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Effect of the addition of polyunsaturated fatty acids to the diet on the growth and fecundity of *Daphnia galeata**

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SUMMARY

1. The importance of long-chain polyunsaturated fatty acids (PUFAs), in particular eicosapentaenoic acid (EPA), for the growth and development of *Daphnia galeata* (Sars) was tested using food types differing in PUFA and EPA contents.
2. Life history experiments of *D. galeata* fed with the cryptophyceans *Rhodomonas lacustris* (Pascher & Ruttner) and *Cryptomonas pyrenoidifera* (Gentler), and the green alga *Scenedesmus acutus* (Meyen), showed that both cryptophycean species were higher in quality than *S. acutus*.
3. Since the cryptomonads contained significant amounts of EPA while no EPA could be detected in *Scenedesmus*, tests were performed to ascertain whether EPA was responsible for the differences in food quality. Feeding daphnids a mixed diet of *Scenedesmus* and emulsion particles that were rich in EPA and DHA, resulted in a significant improvement in the intrinsic population growth rate. The initial difference in food quality between *Scenedesmus* and the cryptomonads was completely compensated for by addition of emulsion to the *Scenedesmus* food.
4. From the observed stimulatory effect of the addition PUFA to the daphnid diet, this study concludes that the presence of such long-chain PUFA improves food quality for daphnids.

Introduction

Biochemical composition is among the major factors that determine the nutritional value of algae and detritus particles for zooplankton, the others being morphology, cell wall digestibility and toxicity (Ahlgren, Gustafsson & Boberg, 1992; Müller-Navarra, 1995a, b). Because several biochemical constituents of animals cannot be synthesized *de nova*, these organisms have a dietary requirement for such compounds, which include essential fatty acids (FA), amino acids and vitamins. Therefore, uptake of carbon, nitrogen or phosphorus, and even total lipids, proteins and carbohydrates may not be an adequate measure of food quality for zooplankton grazers; for instance, even if lipids, proteins or carbohydrates are abundant in food particles, the food quality will be poor if a specific FA or an amino acid is absent. Because the composition of algal FA largely varies with species and growth conditions (Piorreck, Baasch & Pohl, 1984; Wood, 1988), FA are a good starting point for the search for the components determining food quality for zooplankton under natural conditions.

Major FA like palmitic acid (C16 : 0) and oleic acid (C18 : 1ω9) can be synthesized *de nova* from acetate intermediates, and only a limited number of FA are essential for animals (Salati & Goodridge, 1996). FA are dynamic compounds, which can be rapidly metabolized into related FA by the action of specific enzymes: desaturases and elongases. However, such conversions

are limited since this enzyme activity in animal tissues is restricted to the carboxyl part of a FA molecule. The doubl-bond formation at the methyl side, in particular at the ω3 or ω6 position (the number indicates the position of the last double bond counted from the methyl side, which can be seen as the terminus of the molecule), is absent in most animals (Cook, 1996). The need for ω3- and ω6-FA and the inability for *de nova* synthesis results in the essentiality of these FA, although ω6-FA can be synthesized in some animals (Cripps, Blomquist & DeRenobales, 1982). Hence, the ω3- and ω6-FA cannot be converted to one another. Examples of essential FA are linoleic acid (C18 : 2ω6), linolenic acid (C18 : 3ω3), and eicosapentaenoic acid (EPA, C20 : 5ω3), although the last-named can originate from linolenic acid. To date, several papers have reported the importance of polyunsaturated fatty acids (PUFA) for *Daphnia* species, suggesting that the long-chain PUFA, particularly EPA, found in Cryptophyceae algae make them superior in food quality to cyanobacteria and green algae (Ahlgren *et al.* 1990, 1992; Müller-Navarra, 1995a, b). In line with these reports, the present study hypothesizes that dietary EPA and other related long-chain FA can enhance the nutritive value of food for daphnids. This hypothesis is tested by studying the effects on life history of algal diets with and without long-chain PUFAs. Because differences in morphology or biochemical composition other than FA between the algal species can be responsible for changes in food quality, daphnids were also fed a mixture of *Scenedesmus acutus* (which lacks EPA) and a PUFA-emulsion that contained 15% EPA. Life-history traits of *Daphnia galeata* were studied to test whether the addition of this PUFA emulsion enhanced the nutritive value of *Scenedesmus.*

