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Supplemental effects of diet mixing on absorption of ingested organic carbon in the marine copepod *Acartia tonsa*

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ABSTRACT: We investigated increased carbon absorption efficiencies (AEs) as a possible cause for positive effects of diet mixing on copepod egg production rates (EPRs) and hatching success (EHS). Female *Acartia tonsa* were fed $^{14}C/^{51}Cr$ dual-labelled *Dunaliella tertiolecta* (*Dun*), *Amphidinium carterae* (*Amp*), *Phaeocystis globosa* (*Pha*), and 3 pairwise 1:1 mixes of the 3 diets. AEs, derived from the ratios of labels in algae and copepod faecal pellets, were 44% on *Dun*, 37% on *Amp*, and 49% on *Pha*, but increased significantly to 61% on *Dun* + *Amp*. As a result, EPRs remained low in all tested diets except for *Dun* + *Amp*, where it was twice that in the individual diets. Linear multiple regression analysis revealed that EPRs were strongly dependent on the ingestion and absorption of the fatty acids 18:3(n-3) and 22:6(n-3) so that the simultaneous ingestion and absorption of 18:3(n-3) from *Dun* and 22:6(n-3) from *Amp* enhanced EPR in the *Dun* + *Amp* diet. EHS was low with the *Dun* diet, which was devoid of 20:5(n-3) and 22:6(n-3). Multiple regression analysis showed that EHS depended on 16:1(n-7) and any or all of 22:6(n-3), 20:5(n-3), or 18:5(n-3).

KEY WORDS: Copepods · Food quality · Absorption efficiency · Diet mixing · Fatty acid composition

INTRODUCTION

Copepod secondary production is constrained not only by the availability of plankton prey but also by the nutritional quality of the prey. The nutritional composition of the available food is of paramount importance to growth and egg production, and when the species composition of the phytoplankton community fluctuates so does copepod production. The composition of essential nutritional compounds such as fatty acids and amino acids differ considerably among algal species (Jónasdóttir 1994, Kleppel et al. 1998), and copepods may accordingly optimise the nutritional intake through behavioral adaptation of feeding activity. This is possible not only through selective grazing on good quality prey containing all essential nutritional compounds. During periods when poor quality phytoplankton species predominate, as for instance during summer in temperate waters, high copepod production may be sustained by combined ingestion of different phytoplankton species. During these periods, when food quality limits growth and production, diet mixing increases the probability of a nutritionally balanced food intake and consequently higher growth (Kleppel & Burkart 1995).

Previous studies have shown that differences in prey quality induce differences in copepod egg production, longevity, and population development (Schmidt & Jónasdóttir 1997, Payne & Rippingale 2000, Rey et al. 2001, Shin et al. 2003). These differences often arise from varying gross growth efficiencies (GGE) (Rey et al. 2001) not only due to differences between monospecific diets but also due to diet mixing (Kleppel & Burkart 1995).

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Since, in terms of carbon:

$$d = g + r + u \quad (1)$$

and

$$\mathrm{GGE} = \frac{g}{i} \quad (2)$$

where d is the rate of carbon absorption in the gut, g is growth rate, r is respiration rate, u is excretion rate, and i is ingestion rate, it follows that:

$$g = d - (r + u) \quad (3)$$

and

$$\mathrm{GGE} = \frac{d - (r + u)}{i} \quad (4)$$

Increased growth rates or GGE are therefore caused either by lower respiration rates, lower excretion rates, higher absorption rates, or a combination of these, at any given ingestion rate. Respiration is strictly coupled to biosynthesis (Thor 2000) and earlier studies have shown increased rather than decreased respiration rates in copepods feeding on good quality diets as opposed to poor quality diets (Thor et al. 2002). Furthermore, excretion rates of carbon (urea) are very low compared to respiration and egg production rates (Miller & Glibert 1998), and we therefore predict that any enhancing effects of diet mixing may be caused by increased absorption rates in the copepod gut. The objective of the study was therefore to investigate the effect of diet mixing on the efficiency and rate of absorption of ingested carbon and how this affects egg production in *Acartia tonsa*. The chlorophyte *Dunaliella tertiolecta*, the dinoflagellate *Amphidinium carterae* and the prymnesiophyte *Phaeocystis globosa* were chosen as poor quality diets. Previously, these phytoplankton species have been reported to induce low rates of growth and reproduction in marine copepods (Støttrup & Jensen 1990, Koski et al. 1998, Tang et al. 2001).

