω6 ratio and the ratio between 20:5ω3 and 22:6ω3 [hereafter named 20:22]) have also been suggested as an important indicator of metabolic growth and reproduction in crustaceans (Castell 1982, Harrison 1990). The ω3:ω6 ratio was much lower for all ciliates studied (0.4 to 3.1) than for autotrophic prey and the other heterotrophic protozoans (range: 14.8 to 22.5). The highest 20:22 ratio was found in *Rhodomonas salina*, while *Mesodinium pulex* and *Gymnodinium dominans* presented the lowest 20:22 ratio.

The fatty acid profiles of heterotrophic prey fed on different diets are shown in Table 3. In the case of *Strombidium sulcatum*, both diets offered (bacteria or *Marsupiomonas pelliculata*) were poor in 20:5ω3 and 22:6ω3 fatty acids, although *M. pelliculata* presented high values of the fatty acid 18:3ω3 and PUFAs. *S. sulcatum* fed with *M. pelliculata* showed a lipid profile which varied with diet. However, its content in PUFA and HUFA was higher but not significantly different when fed algae compared to when fed on bacteria, suggesting a lack of capability to synthesize such fatty acids or extract them from the diet. The other ciliate studied, *Mesodinium pulex*, showed a fatty acid composition very different from that of *S. sulcatum* and seems to reflect that of its prey with much higher content of the 22:6ω3 fatty acid, PUFAs and HUFAs than *S. sulcatum*. The fatty acid profile of the heterotrophic dinoflagellate *Gymnodinium dominans* also follows that of its prey. *G. dominans* presented a significantly higher ($p < 0.05$) contents of 18:3ω3 fatty acid when fed on *Rhodomonas salina* than when fed *Thalassiosira pseudonana*.

Feeding, fecundity rates and hatching success

Prey concentration, clearance rates, ingestion rates, EPR, and hatching success of *Acartia tonsa* on the different prey are shown in Table 4. Clearance rates were dependent on prey concentration, with similar values for autotrophs and heterotrophs of similar size. EPRs varied between 2 and 51 eggs laid per female per

Table 1. Size, carbon and nitrogen cell contents of the prey used in the experiments. Equivalent spherical diameter (ESD) corresponded to 2% Lugol fixed cells, except for *Rhodomonas salina*, where size was estimated live with Multisizer Coulter Counter. Carbon and nitrogen contents for heterotrophic prey were estimated from the conversion factors provided by: this study (*R. salina*, *Gymnodinium sanguineum*), Mendel-Deuer & Lessard (2000) (*G. dominans*) and Putt & Stoecker (1989) (*Strombidium sulcatum*, *Mesodinium pulex*)

Copepod prey	Specimen	ESD (µm)	C (ng cell^{-1})	N (ng cell^{-1})	C:N (ng:ng)
Autotrophs	*Rhodomonas salina*	6.5	0.055	0.012	4.6
	Gymnodinium sanguineum	28	3.56	0.85	4.2
Heterotrophs	*Strombidium sulcatum* (bacteria diet)	20.9	0.91	0.22	4.1
	S. sulcatum (*Marsupiomonas pelliculata* diet)	23.0	1.22	0.29	4.2
	Mesodinium pulex (*Heterocapsa rotundata* diet)	14.9	0.33	0.079	4.2
	Gymnodinium dominans	14.6	0.23	0.063	3.7

Table 2. Fatty acid composition of copepod prey expressed as fg µm^{-3} (averages of 2 measurements). SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; HUFA, highly unsaturated fatty acids; 20:22 is the ratio between 20:5ω3 and 22:6ω3. Sum of all fatty acids found, included that which are not presented separately, is shown. The diet of the heterotrophs is specified in brackets: M = *Marsupiomonas pelliculata*, B = bacteria, T = *Thalassiosira pseudonana*, R = *Rhodomonas salina*