Materials and methods

Algae and Daphnia *cultures*

Cryptomonas pyrenoidifera (Gentler) and *Rhodomonas lacustris* (Pascher & Ruttner) were obtained from the Norwegian Institute for Water Research (NIVA-2/81 and NIVA-8/82, respectively). *Scenedesmus acutus* (Meyen) and the cryptomonads were grown in 1-l continuous cultures in a medium described in Table 1. Algae were grown at 17 °C on a light : dark cycle of 16 : 8 h. The dilution rate was 0.4 day^{-1} for the two cryptomonads and 1.0 day^{-1} for *Scenedesmus.* Algae were harvested daily, filtered through a 30-μm mesh nylon filter to remove clumps and concentrated by centrifuging for 5 min at 2000 r.p.m. The algal pellet was resuspended in filtered (0.45 μm) Lake Maarsseveen water, which was also used for rearing daphnids (see below). Algal concentration was determined spectrophotometrically using a calibration curve of extinction vs. carbon concentration, which was derived from chemical oxygen demand measurements (Gulati, Siewertsen & Van Liere, 1991).

Table 1 Mineral composition of algal medium

Component	mg ml^{-1}	Component	μg ml^{-1}
$NaNO_3$	170	$MnSO_4$ H_2O	77.0
K_2HPO_4	17	$ZnSO_4$ $7H_2O$	23.0
Na_2SiO_3 $9H_2O$	28	$CuSO_4$ $5H_2O$	5.0
$CaCl_2$ $2H_2O$	37	$(NH_4)_6Mo_7O_{24}$ $4H_2O$	5.0
$MgSO_4$ $7H_2O$	37	$Co(NO_3)_2$ $6H_2O$	15.0
H_3BO_3	2.5	Vitamin B_{12}	0.5
Na_2EDTA $2H_2O$	1.861	Biotin	0.5
$NH_4Fe(SO_4)_2$ $12H_2O$	0.964	Thiamine	100.0

Experiments were carried out with a *D. galeata* clone (DGY1) isolated from IJsselmeer and maintained in cultures at the laboratory. This clone was reared in membrane-filtered (0.45-μm) water from Lake Maarsseveen using a light : dark regime 16 : 8 h at 17.5 °C. The daphnids were fed with *S. acutus*, at a concentration of *c.* 2 mg C l^{-1}, well above the incipient limiting level (Boersma & Vijverberg, 1994).

Lipid and fatty acid analysis

Algae for lipid and FA analysis were concentrated by centrifuging (5 min at 2000 r.p.m.) and freeze-dried. Lipids were isolated from 1 to 5 mg of lyophilized algae using 2 ml chloroform/methanol (2 : 1, v/v) as the extracting solvent. For the isolation of lipids from daphnids, ten to twenty daphnids were collected and freeze dried and extracted in chloroform/methanol (2 : 1, v/v). All extraction procedures were repeated twice, and the solvent was washed twice with 0.88% NaCl according to Folch, Lees & Sloane-Stanley (1956). To prevent autoxidation of PUFA, 0.01% butylated hydroxytoluene (BHT, Sigma) was added to chloroform/methanol mixtures and solvents were flushed with N_2. This extraction procedure was carried out, as far as possible, in a N_2 atmosphere. The lipid samples were stored in chloroform under N_2 atmosphere at –20 °C in the dark. After removing the solvent, lipids were dissolved in hexane and transmethylated in 3%

H_2SO_4 in dry methanol (1 ml) for 4 h (Blau & Darbre, 1993) and the resulting FA methyl esters (FAME) were extracted with 3 ml hexane. Heneicosanoic acid (C21 : 0, Sigma) was used as an internal standard. FAME were analysed on a Hewlett-Packard HP-5890 gas chromatograph (GC) equipped with a BPX70 column (dimensions: 0.22 mm × 25 m, 0.25 μm film thickness, SGE). FAME were detected with a flame-ionization detector interfaced to a PC equipped with Chrom Card software (Fisions Instruments) to allow peak integration. Samples were injected splitless, using He as the carrier gas. FAME were tentatively identified by comparisons with retention times of standards (Sigma, Altech). They were further identified by mass spectral analysis (Hewlett-Packard mass selective detector HP 5970). For all GC analyses the initial oven temperature was set at 50 °C, which rose on injecting to 130 °C at 40 °C min^{-1} and was further programmed to reach 230 °C, increasing 3 °C min^{-1} to separate FAME of interest.