The term assimilation has previously been erroneously used to describe the uptake of nutrients in the gut (Calow & Fletcher 1972). In the present paper, we use the term absorption. For the fraction of ingested carbon absorbed we use the term absorption efficiency (AE) which can be conveniently measured by comparing rates of ingestion (i) and egestion (e):

$$\mathrm{AE} = \frac{d}{i} = \frac{1 - e}{i} \quad (5)$$

Unless otherwise noted in the text, we compare only with previous studies measuring absorption efficiency and not assimilation efficiency (in its true meaning), regardless of what term has been used in those studies.

MATERIALS AND METHODS

Acartia tonsa were reared in culture at Kristineberg Marine Biological Station, Sweden, at 18°C in 0.3 µm filtered seawater (34 PSU) under indirect natural light conditions. The copepods were sustained on a diet consisting mainly of a mixture of *Thalassiosira weissflogii*, *Rhodomonas baltica*, *Tetraselmis impellucida*, and *Isochrysis galbana*.

Dunaliella tertiolecta, *Amphidinium carterae* and *Phaeocystis globosa* were cultured in 34 PSU 0.3 µm filtered and autoclaved seawater at 18°C at a light: dark cycle of 14:10 h. The seawater was enriched with *f*/2 growth medium for *D. tertiolecta* and *A. carterae* and *L*1 medium for *P. globosa* (Guillard 1975), the latter with gentle bubbling. *P. globosa* were filtered through a 30 µm mesh prior to all experiments to exclude colonies.

Prior to all experiments the copepods were acclimatised in 1000 ml glass bottles to the experimental diets at a total algal concentration corresponding to 150 µg C l^{-1} for 48 h on a rotating plankton wheel (0.5 rpm) to allow the copepods to adjust to the experimental food type and concentration.

Ingestion rates, egg production rates, and egg hatching success. Six different diets at a total concentration corresponding to ~150 µg C l^{-1} were prepared. These were *Dunaliella tertiolecta* (*Dun*), *Amphidinium carterae* (*Amp*), *Phaeocystis globosa* (*Pha*), *D. tertiolecta* + *A. carterae* (*Dun* + *Amp*), *D. tertiolecta* + *P. globosa* (*Dun* + *Pha*), and *A. carterae* + *P. globosa* (*Amp* + *Pha*). The mixed diets were prepared as 1:1 mixtures on a carbon concentration basis. Replicate 20 ml samples (n = 3 or 4) were fixed with Lugol's solution for measurements of initial algal cell concentrations. Replicates (n = 3 or 4) of each algal mixture were prepared in 1000 ml glass bottles and 30 *Acartia tonsa* females were added. Likewise replicate controls (n = 3 or 4) without copepods were prepared. All bottles were incubated for 12 to 20 h in dim light conditions on the plankton wheel (0.5 rpm). After the incubation, 20 ml samples were fixed in Lugol's solution for measurements of final algal cell concentrations. Copepod eggs were filtered onto a 30 µm mesh and counted under the dissecting microscope.

Ingestion rates (IR) were measured from the disappearance of particles in the bottles containing copepods compared to the control bottles (Frost 1972). The cell concentrations of the initial and final algal samples were counted using an Elzone 5380 electronic particle counter in the 2 to 12 µm particle size range. This size range was used to avoid false counts due to larger debris in the water. For the mixed diets, numbers of cells of the 2 algal species were estimated separately by fitting 2 normal distribution curves to the two-peaked size frequency distribution output from the particle counter. The fit giving the best correlation between the size frequency distribution output and the sum of the 2 normal distribution curves was chosen by

iteration. The correlation coefficients (r) of these fits were never below 0.98. Algal carbon contents were estimated from several measurements of cell volume with the particle counter using an average volume-to-carbon conversion regression for non-diatom phytoplankton (Montagnes et al. 1994). The calculated weights were 17.1 ± 3.1 pg C cell^{-1} for *Dunaliella tertiolecta*, 37.3 ± 13.0 pg C cell^{-1} for *Amphidinium carterae*, and 7.97 ± 2.06 pg C cell^{-1} for *Phaeocystis globosa*.