	Autotrophs		**Heterotrophs**				
	Rhodomonas salina	*Gymnodinium sanguineum*	*Mesodinium pulex* (H)	*Strombidium sulcatum* (M)	*S. sulcatum* (B)	*G. dominans* (R)	*G. dominans* (T)
14:0	9.2	20.2	13.2	1.6	2.2	13.7	7.9
14:1(ω5)	0.1	3.4	1.5	0.7	1.7	0.0	0.0
15:0	1.0	3.5	2.1	0.7	0.9	2.8	2.3
16:0	22.5	86.1	31.5	9.3	11.3	43.9	30.5
16:1(ω9)	1.7	1.5	8.5	1.2	1.4	0.5	1.9
16:1(ω7)	1.7	3.3	1.3	14.2	28.9	0.8	1.9
17:0	0.7	2.3	1.3	0.4	0.6	1.7	3.2
18:0	15.5	30.3	21.7	4.9	8.3	16.5	14.3
18:1(ω9)	6.4	35.1	9.0	2.0	2.1	8.8	6.6
18:1(ω7)	9.0	11.6	3.8	5.7	8.2	4.3	2.1
18:2(ω6)	2.5	4.8	3.2	0.7	1.6	4.3	1.6
18:3(ω6)	2.0	0.0	0.5	0.5	0.7	0.0	0.0
18:3(ω4)	2.6	0.0	28.0	2.3	4.3	0.0	0.0
18:3(ω3)	27.0	0.0	0.8	2.7	0.2	4.4	0.3
18:4(ω3)	49.5	0.0	4.5	0.0	0.3	0.0	0.0
20:4(ω3)	0.7	0.0	0.0	0.1	0.0	0.0	0.0
20:5(ω3)	15.5	35.3	6.8	0.8	0.2	15.5	9.2
22:5(ω3)	0.3	0.4	0.0	0.1	0.0	0.7	0.0
22:6(ω3)	7.0	36.5	27.8	0.7	0.0	42.9	26.8
24:1(ω9)	0.9	0.0	3.2	1.5	0.5	0.0	0.0
Fatty acid content	183.8	280.9	240.6	56.6	81.8	161.3	111.6
SAFA	48.8	142.5	73.1	17.5	24.1	78.6	58.2
MUFA	19.9	61.3	27.3	25.7	43.1	14.4	15.4
PUFA	112.3	77.1	123.0	11.9	11.5	68.2	38.0
HUFA	23.7	72.2	58.8	2.5	0.9	59.6	36.0
ω3	99.9	72.2	44.8	5.3	1.0	63.9	36.3
ω6	4.8	4.8	26.0	1.7	2.7	4.3	1.6
ω3/ω6	21.0	14.9	1.7	3.1	0.4	14.8	22.5
20:22	2.2	1.0	0.2	1.1	–	0.4	0.3

Table 3. Fatty acid composition (as % of total fatty acids) of the heterotrophic prey offered to copepods, and of the diet offered to those prey. H = *Heterocapsa rotundata*, B = bacteria, M = *Marsupiomonas pelliculata*, R = *Rhodomonas salina*, T = *Thalassiosira pseudonana*

	M. pulex (H)	*H. rotundata*	*S. sulcatum* (B)	Bacteria	*S. sulcatum* (M)	*M. pelliculata*	*G. dominans* (R)	*R. salina*	*G. dominans* (T)	*T. pseudonana*
14:0	5.5	11.7	2.7	2.5	2.9	1.0	8.5	5.0	7.1	11.1
14:1ω5	0.6	0.2	2.0	0.8	1.3	0.1	0.0	0.1	0.0	0.0
15:0	0.9	0.5	1.1	1.9	1.3	0.2	1.8	0.5	2.0	2.1
16:0	13.1	16.9	13.8	19.7	16.4	13.0	27.2	12.2	27.4	29.0
16:1ω9	3.5	3.1	1.7	2.8	2.1	0.9	0.3	0.9	1.7	2.7
16:1ω7	0.5	0.4	35.4	21.8	25.1	0.8	0.5	0.9	1.7	13.4
17:0	0.5	0.3	0.7	1.0	0.7	0.1	1.0	0.4	2.9	1.2
18:0	9.0	2.7	10.2	9.6	8.6	2.0	10.2	8.4	12.8	11.8
18:1ω9	3.8	2.1	2.6	2.9	3.5	23.1	5.5	3.5	5.9	4.5
18:1ω7	1.6	1.1	10.0	16.0	10.1	2.2	2.7	4.9	1.9	1.0
18:2ω6	1.3	2.1	2.0	1.1	1.3	3.3	2.7	1.4	1.4	1.1
18:3ω6	0.2	0.3	0.9	0.9	0.8	0.2	0.0	1.1	0.0	0.0
18:3ω4	11.6	1.9	5.2	5.1	4.0	0.8	0.0	1.4	0.0	0.0
18:3ω3	0.3	1.5	0.3	0.0	4.7	40.5	2.7	14.7	0.3	0.0
18:4ω3	1.9	17.1	0.3	0.0	0.0	0.1	0.0	26.9	0.0	0.0
20:4ω3	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.4	0.0	0.0
20:5ω3	2.8	0.3	0.2	0.2	1.3	0.1	9.6	8.4	8.3	19.0
22:5ω3	0.0	0.2	0.0	0.0	0.2	0.3	0.4	0.1	0.0	0.0
22:6ω3	11.5	13.8	0.0	0.0	1.2	0.4	26.6	3.8	24.0	3.1
24:1ω9	1.3	0.6	0.7	0.0	2.7	0.3	0.0	0.5	0.0	0.0
SAFA	30.4	32.4	29.5	35.4	30.9	16.6	48.8	26.5	52.2	55.2
MUFA	11.4	9.6	52.8	44.3	45.4	27.5	8.9	10.8	13.8	21.7
PUFA	51.1	56.1	14.1	14.8	21.0	54.4	42.3	61.1	34.0	23.1
HUFA	24.4	26.6	1.1	1.8	4.5	0.9	36.9	12.9	32.3	22.1

day, this maximum being similar to maximum EPRs previously reported for this copepod species in the laboratory (Kiørboe et al. 1985, Støttrup & Jensen 1990).