Grazing on emulsion particles

Emulsion particles were used as food additives in life history experiments (see below). The emulsions were: emulsion-A [International Council for Exploration of Sea (ICES) 30/0.6/C] and emulsion-B (ICES 0/-/C), both supplied by the Artemia Reference Centre (Ghent, Belgium). Both emulsions contained emulsifier and anti-oxidants as well as FA. They differed in their FA composition and particle size: emulsion-A was rich in long-chain PUFA and had a particle diameter of *c.* 2 μm and emulsion-B had a smaller particle diameter (*c.* 1 μm) and contained mainly saturated (SAFA) and monounsaturated fatty acids (MUFA) (Table 2). Before using the emulsions in life history experiments, the ability of daphnids to feed on the emulsion particles was tested. Uptake of emulsion particles by daphnid grazing was measured on the basis of decrease in the turbidity of the incubation medium due to the presence of emulsion particles. Decrease of turbidity was measured with a Spectrophotometer (Pye Unicam PU 8600, UV/VIS) using a 5 cm cuvet at $\lambda = 750$ nm. Ten adults or twenty-five juvenile daphnids were placed in 50-ml incubation vessels containing lake water and emulsion. A starting emulsion concentration of 25 mg C l^{-1} was used; this high concentration was needed for optimal spectrophotometric measurements. Emulsion suspension was prepared as follows: 50 mg of emulsion was weighed on a balance and 2 ml of lake water was added and then vigorously shaken. From this stock solution, a known volume was added to the lake water. The daphnids were incubated for 24 h in the emulsion solution, removed and the emulsion solution was filtered (30 μm) to remove large particles, after which the emulsion turbidity of the filtrate was measured. The decrease in the amount of emulsion due to *Daphnia* grazing was calculated using a calibration curve between extinction and emulsion concentration, as for measuring algal concentration.

Life history experiments

To investigate the importance of dietary long-chained PUFA, five food types were tested in life history experiments with *D. galeata*. Diet 1 (*S. acutus*) which lacked EPA and docosahexaenoic acid (DHA), was used as the standard food type. Diet 2 (*Scenedesmus* supplemented with emulsion-A) was enriched with large amounts of C20/22 PUFA because of the emulsion (see Table 2 for FA compositions). Diet 3 was a mixture of *Scenedesmus* food and emulsion-B composed primarily of C14 : 0, C16 : 0, C18 : 1 and C18 : 2 (see Table 2). This food type was used as a control for the use of emulsions to rule out the possibility that emulsifier and anti-oxidants were responsible for changes in food quality. Diet 4 was *Cryptomonas pyrenoidifera*, which contained high amounts of EPA and DHA. Diet 5 was *Rhodomonas lacustris,* an algae that contained high amounts of EPA and DHA.

Newborns from the *D. galeata* stock culture were collected within about 12 h of birth, and transferred to 100-ml test tubes containing membrane-filtered lake water. Algal concentration was kept at 2.0 mg C l^{-1} and the temperature maintained at 17.5 °C. The medium was refreshed daily to reduce bacterial densities. The food mixtures contained 2 mg C l^{-1} of *Scenedesmus* algae and 0.5 mg C l^{-1} of emulsion. The selected newborn daphnids were adapted to the food types until they produced the second brood. Newborns from this second brood were selected within 12 h and their life history characteristics were measured until they reached the fourth adult instar stage. The following life history characteristics were determined for each of the first four adult instars: (i) daphnid length after moulting (measured from the anterior side of the eye to the base of the tail spine); (ii) number of eggs; and (iii) number of new newborns. Newborns were

Table 2 Fatty acid (FA) contents of algae and emulsions (μg mg^{-1} DW); the content as percentage of total FA is also given in parentheses. Emulsion-A also contained the following minor FA: C14 : 1ω5, C15 : 0, C15 : 1ω5, C17 : 0ω7, C20 : 0, C20 : 1ω9/7, C20 : 3ω6/3, C20 : 4ω3, C22 : 1ω9/7, C21 : 5ω3, C22 : 4ω6, C22 : 4ω3 and C22 : 5ω3 (< 1% of total FA)