Egg production rates (EPR) were calculated from the total number of eggs produced during the incubation period. Carbon specific rates were estimated using a specific egg mass of 45.7 ng C egg^{-1} (Kiørboe et al. 1985). Produced eggs were kept in petri dishes at the experimental temperature for 48 h and hatched nauplii and unhatched eggs were counted to calculate egg hatching success (EHS). Gross growth efficiencies (GGE) were calculated as EPR/IR × 100 %.

Ten copepods from each experiment were treated with 4 % formaldehyde, frozen and analysed for carbon weight using high temperature combustion. Rates of ingestion and egg production were normalized to the copepod's body carbon weight.

Absorption efficiencies. Absorption efficiency (AE) was measured using a dual tracer technique with ^{14}C as the absorbable tracer and ^{51}Cr as the inert tracer (Calow & Fletcher 1972). *Dunaliella tertiolecta*, *Amphidinium carterae*, and *Phaeocystis globosa* were incubated with 500 µCi $NaH^{14}CO_3$ l^{-1} and grown for at least 5 cell divisions prior to the experiments to ensure uniform labelling. One day before the experiments 100 µCi $^{51}CrCl_3$ l^{-1} were added. The $^{51}Cr^{3+}$ was kept dissolved in 0.5 M HCl in the stock solution. When introduced into the algal culture medium (pH ~ 8.5) the $^{51}Cr^{3+}$ adsorbs to particles. Prior to the incubations with copepods, the labelled algae were rinsed by centrifugation and resuspending in 0.3 µm filtered seawater. The centrifugal force was chosen so that algal cells were alive and motile after the process. *D. tertiolecta* were centrifuged at 500 × *g* for 5 min and *A. carterae* and *P. globosa* at 1000 × *g* for 5 min. As a further precaution, after one experiment it was ensured that all 3 algal cultures grew well after the centrifugation process.

Algal mixtures were prepared as for the IR and EPR measurements from the labelled algal stocks. Samples of 200 to 500 ml from these mixtures were fixed in Lugol's solution. Replicate 10 ml subsamples (n = 5) were filtered onto GF/F filters and the isotopic activities were measured by liquid scintillation counting (Beckman LS5000TD liquid scintillation counter). Auger electrons (4.5 keV) and X-rays (5 keV) from ^{51}Cr was counted from channel 0 to 270 and β-electrons from ^{14}C (156 keV) were counted from channel 270 to 1000 on the liquid scintillation counter (cf. Sheppard & Marlow 1971), both corrected for counting efficiencies and quenching. As for the IR and EPR measurements, 3 or 4 replicates of each algal mixture were prepared in 1000 ml glass bottles, 30 copepods were added, and the bottles were incubated for 6 hr in dim light conditions on the plankton wheel. Subsequently, faecal pellets were filtered onto a 30 µm mesh, washed with 0.3 µm filtered seawater 3 times, and rinsed into petri dishes. All intact faecal pellets were carefully mouth pipetted into scintillation vials using drawn Pasteur pipettes. Isotopic activity of the faecal pellets was measured as above. AEs were calculated as:

$$AE = \left(1 - \frac{R_{faeces}}{R_{algae}}\right) \times 100\% \quad (6)$$

where R_{faeces} and R_{algae} are the ratios between the isotopic activities of ^{14}C and ^{51}Cr in faecal pellets and algae. Absorption rates (AR) were calculated as IR × AE.

Fatty acid composition. Approximately 20 ml of each algal culture was filtered onto pre-combusted GF/F filters. The filters were frozen at −80°C and stored for fatty acid composition analysis by gas chromatography. Lipids were extracted by ultrasound into CH_2Cl_2-methanol (2:1 v/v) and the fatty acids transmethylated with BF_3-methanol to form fatty acid methyl esters (FAME). Identification of fatty acids was accomplished by comparison with retention times of several FAME standards: Larodan PUFA, fatty acid from the dinoflagellate *Prorocentrum mimimum* to locate 18:5(n-3), Matreya PUFA3, and Supelco FAME mix 18919.

Statistics. IRs, EPRs, and AEs were compared between diets by ANCOVA with algal concentration as the covariate. This approach was chosen since algal concentrations were not entirely equal between experiments (Table 1). Subsequent Bonferroni post-hoc tests were used to test for differences between specific diets.