Fig. 1a,b shows the relationship between ingestion rate and EPR of *Acartia tonsa* expressed in carbon units. For the *Rhodomonas salina* diet, feeding and fecundity showed a significant linear relationship (r^2 = 0.72, $p < 0.001$), with an EPE estimated from the slope as 21%. The observations from the *Gymnodinium sanguineum* diet were conspicuously different from the *R. salina* data set; however, the scarcity of data and the narrowness of its range precluded regression analysis, so that EPE was estimated simply as the quotients EPR/ingestion rate. The estimated EPE for *G. sanguineum* ranged from 43 to 48%. A comparison of both procedures to calculate EPE for the *R. salina* data set

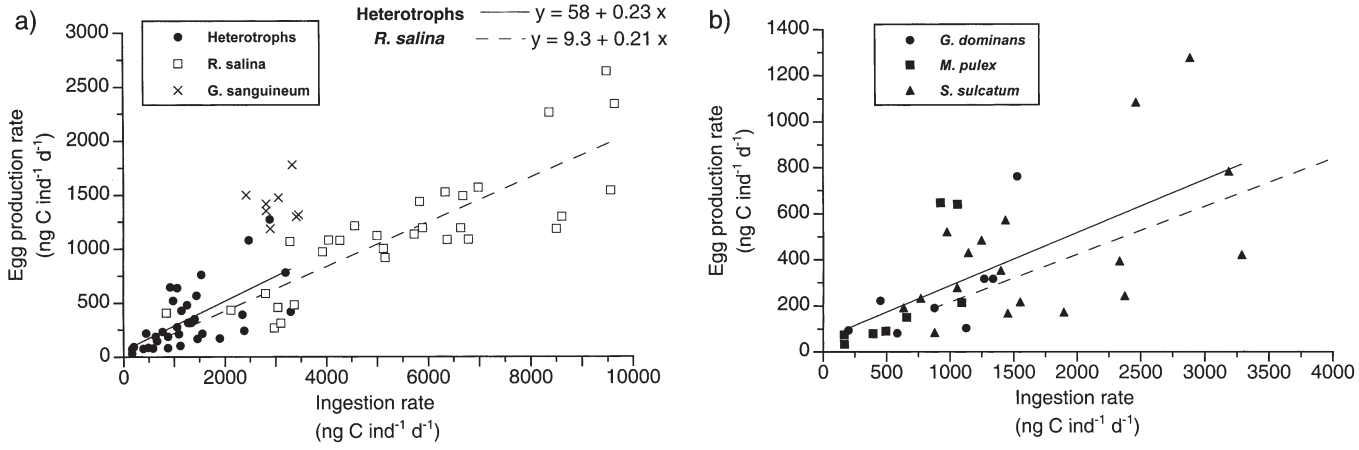

Fig. 1. *Acartia tonsa.* Egg production and ingestion rates of expressed in ng C ind.$^{-1}$ d^{-1}. (a) Diets on the autotrophic prey *Rhodomonas salina* and *Gymnodinium sanguineum* and on heterotrophic prey (all pooled in a single category, Heterotrophs). Linear fits for Heterotrophs and *R. salina* are shown. (b) Detail showing the heterotrophic prey of the category 'Heterotrophs'

Table 4. Average prey concentration in the experimental bottles, clearance rates, ingestion rates, egg production rates (EPR), egg production efficiency (EPE) in carbon and nitrogen units, and hatching success of the copepod *Acartia tonsa* fed on autotrophic and heterotrophic diets. Average values ± SE are shown. nd = not determined