Fatty acid	*Scenedesmus*	*Cryptomonas*	*Rhodomonas*	Emulsion-A	Emulsion-B
C14 : 0	0.4 (0.4)	0.81 (1.1)	4.5 (5.4)	52.5 (6.1)	149.4 (39.5)
C16 : 0	12.3 (12.7)	13.2 (18.2)	9.4 (11.3)	126.0 (14.5)	86.8 (22.9)
C16 : 1ω7	3.1 (3.2)	0.9 (1.3)	1.1 (1.3)	62.1 (7.2)	0.4 (0.1)
C17 : 0	0.2 (0.2)	0.2 (0.3)	–	11.8 (1.4)	–
C16 : 3	4.0 (4.1)	–	0.9 (1.1)	–	–
C16 : 4ω3	22.5 (23.2)	0.3 (0.4)	–	–	–
C18 : 0	0.1 (0.1)	0.4 (0.6)	–	43.6 (5.0)	25.2 (6.7)
C18 : 1ω9	5.0 (5.2)	0.7 (0.9)	1.0 (1.2)	98.5 (11.4)	64.7 (17.1)
C18 : 1ω7	0.06 (0.06)	0.5 (0.7)	2.9 (3.5)	27.5 (3.2)	43.6 (11.5)
C18 : 2ω6	5.5 (5.7)	1.4 (1.9)	–	43.2 (5.0)	43.6 (11.5)
C18 : 3ω3	38.9 (40.2)	12.0 (16.6)	18.8 (22.6)	13.6 (1.6)	4.3 (1.1)
C18 : 4ω3	4.1 (4.2)	26.2 (36.2)	23.6 (28.3)	17.5 (2.0)	–
C20 : 1ω9	0.1 (0.1)	–	–	11.0 (1.3)	0.5 (0.1)
C20 : 4ω6	–	–	–	8.5 (1.0)	–
C20 : 5ω3	–	10.5 (14.5)	20.1 (24.1)	131.3 (15.1)	–
C22 : 5ω3	–	–	–	20.7 (2.4)	–
C22 : 6ω3	–	4.4 (6.1)	5.9 (7.0)	96.8 (11.2)	–
C24 : 0	–	–	4.3 (5.1)	–	–
Total FA	96.8	72.4	83.3	867.6	378.1

subsequently removed from the test tubes. For each food type, ten replicates were used. For measuring the dry weight increase, ten newborns were freeze dried, transferred to a pre-weighed aluminium boat, and weighed on a microbalance (Sartorius). The dry weight of the adult daphnids was measured at the beginning of the fifth adult instar. The somatic instantaneous growth rate g_s (day^{-1}) was calculated from the equation:

$$g_s = \frac{\ln W_t - \ln W_0}{t}$$

where W_t is the dry weight of adults after time t (days) including the dry weight of the total number of newborns, and W_0 is the average dry weight of a newborn. Population growth rate of the daphnids was estimated using the Euler equation (Stearns, 1992):

$$1 = \sum_{x=0}^{n} e^{-r \cdot x} \cdot l_x \cdot m_x$$

where r = per capita rate of increase for the population (day^{-1}); x = age class; l_x = probability of surviving to age class x; and m_x = fecundity at age class x. The standard error of r was calculated using the jacknife method (Meyer *et al*., 1986).

Results

Algal fatty acid compositions

The FA profile of *S. acutus* differed greatly from those of the two cryptophytes *C. pyrenoidifera* and *R. lacustris*, particularly in EPA (C20 : 5ω3) and DHA (C22 : 6ω3) content. *Scenedesmus acutus* contained large amounts of unsaturated FA, most with a carbon chain length of sixteen or eighteen (Table 2). Major PUFA were C16 : 4ω3 and C18 : 3ω3 (linolenic acid), comprising more than 60% of total FA, while 13.5% of the FA were composed of low quality (saturated) fatty acids. Neither EPA nor DHA were detected in *Scenedesmus* (detection limit = 0.01 μg mg^{-1} DW). In contrast, PUFA comprised about 30% of total FA in both *Cryptomonas* sp. and *Rhodomonas* sp. Moreover, in both cryptophytes C18 : 4ω3 was the most abundant FA.