Table 1. Algal concentrations in the different experiments: absorption efficiency experiments (AE), ingestion rate experiments (IR), and egg production rate experiments (EPR) in µg C l^{-1}. *Dun* = *Dunaliella tertiolecta*, *Amp* = *Amphidinium carterae*, *Pha* = *Phaeocystis globosa*. *Dun* + *Amp*, *Dun* + *Pha*, and *Amp* + *Pha* denote mixed diets. Numbers are means ± SD of 3 or 4 replicates

Diet	AE		IR & EPR	
Dun	137	±15	173	± 1
Amp	230	±13	142	±57
Pha	202	±20	168	±44
Dun + *Amp*	183	± 1	151	±17
Dun + *Pha*	169	±17	151	±19
Amp + *Pha*	216	± 5	162	± 2

To test whether overall carbon ingestion and the fatty acid composition of the diet could explain variations in EPR and EHS we calculated IRs of specific fatty acids on all diets from the fraction of individual fatty acids and the carbon ingestion rates assuming that total fatty acids constitute 13% of the carbon mass of algal cells (Arendt et al. 2005). Likewise, to test the extent to which variations in EPR could be explained by the overall carbon AE and fatty acid composition of the diet we calculated estimated absorption rates (ARs) of specific fatty acids as IR of the fatty acids × AE. These ARs are an approximation since they do not explain the actual absorption rates of specific fatty acids. IRs of specific fatty acids were compared using Pearson product moments correlation analysis. EPRs and EHS were compared to IRs and ARs of specific fatty acids by linear multiple regression analysis.

RESULTS

Ingestion rates, egg production rates, and egg hatching success

Ingestion rates, egg production rates, and egg hatching success on the 6 diets are shown in Table 2. There were no significant differences in IR between diets (1-factor ANCOVA: $F_{5,11} = 3.122$, $p = 0.054$) but the power of the test was only 0.477 so the negative result should be interpreted cautiously. From the data it is obvious that any differences would arise from the lower ingestion rates on the *Dun* diet. EPRs were significantly different between diets (1-factor ANCOVA: $F_{5,26} = 7.23$, $p < 0.001$). This difference was caused by significantly higher EPRs on the *Dun* + *Amp* diet (Bonferroni post hoc test: $p < 0.0001$). On the other hand, EPRs in the *Dun* + *Pha* and *Amp* + *Pha* treatments were not significantly different from those in the corresponding monospecific diet treatments. Gross growth efficiencies (GGEs) averaged 37% for *Dun* + *Amp*, whereas it was below 25% on all other diets (Table 2). EHS were significantly different (1-factor ANOVA: $F_{5,13} = 24.8$, $p < 0.001$) due to the low hatching success of eggs produced on the *Dun* diet (Bonferroni post-hoc test: $p < 0.001$) (Table 2). Algal cell concentrations were lowered by 20–30% during incubations so we are confident with the IR measurements.

Absorption efficiencies

Diets had significant effects on AEs (Table 2; 1-factor ANCOVA on arcsine-square root transformed AEs: $F_{5,12} = 182.9$, $p < 0.001$). The AE of the *Dun* + *Amp* diet was significantly higher than on both monospecific diets (Bonferroni post-hoc test: $p < 0.001$). This enhancing effect was not seen with the 2 other mixed diets: *Dun* + *Pha* induced lower and *Amp* + *Pha* unchanged AEs. The absorption rate (IR × AE) increased more than 3-fold on *Dun* + *Amp* compared to the corresponding monospecific diets (Table 2).

Relationship between ingestion and absorption of fatty acids, and EPR or EHS

The fatty acid composition was different between the 3 algal species (Fig. 1). There was extensive intercorrelation between the IRs of the different fatty acids (Pearson product moment analysis, $p < 0.05$) with 3 distinct intracorrelating groups:

Table 2. *Acartia tonsa*. Ingestion rates (IR), absorption efficiencies (AE), absorption rates (AR), egg production rates (EPR), gross growth efficiencies (GGE), and egg hatching success (EHS) of *A. tonsa* fed 6 different algal diets. See Table 1 for abbreviations of diets. Numbers are mean ± SD of 3 to 4 replicates