Prey	Prey diet	Prey concentration (cell ml^{-1})	Clearance (ml ind.$^{-1}$ d^{-1})	Ingestion (cell ind.$^{-1}$ d^{-1}) (×10^3)	EPR (eggs ind.$^{-1}$ d^{-1})	EPE Carbon	EPE Nitrogen	Hatching success (%)
Autotrophic diets								
Rhodomonas salina	–	1723 ± 1	19 ± 10	32 ± 18	7 ± 2	0.27 ± 0.2	0.25 ± 0.2	nd
R. salina	–	2380 ± 14	22 ± 2	52 ± 5	11 ± 1	0.17 ± 0.03	0.16 ± 0.03	nd
R. salina	–	2917 ± 4	18 ± 0.2	51 ± 0.4	8 ± 2	0.12 ± 0.02	0.11 ± 0.02	nd
R. salina	–	4644 ± 69	14 ± 2	63 ± 8	23 ± 0.1	0.28 ± 0.03	0.26 ± 0.03	nd
R. salina	–	5913 ± 36	11 ± 0.2	67 ± 1	22 ± 1	0.25 ± 0.01	0.23 ± 0.01	nd
R. salina	–	7997 ± 72	10 ± 0.5	80 ± 4	25 ± 1	0.24 ± 0.02	0.22 ± 0.02	nd
R. salina	–	8896 ± 44	10 ± 0.6	91 ± 5	22 ± 2	0.24 ± 0.02	0.17 ± 0.01	nd
R. salina	–	14703 ± 248	9 ± 2	128 ± 31	31 ± 1	0.20 ± 0.04	0.18 ± 0.04	nd
R. salina	–	15456 ± 85	6 ± 0.3	99 ± 5	33 ± 0.5	0.22 ± 0.01	0.14 ± 0.02	95
R. salina	–	16324 ± 58	9 ± 0.4	153 ± 7	51 ± 2	0.25 ± 0.01	0.21 ± 0.01	91
R. salina	–	18070 ± 101	7 ± 0.9	127 ± 16	25 ± 0.1	0.15 ± 0.02	0.24 ± 0.01	67
R. salina	–	18666 ± 61	5 ± 0.4	92 ± 6	23 ± 2	0.19 ± 0.003	0.18 ± 0.003	nd
R. salina	–	29912 ± 36	4 ± 0.02	113 ± 0.9	27 ± 4	0.18 ± 0.03	0.17 ± 0.03	nd
R. salina	–	36574 ± 125	3 ± 0.5	125 ± 19	25 ± 2	0.15 ± 0.01	0.14 ± 0.01	nd
Gymnodinium sanguineum	–	7 ± 0.3	119 ± 9	0.9 ± 0.03	29 ± 1	0.48 ± 0.04	0.50 ± 0.04	70
G. sanguineum	–	10 ± 0.3	96 ± 5	0.9 ± 0.02	31 ± 2	0.43 ± 0.04	0.44 ± 0.04	69
Heterotrophic diets								
Strombidium sulcatum	Bacteria	49 ± 1.5	68 ± 6.1	3.2 ± 0.2	19 ± 4	0.30 ± 0.07	0.39 ± 0.1	36
S. sulcatum	Bacteria	14 ± 0.6	59 ±11.8	0.8 ± 0.1	3 ± 1	0.19 ± 0.10	0.25 ± 0.1	20
S. sulcatum	*Marsupiomonas pelliculata*	38 ± 1.0	35 ± 6.9	1.3 ± 0.2	7 ± 2	0.23 ± 0.09	0.29 ± 0.1	62
S. sulcatum	*M. pelliculata*	11 ± 0.8	134 ± 29	1.4 ± 0.2	6 ± 1	0.20 ± 0.07	0.25 ± 0.1	27
S. sulcatum	*M. pelliculata*	20 ± 1.3	49 ± 9	0.9 ± 0.1	8 ± 2	0.32 ± 0.03	0.41 ± 0.04	52
Mesodinium pulex	*Heterocapsa rotundata*	56 ± 2.3	63 ± 8.8	3.5 ± 0.4	9 ± 3	0.41 ± 0.10	0.53 ± 0.2	83
M. pulex	*H. rotundata*	31 ± 1.3	38 ± 11.6	1.1 ± 0.3	2 ± 0.3	0.25 ± 0.06	0.31 ± 0.1	85
Gymnodinium dominans	*R. salina*	43.2	49	0.2	3	0.19	0.13	71
G. dominans	*Thalassiosira pseudonana*	74 ± 3.7	60 ± 9.1	4.3 ± 0.5	8 ± 3	0.29 ± 0.06	0.21 ± 0.05	96

resulted in similar estimates (slope: 21%; quotients: 22 to 25%).

For *Acartia tonsa* on heterotrophic diets, the narrow range of values and the variability among diets and among experiments for the same diet precluded linear regression for each diet independently. Since the quotient egg production/ingestion did not vary significantly among heterotrophic diets (1-way ANOVA, $p = 0.9$), all data were pooled and 1 single linear regression analysis was conducted for heterotrophic diets to give a more robust estimate. EPE for heterotrophic prey estimated as the slope of this linear relationship was 23%, significantly different from 0 ($r^2 = 0.41$, $p < 0.001$). Calculations done in terms of nitrogen resulted in a EPE of 21% for heterotrophs and 23% for *Rhodomonas salina*.

When all prey are considered together, there appears to be a positive relationship between the ingestion of certain fatty acids and the EPR of *Acartia tonsa* (Fig. 2). However, when considered individually, the different prey are associated with different patterns. Thus, there is absence of correlation considering only heterotrophic prey, while *Rhodomonas salina* always show the highest significant correlation. *Gymnodinium sanguineum* tends to appear as a different case.

However, because ingestion of fatty acid and ingestion of carbon are not independent variables ($r^2 = 0.9$, $p < 0.001$), part of the significant correlation obtained between the ingestion of specific fatty acids and the EPR when all prey are considered together, may reflect variations in carbon ingestion and, eventually, in food availability. To attempt a better discrimination of the effects of fatty acid contents on EPRs, independent of food availability, we searched for a relationship between the specific fatty acid content of the prey (as pg µm^{-3}) and the residuals from the regression analysis between EPR (eggs ind.$^{-1}$ d^{-1}) and carbon ingestion rate (ng C ind.$^{-1}$ d^{-1}). No significant correlation was observed between the specific prey content of the

PUFA and ω3-type fatty acids and the residuals from the egg production carbon ingestion regression analysis (Fig. 3a,b). However, in the case of the fatty acids 20:5ω3 and 22:6ω3, a weak but significant correlation was observed (Fig. 3c,d). Again, *Gymnodinium sanguineum* appears to be a different case, and its exclusion from the regression analysis resulted in no significant correlation for any type of fatty acid considered.