The amount of total FA in the three algal genera was similar, varying between 72.4 and 96.8 μg mg^{-1} DW. Because EPA and DHA were not found in *Scenedesmus*, the importance of these long-chained PUFAs for *Daphnia* food quality was studied by comparing the food quality of the three unialgal diets. An artificial FA supplement was also used. The Artemia Reference Centre from the University of Ghent (Belgium) kindly

Table 3 Uptake of emulsion particles by adult and juvenile daphnids during a 24-h period. The initial emulsion concentration was 25 mg C ml^{-1} DW and ten adults or twenty-five juveniles were used in each experiment

Animal age/emulsion	Decrease of particles (%)
Adult + emulsion-A	88.3
Juvenile + emulsion-A	75.0
Adult + emulsion-B	47.0
Juvenile + emulsion-B	21.2

supplied two types of emulsions for the present study: (A) containing 15.1% EPA and 11.2% DHA; and (B) that lacked these long-chained PUFAs. Instead, emulsion-B had a high content of SAFA: myristic acid (C14 : 0), palmitic acid (C16 : 0) and stearic acid (C18 : 0). The detailed FA profiles of both emulsions are summarized in Table 2.

Grazing on emulsions

The diameter of the emulsion particles which ranged between 1 and 2 μm is close to the lower retention limit of the filtering apparatus of *D. galeata* (Geller & Muller, 1981). The test grazing experiment in the present study showed that juveniles and adults were able to ingest both kinds of emulsion particles. Some differences were found, however, between juveniles and adults, as well as between the two emulsions (Table 3). Adult daphnids caused a greater decrease of the emulsion particles than juveniles, which can be explained by higher clearance rates of larger animals. In addition, particles in emulsion-A were cleared more effectively than those in emulsion-B, which is probably because the particle diameter in emulsion-B was smaller. Nevertheless, both juveniles and adult daphnids were able to clear the particles of both emulsions. This indicates that the emulsions can be used as 'additives' in food quality experiments with daphnids.

In daphnids fed on emulsion-A or on the *Cryptomonas* species for 1 week the level of EPA increased from 0.5–2% (percentage of total FA) to *c.* 10% (see also Weers, Siewertsen & Gulati, in press). Thus, the daphnids were not only able to filter the emulsion particles but also to ingest them and to incorporate the emulsion FA into their body lipids.

Life history experiments with different food types

The length increase was greatest in *Daphnia* fed *Rhodomonas* (diet 5) or *Cryptomonas* (diet 4), although the differences were moderate (Fig. 1). The daphnids fed diet 3 (mixture of *Scenedesmus* and emulsion-B) were smaller than daphnids fed the other food types. Daphnids fed *Cryptomonas*, *Rhodomonas*, or the mixture of *Scenedesmus* and emulsion-A showed higher clutch sizes (Fig. 2), higher cumulative number of newborns produced per *Daphnia* from three clutches and higher somatic and population growth rates (Table 4), than those fed *Scenedesmus* alone or *Scenedesmus* with emulsion-B.

Based on the life history traits the food quality of the five food types could be divided into two groups: (i) high food quality, diets 2, 4 and 5, all of which contained long-chained PUFA; and (ii) lower food quality, diets 1 and 3, which contained no long-chained PUFA (Fig. 2). Clearly, the quality of *Scenedesmus* food was ameliorated by addition of emulsion-A (food type ii) to the level observed for *Cryptomonas* or *Rhodomonas* (Table 4). The positive effect of emulsion-A on *Scenedesmus* was presumably caused by the presence of C20/22 PUFA in this emulsion. In contrast, the use of emulsion-B (composed of low quality FA) in combination with *Scenedesmus* (diet 3) did not improve algal food quality. Tests (*t*-tests, $P < 0.05$) of the intrinsic rate of population growth (r) showed that the high food quality diets differed significantly from the lower food quality diets, and that the diets within each group did not differ significantly.