Diet		IR ($\mu g C\ \mu g C^{-1}\ d^{-1}$)	AE (%)	AR ($\mu g C\ \mu g C^{-1}\ d^{-1}$)	EPR ($\mu g C\ \mu g C^{-1}\ d^{-1}$)	GGE (%)	EHS (%)
Dun		0.099 ± 0.063	43.9 ± 2.3	0.043	0.022 ± 0.011	22	5.7 ± 0.7
Amp		0.372 ± 0.081	40.6 ± 1.1	0.151	0.034 ± 0.022	9	43.9 ± 3.9
Pha		0.261 ± 0.131	49.2 ± 1.6	0.128	0.034 ± 0.002	13	35.3 ± 3.7
Dun + *Amp*	*Dun*	0.121 ± 0.021	61.1 ± 1.5	0.253	0.153 ± 0.029	37	36.4 ± 4.9
	Amp	0.293 ± 0.147					
	Sum	0.414 ± 0.168					
Dun + *Pha*	*Dun*	0.218 ± 0.071	29.3 ± 0.4	0.140	0.067 ± 0.007	14	37.3 ± 2.1
	Pha	0.261 ± 0.020					
	Sum	0.479 ± 0.087					
Amp + *Pha*	*Amp*	0.280 ± 0.131	43.9 ± 0.3	0.150	0.058 ± 0.020	17	34.6 ± 2.6
	Pha	0.061 ± 0.019					
	Sum	0.340 ± 0.123					

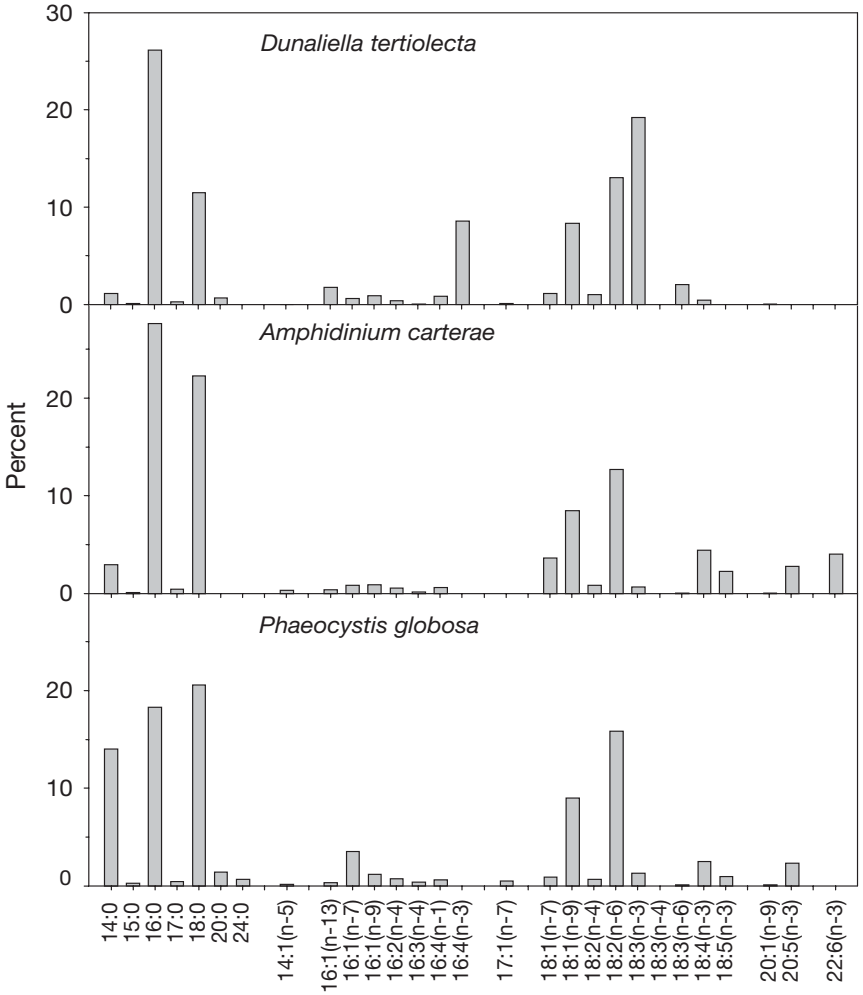

Fig 1. Distribution of fatty acids in the 3 algal diets as a percent of the total mass of fatty acids