There was an even scatter in the hatching success of *Acartia tonsa* from being poor (20 to 40% on *Strombidium sulcatum* when fed on bacteria) to high (>80% for several prey diets) (Table 4). Actually, bacteria-fed *S. sulcatum* showed the lowest rates of hatching (28% on average), while hatching was much higher (47% on average) when the same species was fed on *Marsupiomonas pelliculata.* There were asymptotic relationships between both the ingestion rate of PUFA (Fig. 4a) and EFAs (sum of 20:5ω3, 22:6ω3 and 18:3ω3) (Fig. 4b) and egg hatching success. It appears that for *A. tonsa* the ingestion of <0.1 µg of EFA in its daily ration is associated with a decline on hatching success. In our experiments, this very low hatching success was observed only when *A. tonsa* fed exclusively on *S. sulcatum*; it is noticeable, however, that the other heterotrophic prey provided sufficient EFA ingestion for successful egg hatching. Hatching success was also correlated with the fatty acid ratio ω3:ω6 ($r^2 = 0.42$, $p < 0.01$), but not with the fatty acid ratio 20:22.

DISCUSSION

We examined the nutritional value of heterotrophic prey for copepod reproduction, studying both the EPE and the egg viability of the copepod *Acartia tonsa* under mono-specific diets, and explored their relationship with prey fatty acid composition. The heterotrophic diets were compared with autotrophic ones known to be suitable for copepod growth, such as the cryptophyte *Rhodomonas salina* and the naked

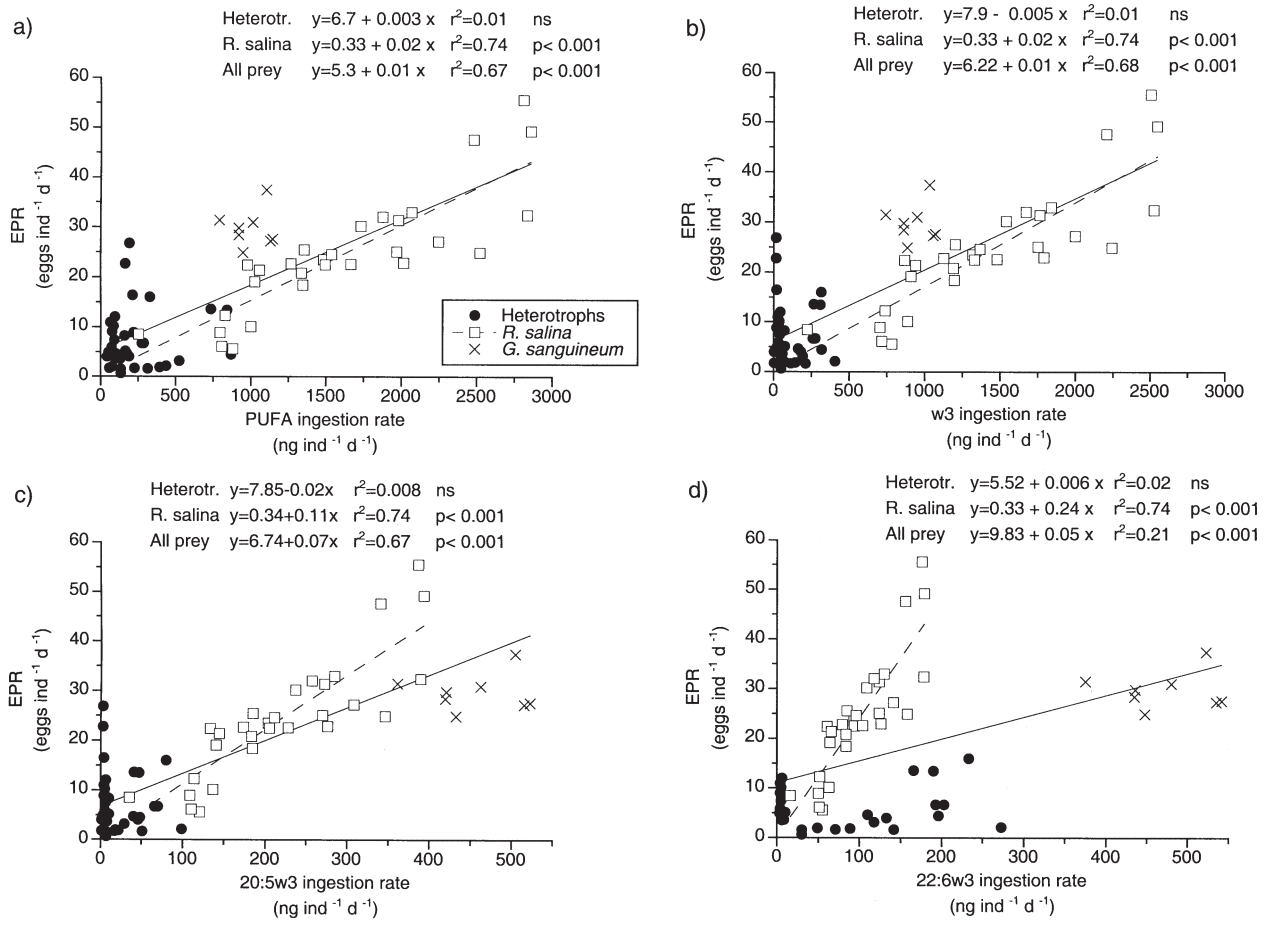

Fig. 2. *Acartia tonsa.* Egg production rates (egg ind.$^{-1}$ d^{-1}) as function of fatty acid ingestion (ng ind.$^{-1}$ d^{-1}). Regression analysis for diets on heterotrophic prey, *Rhodomonas salina* or all diets together are shown. Regression lines only shown for *R. salina* (dashed) and all diets (continuous). (a) Polyunsaturated fatty acids (PUFAs), (b) ω3, (c) 20:5ω3, (d) 22:6ω3