Discussion

To date knowledge about the FA requirements of zooplankton is rather limited. So far, it is common knowledge that essential FA should be present in the ingested food. If the algae that are the major food sources for zooplankton are analysed for their FA, it becomes evident that many of these algae differ in both quality and quantity of FA. Because PUFA play a role in the photosynthetic reactions of algae, almost all of the algae species, including those of prokaryotic cyanobacteria (blue-green algae), contain significant amounts of PUFA. Some cyanobacteria contain primarily bacterial FA (SAFA and MUFA), whereas others, especially the filamentous forms like *Oscillatoria* (Kenyon & Stanier, 1970), contain substantial amounts of PUFA. Moreover, unicellular cyanobacteria can be divided into groups lacking polyenoic fatty acids (several *Synechococcus* strains) and algae containing polyenoic ω3-FA (other *Synechococcus* strains) or ω6-

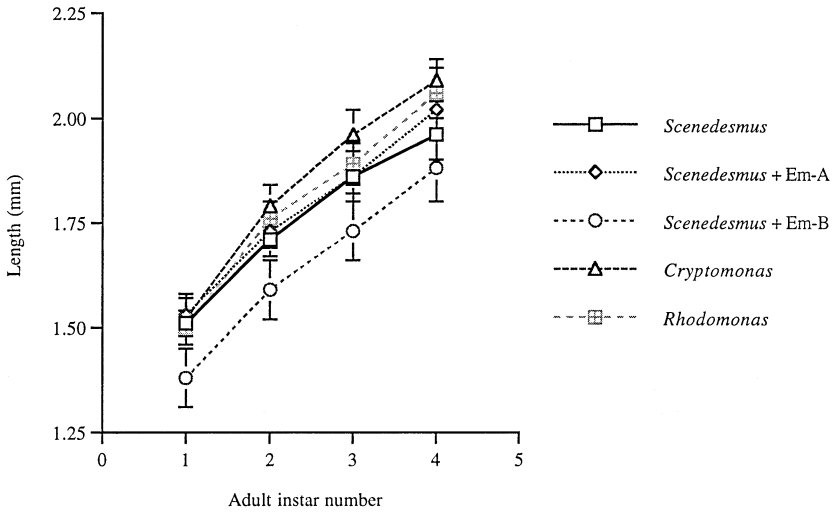

Fig. 1 Mean length of *Daphnia galeata* during the adult development. After every moult the daphnids were measured (bars indicate 2 × SE, 95% CL). Daphnids were fed with five different food types: *Scenedesmus*, *Scenedesmus* supplemented with emulsion-A or emulsion-B, *Cryptomonas* and *Rhodomonas*.

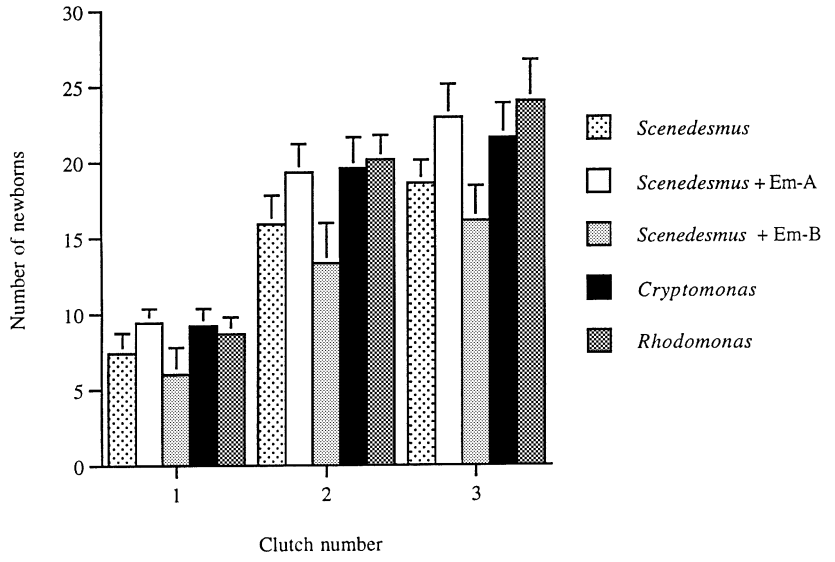

Fig. 2 Clutch sizes of adult daphnids fed with, from left to right and increasing dot density: *Scenedesmus*, *Scenedesmus* supplemented with emulsion-A, *Scenedesmus* plus emulsion-B, *Cryptomonas* and *Rhodomonas*. The bars indicate 2 × SE (95% CL).