(1) 18:3(n-3), 16:4(n-3), 16:1(n-13), and 18:3(n-6)
(2) 22:6(n-3), 20:5(n-3), and 18:5(n-3)
(3) 16:1(n-7)

The linear multiple regression analysis showed a significant positive correlation between EPRs and IRs of the 18:3(n-3) and 22:6(n-3) groups (Table 3). Using this regression we were able to explain 40% of the variation in EPRs. Multiple regression tests are only possible with independent variables (fatty acid groups) with no significant intercorrelation, but judging from the size of the variance inflation factors the intercorrelation was low (Table 3). The correlation between EPRs and fatty acids became stronger when applying the AR data. Here we were able to explain 70% of the variation in EPRs with variations in fatty acid ARs (Table 3).

EHS varied significantly with IRs of the 16:1(n-7) and 22:6(n-3) groups and the regression could explain as much as 82% of the variation (Table 4). Unlike the regression of EPR we did not find stronger correlations when applying the AR data.

DISCUSSION

In the present study we demonstrated that EPRs in *Acartia tonsa* feeding on a mixed diet of the chlorophyte *Dunaliella tertiolecta* and the dinoflagellate *Amphidinium carterae* were higher than EPRs on any of the 2 species alone. Concurrently, we found significantly higher AE on this diet and we were able to explain 80% of the variations in EPR between the diets by variations in estimated ARs of 3 groups of unsaturated fatty acids in the gut.

Given the right food conditions *Acartia tonsa* can achieve EPRs of 50% body C d^{-1} (recalculated from Jónasdóttir 1994). Considering the average female biomass of 4.6 µg C, the egg production rates in our study

Table 3. *Acartia tonsa*. Linear multiple regression analysis of EPR vs. ingestion rates (IR) and absorption rates (AR) of specific fatty acid groups. VIFs are variance inflation factors, a measure of the intercorrelation between variables

	Coefficient ± SD	p	VIF	Global R^2
$IR_{constant}$	−2.76 ± 0.45	<0.001		
$IR_{18:3(n-3)}$	44.1 ± 18.0	0.027	1.35	
$IR_{16:1(n-7)}$	89.6 ± 85.6	0.312	1.32	
$IR_{22:6(n-3)}$	115.1 ± 46.9	0.027	1.41	0.400
$AR_{constant}$	−0.02 ± 0.07	0.796		
$AR_{18:3(n-3)}$	24.3 ± 6.75	0.003	1.21	
$AR_{16:1(n-7)}$	28.6 ± 35.9	0.437	1.45	
$AR_{22:6(n-3)}$	78.3 ± 17.9	<0.001	1.21	0.702

Table 4. *Acartia tonsa.* Linear multiple regression analysis of EHS vs. ingestion rates (IR) and absorption rates (AR) of specific fatty acid groups. VIFs are variance inflation factors

	Coefficient ± SD	p	VIF	Global R^2
$IR_{constant}$	0.04 ± 0.04	0.344		
$IR_{18:3(n-3)}$	−0.11 ± 1.63	0.948	1.35	
$IR_{16:1(n-7)}$	53.6 ± 7.73	<0.001	1.32	
$IR_{22:6(n-3)}$	28.9 ± 4.24	<0.001	1.41	0.828
$AR_{constant}$	0.11 ± 0.04	0.018		
$AR_{18:3(n-3)}$	−8.66 ± 3.89	0.042	1.21	
$AR_{16:1(n-7)}$	116.4 ± 20.7	<0.001	1.45	
$AR_{22:6(n-3)}$	57.0 ± 10.3	<0.001	1.21	0.747

were all below 10% body C d^{-1} on the monoalgal diets, comparable to other studies using similar poor quality diets (Verity & Smayda 1989, Støttrup & Jensen 1990, Kleppel & Burkart 1995, Tang et al. 2001). The transformation of the ingested energy from the monoalgal diets was poor, with GGEs as low as 10% on all 3 monoalgal diets, proving the less than optimal quality of these diets. Turner et al. (2002) arrived at the same conclusion for *Phaeocystis* sp. eaten by *Temora stylifera* and *Calanus helgolandicus*. Discussing data from their own and 2 other studies (Verity & Smayda 1989, Tang et al. 2001), Turner et al. (2002) concluded that copepods may well ingest *Phaeocystis* sp. at relatively high rates but the resulting egg production, and thus GGE, remains low; they also speculated that *Phaeocystis* sp. constitutes a poor food source due to the lack of certain fatty acids. The same has been suggested for *Dunaliella tertiolecta* (Støttrup & Jensen 1990).