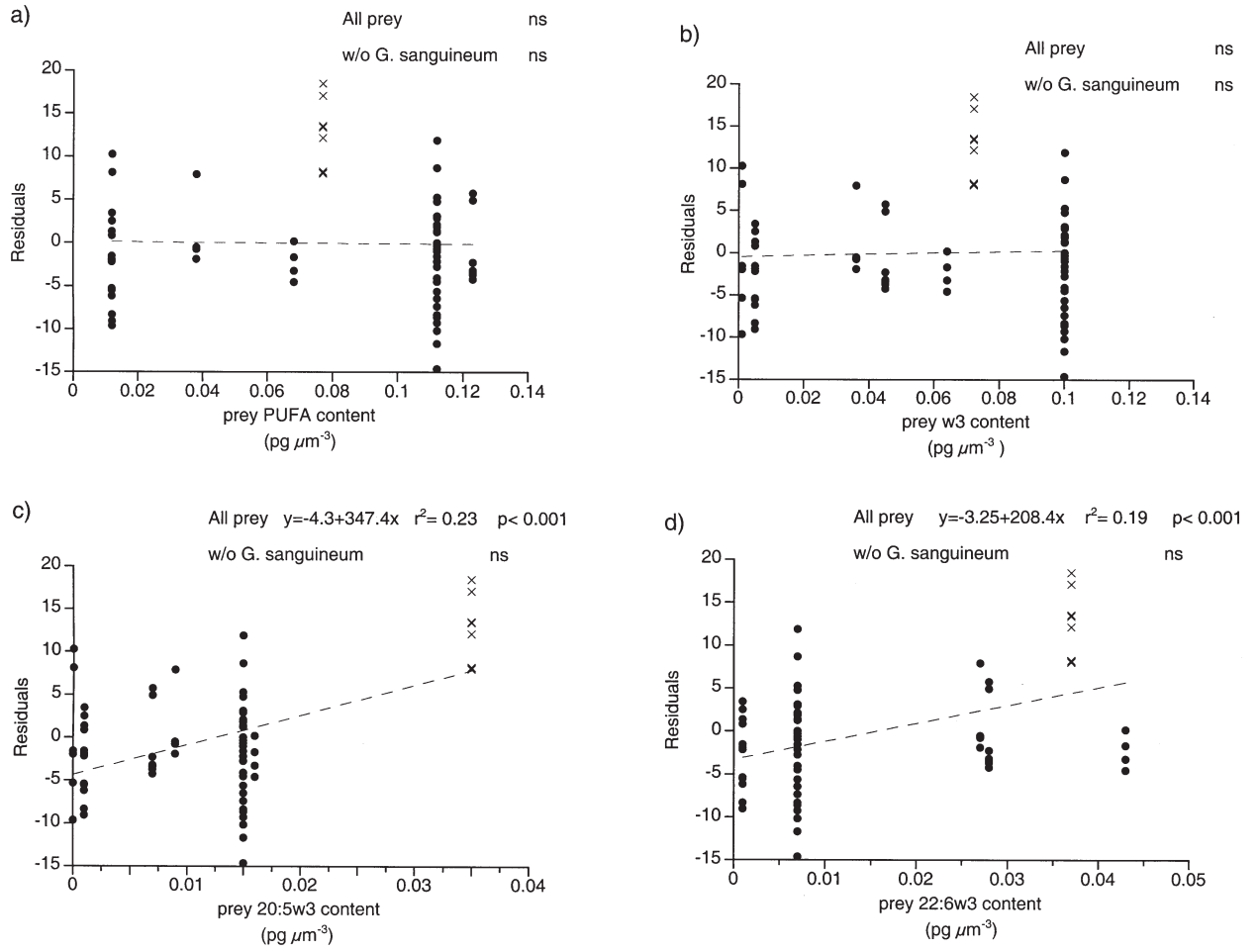

Fig. 3. *Acartia tonsa*. Relationship between the residuals from the egg production-carbon ingestion regression analysis (Fig. 1) and the fatty acid contents of the prey (as pg μm^{-3}). (a) Polyunsaturated fatty acids (PUFAs), (b) ω3, (c) 20:5ω3, (d) 22:6ω3. Crosses are for the dinoflagellate *Gymnodinium sanguineum*. Dashed line represents all prey fit. Results of regression analyses for all prey or excluding *G. sanguineum* are shown

dinoflagellate *Gymnodinium sanguineum*. Clearly, laboratory studies under such conditions are not representative of the situation that copepods encounter in the field. As copepods are typically omnivorous, any deficiency resulting from a nutritionally poor diet could be compensated in the field by feeding on a wider spectrum of prey. However, monospecific experiments are always a first-step approach to exploring the contribution of a potential prey to the diet and nutrition of a predator, and thereby, providing valuable information.