Table 4 Life history characteristics of *Daphnia galeata*: total numbers of newborns produced from the first three clutches, instantaneous somatic growth rate g_s (day^{-1}) calculated from birth of the animals to their fifth adult instar, and intrinsic rate of population growth r (day^{-1}). The figures in parentheses are 2 × SE (95% CL)

Diet	Newborns	g_s	r
Scenedesmus	41.9 (4.2)	0.282 (0.011)	0.310 (0.009)
Scen. + emulsion-A	51.6 (3.2)	0.312 (0.012)	0.350 (0.007)
Scen. + emulsion-B	37.3 (3.2)	0.274 (0.015)	0.290 (0.005)
Cryptomonas	51.0 (3.5)	0.310 (0.009)	0.351 (0.011)
Rhodomonas	53.4 (3.9)	0.310 (0.007)	0.345 (0.005)

FA(*Microcystis*) (Kenyon, 1972). Members of the Chlorophyceae (*Chlamydomonas*, *Scenedesmus* and *Chlorella* species) contain large amounts of PUFA, most with a chain length of sixteen and eighteen C atoms (Piorreck *et al.*, 1984; Giroud, Gerber & Eichenberger, 1988; Wood, 1988; Ahlgren *et al.*, 1990). Cryptomonads and diatoms usually contain significant amounts of long-chain PUFA, in particular EPA and DHA (Beach, Harrington & Holz, 1970; Ahlgren *et al.*, 1990). In contrast, these FA are completely absent in blue-greens, and can be minor constituents or completely lacking in green alga species (Cranwell, Jaworski & Bickley, 1990; Ahlgren *et al.*, 1992; Renaud, Parry & Luong-Van Thinh, 1994).

The FA composition of *S. acutus*, *R. lacustris* and *C. pyrenoidifera* found in the present study is in full accordance with the above-mentioned trends of algal FA patterns. *Scenedesmus acutus* contained mainly C16 : 4ω3 and linolenic acid (C18 : 3ω3), while major

FA of the two cryptophytes were linolenic acid, C18 : 4ω3, EPA and DHA. Food quality studies have shown that cryptomonads are superior to green algae and cyanobacteria, perhaps because of the presence of EPA and DHA. To date, only a limited number of published papers on the importance of C20/22 PUFAs for freshwater zooplankton grazers exist, most of which indicate the importance of EPA and other PUFAs for freshwater ecosystems (Ahlgren *et al*., 1990, 1992; Ahlgren, 1993; Müller-Navarra, 1995a, b). The present study has demonstrated that addition of PUFA-emulsion containing EPA and DHA to the *Scenedesmus* diet results in an improvement of food quality. The intrinsic rate of population growth (r) increased discernibly from 0.31 day^{-1} (for *Scenedesmus* alone) to 0.35 day^{-1} (*Scenedesmus* + the PUFA emulsion-A). Such an increase was also obtained when the daphnids were fed with high quality cryptomonad algae. The increase in the population growth by adding emulsion-A is relatively small (*c.* 10%), and *c.* 20% more newborns are produced. This relatively small improvement can be explained by the good quality of *Scenedesmus* food; daphnids fed with *Scenedesmus* show a population growth rate which is rather close to the maximum population growth rate (r_{max}), assuming that the maximum growth rate is obtained in feeding daphnids with cryptophytes. Thus, adding the PUFA emulsion-A can only lead to a modest increase in r_{max}. Because addition of control emulsion-B to *Scenedesmus* did not enhance food quality, the improvement of food quality by emulsion-A can be attributed to the PUFAs. The most important FA in the PUFA emulsion are EPA and DHA, even though a variety of other PUFAs are also present. Therefore, it would be premature to conclude that EPA alone is responsible for the improvement in food quality, because other PUFAs may be involved. DHA probably does not play an important role in daphnid FA requirements because it is not incorporated into the daphnids (Weers *et al*., 1997).