From the energy balance equation it is evident that the low EPRs on the 3 monoalgal diets were a direct consequence of AEs below 50%. In comparison, Conover (1966a) reported AEs of natural zooplankton assemblages ranging from 41 to 87%. Tang & Dam (1999) reviewed literature data on carbon AE of herbivorous copepods and found AEs for carbon mostly above 60 to 70%. More importantly, Conover (1966b) engaged in the first study of food quality effects on copepod AE. Here *Calanus hyperboreus* experienced AEs ranging from 40 to 72% on a range of different phytoplankton diets. A few other studies describe food quality effects on AE in copepods. Pagano & Gaudy (1986) fed *Eurytemora velox* different diets consisting of natural plankton assemblages and a range of different algal species. Analogous to Conover (1966b), they found a negative relationship between AEs and ash content of the algal diets. However, one assumption for the method used in those studies is that only the organic (or non-ash) fraction of the food is affected by the digestive processes. Thus, the ash content is used directly in the calculation of AEs and any regressions between AEs and ash content will carry an inherent element of autocorrelation. Besiktepe & Dam (2002) extracted AEs by comparing ingestion and egestion rates of carbon established by carbon:volume ratios of algal cells and faecal pellets and found AEs in *Acartia tonsa* around 10% on the heterotrophic dinoflagellate *Oxyrrhis* sp. and the diatom *Thalassiosira weissflogii*, while the ciliate *Uronema* sp., and the autotrophic dinoflagellate *Prorocentrum minimum* generated AEs at 85%.

Interestingly, both Conover (1966b) and Besiktepe & Dam (2002) found AEs above 85% on *Dunaliella* sp., much higher than our values around 45%. It is difficult to explain why AE values would deviate so significantly between studies but it may depend on differences in methodology. Ingestion and egestion rates of *Acartia tonsa* feeding on *Dunaliella tertiolecta* are often quite low, making accurate measurements of these parameters difficult (Støttrup & Jensen 1990, Besiktepe & Dam 2002, Thor et al. 2002). Furthermore, calculations of AEs on the basis of ash may be biased since *Dunaliella* sp. contains only small amounts of ash (Conover 1966b). On the other hand, radiotracers can be used to follow the fate of organic carbon down to quantities in the nanogram range. In all experiments the measured isotopic activities were well above background levels and highly reproducible. There is therefore no reason why we should refrain from using the data for dietary comparisons in our study.

Egg production rates increased by 100% on the *Dun + Amp* diet relative to the monoalgal diets. This kind of supplemental effect of mixed diets on copepod egg production has been observed previously (Kleppel & Burkart 1995, Schmidt & Jónasdóttir 1997) and a parallel study showed EPRs increasing from 0.03 to 0.10 d^{-1} in *Temora longicornis* when switching from the monoalgal diets to a mixture of *Dunaliella tertiolecta* and *Amphidinium* sp. (Koski et al. 2006). Since the ingestion rates in our study were not significantly higher on the *Dun + Amp* diet than on the monoalgal diets, the GGE on the *Dun + Amp* diet was over 3 times that on the monoalgal diets. This enhancement of GGE coincided with considerably higher AE and AR. It therefore seems plausible that EPRs increased at least in part as a result of increased absorption of organic carbon, and it appears that the effects of diet mixing set in already during absorption in the gut. This notion has been put forward previously by Mayzaud et al. (1998): They fed *Acartia tonsa* diatom (*Thalassiosira weissflogii*) and diatom detritus diets and recorded decreased AEs on the latter. Mayzaud et al. (1998) speculated that gut absorption in '*Acartia*-type' copepods may be adapted to essential nutrients, in this case nitrogen/protein. Obviously this could also apply for other nutritional components like fatty acids.