The nutritional value of a prey item has been frequently described with stoichiometric ratios (e.g. C:N). Actually, protozoans have been suggested to be qualitatively important as nourishment for zooplankton because of their low C:N ratios compared to phytoplankton (Stoecker & Capuzzo 1990). In our study, C:N ratios of *Rhodomonas salina* and *Gymnodinium sanguineum* were lower than the ratio of 6:1 commonly accepted for phytoplankton (Parsons et al. 1984), but were similar to the ratios for heterotrophic prey (except for *G. dominans*, which showed the lowest C:N ratio), indicating that prey items, from a C:N stoichiometric point of view, differed little. However, although stoichiometric analysis can be indicative, there is evidence that C:N ratios are not adequate predictors of nutritional quality for crustaceans (Cahoon 1981, De Biase 1990) and recent attempts to obtain a better description of food quality have focused on different biochemical compounds, for example fatty acids (Ederington et al. 1995, Kleppel et al. 1998). Fatty acids are the principal form of stored energy in many organisms. Certain types of fatty acids are considered essential (EFA) as they cannot be easily synthesized by the organism and must be obtained in sufficient quantity from the food to maintain growth and survival (Sargent & Falk-Petersen 1988, Harrison 1990, Olsen 1998). EFA can be obtained directly from phytoplankton, but as our

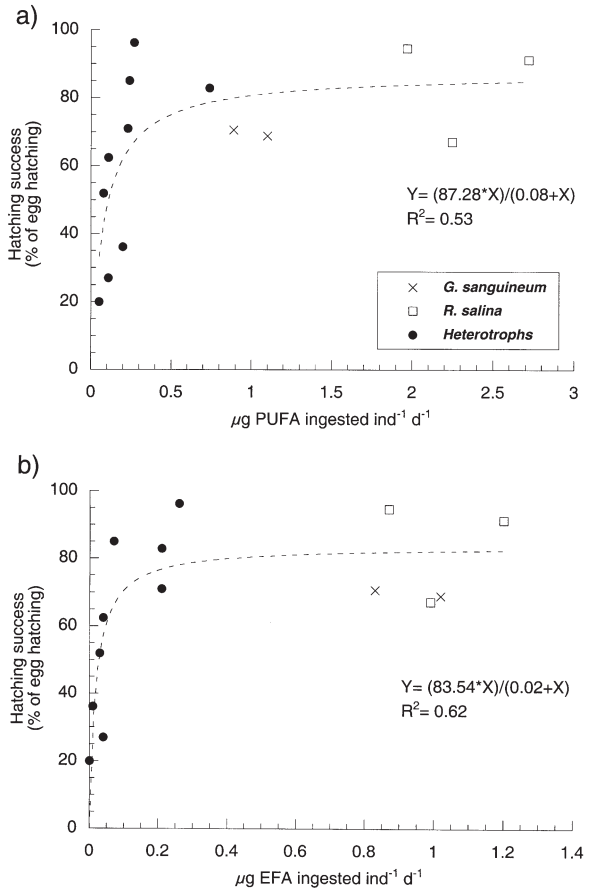

Fig. 4. *Acartia tonsa*. Relationship between hatching success and ingestion rates of fatty acids. (a) Polyunsaturated fatty acids (PUFAs), (b) essential fatty acids (EFAs) (18: 3ω3 + 20:5ω3 + 22:6ω3). Asymptotic curves are shown: Y = hatching success, X = ingestion of fatty acids

analysis and others show (e.g. Brown et al. 1998, Volkman 1989), the different protists vary greatly in their EFA content. PUFAs seem to play an important role in cell membrane activity as precursors of prostaglandins, the hormones regulating ionic fluxes, oocyte maturation and egg production in invertebrates (Harrison 1990).

Taxonomic differences in fatty acid composition have been well established in the case of autotrophic phytoplankton (e.g. Volkman 1989). Our study and that of Klein Breteler et al. (1999) revealed that heterotrophic dinoflagellates are particularly rich in the fatty acid 22:6ω3 and that their fatty acid composition does not differ significantly from that of autotrophic dinoflagellates (Volkman 1989). In the case of ciliates, previous studies on marine ciliates reported very low contents of PUFA for bacterivorous ciliates (*Euplotes crassus*, <25%, Zhukova et al. 1999; *Pleuronema* sp., <15%; Ederington et al. 1995) and for the ciliate *Fabrea salina* fed on the prymnesiophyte *Isochrysis galbana* (Harvey et al. 1997). In contrast, Claustre et al. (1988) found very high PUFA contents for the herbivorous tintinnid *Stenosemella ventricosa* (59%).