Müller-Navarra (1995a, b) observed that EPA possibly determines food quality for *Daphnia*. This is based on a study in which she monitored seasonal changes in seston food composition in Schöhsee and found a strong correlation between EPA and *D. galeata* body growth (Müller-Navarra, 1995a). She also compared *S. acutus* and *Cyclotella meneghiniana* (Kütz) grown under various phosphorus concentrations, and attributed the higher quality of *Cyclotella* to the presence of high amounts of EPA (Müller-Navarra, 1995b). However, the response of algae to nutrient changes is not manifest in changes in FA alone but can include changes in other biochemical compounds or in morphology (Van Donk & Hessen, 1993). The results of the present study show that long-chain PUFA improve algal food quality for *D. galeata*, although the enhanced quality is small (*c.* 10% as measured in *r*). Others showed more pronounced effects by adding PUFA emulsions (DeMott & Müller-Navarra, 1997; Sundbom & Vrede, 1997). EPA is considered an essential FA, but its presence in food does not seem to be indispensable for growth and reproduction of *Daphnia* because the animals fed exclusively *Scenedesmus* (and lacking EPA), develop normally and without timelag. Development in *Daphnia* is delayed if animals are fed algae at concentrations below the incipient limiting level (Boersma & Vijverberg, 1994) or P-limited algae (Weers & Gulati, in press). The high content of EPA precursors in many green algae, especially linolenic acid, and the ability of daphnids to elongate and desaturate these precursors into long-chain PUFAs might be responsible for EPA not being strictly an essential compound for *D. galeata*. For *D. magna* the ability to convert linolenic acid into EPA has been suggested because incubation of daphnids in the presence of [^{14}C]acetate results in the incorporation of radioactivity into EPA (Farkas, Kariko & Csengeri, 1981). The present study confirmed this also in *D. galeata*, which is apparently able to form EPA out of ω3-FA even though the conversion activity was very low (Weers & Gulati, in press). Nevertheless, it will be advantageous for maximum body growth and high fecundity if daphnids ingest food particles with a biochemical composition that matches their needs, so avoiding the extra energy and time involved in bioconversions.

Variations in algal food quality are undesirable, especially in toxicity bioassays with *D. magna* (Straus), a species often used to test toxic compounds. Food enrichment for daphnids using microcapsules was attempted by Elendt (1990), who investigated the possibility of replacing algae by an artificial diet in order to circumvent the fluctuations in the nutritive value of diet. The microcapsules contained lipids, proteins and a mixture of minerals and vitamins, and had a capsule wall composed of cross-linked proteins. Interestingly, the lipid fraction contained 6% EPA and 2.6% DHA. Total replacement of *Scenedesmus* food by microcapsules caused a deterioration of food quality for *D. magna* as indicated by higher mortality rates, decrease in

length and fecundity, increase in time to reach maturity, and alteration of midgut ultrastructure. On the other hand, supplementing *Scenedesmus* food with microcapsules did not result in differences in food quality for *D. magna*. The mixture had a quality comparable with that of *Scenedesmus* alone. Despite the presence of EPA and DHA in the microcapsules offered to *D. magna*, the nutritive value of the food did not improve, perhaps because of a lower digestion rate of microcapsules, their high sedimenting rates, or the deterioration of the gut caused by microcapsules. In contrast to microcapsules, the emulsions used in the present study do not have protein cell walls and remain in suspension (see Coutteau & Sorgeloos, 1997) and thus were easily ingested and assimilated by daphnids. This was verified by the high EPA content of the daphnids fed emulsion particles. The lack of an enhancement of food quality by the addition of microcapsules containing EPA might also be because of differences between *D. magna* and *D. galeata*. Because the emulsion was composed primarily of lipids, it could only supplement algal food, rather than completely replace it. In marine aquaculture long-chain PUFA are known for their high nutritive value. *Artemia* nauplii and rotifers, which are frequently used as a food source for marine fish, crabs, shrimps and prawns, can be enriched with EPA and DHA using artificial diets (Langdon, Levine & Jones, 1985). This EPA and DHA enrichment of food clearly improves the nutritive value of the food for marine aquaculture (Langdon & Waldock, 1981; Watanabe, Kitajima & Fujita, 1983; Levine & Sulkin, 1984; Sorgeloos & Léger, 1992). In contrast to freshwater fish, marine fish have limited ability to synthesize EPA and DHA from linolenic acid (see review by Henderson & Tocher, 1987). This limited capacity for EPA and DHA synthesis might be among the main reasons for the importance of EPA/DHA emulsions and microcapsules in marine aquaculture.

The experiments of the present study with *Scenedesmus* show that EPA is not essential in the diet of *D. galeata*. Perhaps this can be explained by the high content of EPA precursors in *Scenedesmus* or by low metabolic demands. Algae low in content of ω6- and ω3-FA might be a very poor food source, as demonstrated by DeMott & Müller-Navarra (1997). Therefore, the quantity and quality of essential FA in algae will probably influence daphnid food quality. To establish the importance of FA in zooplankton diet, much more research is needed, both using well controlled laboratory experiments and field experiments.

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