Highly unsaturated fatty acids (HUFAs), especially the omega-3 (n-3) HUFAs, are important for the functioning of the cell membrane. Since these are not synthesised de novo by copepods they must be obtained from the diet. Of these HUFAs, the 22:6(n-3) and 20:5(n-3) fatty acids are essential for egg production in calanoid copepods (Jónasdóttir 1994, Shin et al. 2003, Arendt et al. 2005), hence the significant correlation of EPRs to IR and AR of the 22:6(n-3) fatty acid group. However, though *Amphidinium carterae* contained both 20:5(n-3) and 22:6(n-3), EPRs remained low on this diet; this suggests that EPRs in *Acartia tonsa* depend on additional food components. Based on the EPRs with the mixed diets it appears that *Dunaliella tertiolecta* could supplement *A. carterae* but not *Phaeo-*

cystis globosa. A comparison of the algal fatty acids (Fig. 1) shows that this additional component could be either 18:3(n-3) or 16:4(n-3). These 2 fatty acids belonged to the same intracorrelating group with respect to IR, and the multiple regressions showed that ingestion and absorption of fatty acids from the 18:3(n-3) group was highly important for egg production. This is consistent with field observations by Hazzard & Kleppel (2003). Interestingly, even though *D. tertiolecta* contained high amounts of 18:3(n-3), the *Dun* diet did not induce high EPRs and the multiple regression showed the 22:6(n-3) group to be equally important for EPR. This group contained 20:5(n-3), 18:5(n-3) and 22:6(n-3). *A. carterae* contained both 22:6(n-3) and 20:5(n-3) whereas *P. globosa* contained only 20:5(n-3). Both algae contained 18:5(n-3). Thus, if indeed EPRs were enhanced by differences in the fatty acid contents of the diets, the logical extension is that simultaneous ingestion and absorption of 18:3(n-3) or 16:4(n-3) from *D. tertiolecta* and 22:6(n-3) from *A. carterae* caused the higher EPRs.

The copepod gut is probably evolutionarily adapted to absorption of essential nutrients, including essential fatty acids, and a higher content of these fatty acids in the diet could stimulate overall carbon AE. A study of the freshwater cladoceran *Daphnia magna* has shown that a higher content of nutrients, in this case phosphorous, may indeed stimulate overall carbon AE (DeMott et al. 1998). Experiments with dual-labelled ($^{14}C/^{32}P$) *Scenedesmus acutus* showed that carbon AE declined as the C:P ratio of the diet increased. Adding unlabelled P-deficient algae reduced the carbon AE for labelled P-sufficient algae, whereas adding unlabelled P-rich algae improved the carbon AE for labelled P-deficient algae (DeMott et al. 1998). If this also applies for fatty acids in copepods, the increased EPR observed in our study could have been caused not only by fatty acid supplementation for biosynthesis, but also by the increased carbon AE triggered by ingestion of essential fatty acids on the *Dun* + *Amp* diet.

Egg hatching has been shown to depend on the availability of several fatty acids, particularly 20:5(n-3), 22:6(n-3), 18:3(n-3) and 18:4(n-3) (Lee et al. 1999). *Dunaliella tertiolecta* was devoid of both 20:5(n-3) and 22:6(n-3) and it is not surprising that egg hatching success was significantly lower on the *Dun* diet. With the multiple regression analysis we found that EHS depends on the 22:6(n-3) group and 16:1(n-7). Since EHS was significantly higher on all other than the *Dun* diet it is difficult to judge which of the fatty acids from the 22:6(n-3) group were responsible. Interestingly, we did not find any positive correlation between EHS and the IR or AR of the 18:3(n-3) group fatty acids. EHS may thus depend on any of 22:6(n-3), 20:5(n-3), or 18:5(n-3) and on 16:1(n-7).

The present study is the first of its kind, combining measurements of absorption with analysis of dietary fatty acid content. Since absorption measurements of specific fatty acids would involve individually labelled fatty acids, we used theoretical fatty acid ARs. Even though dietary fatty acids are not always absorbed equally well in the copepod gut (Hazzard & Kleppel 2003) it is reasonable to assume that essential fatty acids would be preferentially absorbed in the gut. We found that 18:3(n-3) (or 16:4[n-3]) and 22:6(n-3) are essential for egg production and previous studies have shown that these are incorporated into copepods with high efficiency (recalculated from Hazzard & Kleppel 2003). We therefore believe that any differential absorption would only strengthen the relationship we found between fatty acid ARs and EPRs. Thus, applying the estimated AE of fatty acids, has brought us one step closer to the elusive connection between diet composition in terms of fatty acids and egg production and hatching in copepods.

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