In our study, we have dealt with heterotrophic ciliate genera commonly found in pelagic marine ecosystems (Vaqué et al. 1997, Pitta & Giannakourou 2000). Lipid profiles of ciliates have been reported to reflect the fatty acid composition of their diet (Harvey et al. 1997). In this regard, bacteria are particularly poor in PUFA, while in general, algae tend to have higher contributions of PUFA (cited in Desvilettes et al. 1997). Our results show that heterotrophic prey also follow the trend by having similar profiles of fatty acid composition compared to their diets. However, the differences between heterotrophs fed with different diets were not always significant, which suggests that individual species may tend to maintain a certain stoichiometry in their fatty acid composition, regardless of diet. Thus, it seems that the ciliate *Strombidium sulcatum* does not have the necessary fatty acid precursor to elongate HUFA when feeding on bacteria, and indeed appears to be particularly lacking in them. However, when feeding on *Marsupiomonas pelliculata*, *S. sulcatum* seems to be able to elongate 18:3n3 to EPA and DHA (pathways shown in Castell 1982). A similar trend can be observed for *Gymnodinium dominans*.

Although the heterotrophs we studied appeared to be very different in terms of fatty acid content and composition both within the group and also compared to autotrophic species, our results show that EPE of *Acartia tonsa* was similar when fed on heterotrophs and on autotrophs, and fell within the range of previous estimates for planktonic protozoans and metazoans (Straile 1997). In fact, the differences in EPE between the examined autotrophic prey were larger than those observed among the studied heterotrophic prey. Hence, the autotrophic dinoflagellate *Gymnodinium sanguineum*, which contained very high amounts of the 20:5ω3 and 22:6ω3 fatty acids but not higher PUFA contents than those of *Rhodomonas salina* or *Mesodinium pulex*, exhibited the highest EPE among the diets studied. Among studies investigating fatty acid composition there are some discrepancies regarding the importance of these components for the growth of copepods. For some copepods, the 20:5ω3 (EPA) and 22:6ω3 (DHA) fatty acids appear to be particularly important and have been correlated with copepod growth and development (Jónasdóttir 1994, Jónasdóttir & Kiørboe 1996, Pond et al. 1996, Støttrup et al. 1999). However, Støttrup & Jensen (1990) observed that the EPE of *A. tonsa* fed on *Isochrysis galbana* was higher than when fed *Thalassiosira weissflogii*, despite the fact that *T. weissflogii* was richer in 20:5ω3 and 22:6ω3 fatty acids. Lee et al. (1999) found no significant correlation between the egg

production of the copepod *Pseudocalanus newmani* and either 20:5ω3 or 22:6ω3 concentrations in the diet.

To date, most studies have only attempted to find relationships between fecundity rates and fatty acid content of the diet, independently of actual fatty acid ingestion rates. Our correlation of specific fatty acid ingestion with EPR shows a possible dependence of egg production on these biochemical compounds. However, considering that fatty acids are only a fraction of the total carbon pool and that the carbon-based ingestion also showed the same high correlation with EPR, it was difficult to discern if the effect on EPR was due to the carbon ingestion or to the lipid ingestion. The inconclusive result of the residual analysis could be either the consequence of a species-specific difference in fatty acid requirement, or of a need for other essential nutritional components not measured here (e.g. sterols, amino acids and proteins), which appear to be implicated in regulating copepod egg production (for sterols, e.g. Ederington et al. 1995, Klein Breteler et al. 1999; for amino acids, e.g. Kleppel et al. 1998, Guisande et al. 2000; for proteins, e.g. Jónasdóttir 1994, Kleppel & Hazzard 2000).

However, we should not forget that recruitment (i.e. reproduction success) to copepod populations depends not only on fecundity rates but also on the viability of eggs. There is little literature available on the hatching success of heterotrophic diet-fed copepods. In our experiments, food quality seems to affect the viability of the eggs more than the EPR or EPE. Egg hatching success was found to be influenced by the ingestion of PUFA and the sum of the EFAs 18:3ω3, 20:5ω3 and 22:6ω3 (Fig. 4). It seemed that a minimum amount of EFAs was needed to reach >60% hatching success. Thus, fatty acid content and composition appear to be important factors in determining egg hatching success of *Acartia tonsa*. Our results are in agreement with general patterns of PUFA dependence of hatching success and embryonic development in crustaceans (Jónasdóttir & Kiørboe 1996, Tang et al. 2001). Similarly, the study of Ederington et al. (1995) showed equally high hatching success for the copepod *A. tonsa* fed either diatoms or bacterivorous ciliates. While these diets differed greatly in their fatty acid profile, they had same relative concentration of PUFA, qualitatively agreeing with our study.

In conclusion, our results on fatty acid composition and its relationship with EPR do not support the notion that ciliates or heterotrophic dinoflagellates are nutritionally different from other prey, e.g. autotrophic ones, for copepods. However, further research is needed in order to complete the description of the biochemical composition of heterotrophs, because biochemical compounds other than fatty acids can be also essential for copepod growth and egg viability. From an ecological point of view, these results imply that if the preference for ciliates and heterotrophic dinoflagellates exhibited by copepods in many field studies is not directly related to their higher nutritional quality, other factors like their size, shape, motility and patch behavior can very likely play a major role in favoring this predator–prey interaction.

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