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Food quality for *Daphnia* in humic and clear water lakes

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SUMMARY

1. Growth and reproduction of *Daphnia* fed lake seston were measured in two categories of meso- to eutrophic lakes differing with respect to terrestrial organic matter influence (humic and clear water lakes). The content of highly unsaturated fatty acids (HUFA), P and N, as well as the taxonomical composition of seston were analysed.
2. Seston HUFA and C : P ratios were similar between lake categories, whereas C : N ratios were lower in the clear water lakes in both spring and summer. Despite the similarity in HUFA and P content of seston, *Daphnia* growth rate, clutch size and the proportion of gravid females were, respectively, about 1.5, 3 and 6 times higher in the clear water lakes.
3. Differences in growth and reproduction were related to a combination of higher N content and good fatty acid quality of the seston in the clear water lakes. Relatively high biomass of edible algae, such as *Rhodomonas* sp. and *Cryptomonas* sp., in the clear water lakes, and differences in water pH likely contributed to the observed differences in *Daphnia* growth and reproduction between lake categories. Additionally, it is possible that *Daphnia* was energy limited in the humic lakes despite high particulate organic carbon (POC) concentrations, as the contribution of non-algal and detrital C to the POC pool was high.
4. Our results suggest that dietary HUFA content has the potential to improve herbivore growth and reproduction if N and P are not limiting. N merits more attention in studies of zooplankton nutrition.

Keywords: *Daphnia*, fatty acids, nitrogen, phosphorus, seston

Introduction

Higher respiration than production in the epilimnion is a common feature in lakes and implies utilisation of additional carbon sources, other than autochthonous production, to subsidise the metabolism of the plankton community (del Giorgio & Peters, 1994; Cole, 1999). In humic lakes, the food web is partly fuelled by the abundant terrestrial carbon, and the strong relationship between edible phytoplankton and zooplankton production often found in clear water lakes is not as obvious (Hessen, 1989). It has been shown, for instance, that as much as 50% of zooplankton carbon can be derived from allocthonous carbon (Grey, Jones & Sleep, 2001; Karlsson *et al*., 2003; Pace *et al*., 2004), through ingestion of detritus, bacteria and phagotrophic flagellates. Detritus can make up more than 75% of the particulate organic carbon pool in humic lakes (Hessen, Andersen & Lyche, 1989, 1990), and is, therefore, a quantitatively important C source for zooplankton.

However, detritus is poor in essential minerals and biochemical compounds important for zooplankton growth and reproduction, and is assimilated with low efficiency (Hessen, 1998). Even though algae can make up only a small portion of the particulate C pool in humic lakes, their role in zooplankton nutrition may be crucial (Hessen, 1998). Algae are the main sources of highly unsaturated fatty acids (HUFA) for zooplankton and several studies have shown HUFA to promote zooplankton growth and reproduction under non-limiting phosphorus conditions (Sundbom &

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Vrede, 1997; Sterner & Schulz, 1998; Wacker & Von Elert, 2001; Becker & Boersma, 2003). HUFA are vital components of the cell membranes and can be precursors to several animal hormones and other classes of biochemicals (such as eicosanoids) related to animal growth and reproduction (Brett & Müller-Navarra, 1997; Gulati & DeMott, 1997). Examples of important HUFA for zooplankton are the eicosapentaenoic acid (EPA 20:5ω3) and the docosahexaenoic acid (DHA 22:6ω3) acid. Even though bacteria can be important sources of elemental nutrients for zooplankton, because of their low carbon to phosphorus ratios compared with phytoplankton, most bacteria are deficient in HUFA (Lechevalier & Lechevalier, 1988). For this reason and because zooplankton cannot synthesise HUFA *de novo*, zooplankton partly depend on their algal food to obtain such fatty acids (Brett & Müller-Navarra, 1997).

Different groups of algae differ in fatty acid composition and thus food quality. Hence, how well zooplankton grow and reproduce will depend not only on algal quantity, but also on the algal community composition (Brett, Muller-Navarra & Park, 2000; Ravet & Brett, 2006). There seems to be no planktonic species that are clearly unique to humic lakes, although some species are more commonly found in humic lakes (Jones, 1998). For instance small, soft-bodied and mixotrophic species, such as the HUFA-rich cryptophytes (Ahlgren *et al*., 1990), may be highly abundant in coloured lakes (Sarvala *et al*., 1999).

The fatty acid content of seston and its quality for zooplankton growth and reproduction in humic lakes is still unexplored. On the one hand, the high contribution of detritus to the particulate organic carbon pool may negatively affect the overall quality of the food. On the other hand, the common occurrence of HUFA-rich algae in humic lakes may result in better food quality for zooplankton relative to clear water lakes of similar trophy (Sarvala *et al*., 1999). Indeed, our data from oligotrophic humic lakes indicated higher concentrations of EPA in summer than in clear water lakes of similar trophic state (Gutseit, Berglund & Granéli, 2007). In order to assess possible differences in zooplankton growth or reproduction related to biochemical differences between lake types, we measured growth rates and reproduction of *Daphnia magna* Straus fed seston from lakes with similar total P and Chl *a* concentrations but differing with respect to terrestrial organic matter influence (humic versus clear water lakes). We hypothesised that if the fatty acid quality of the seston differs between clear water and humic lakes this would result in differences in *Daphnia* growth and reproduction.

Methods

Water from three clear water lakes (Havgårdsjön, Krankesjön and Lyngsjön) and from three humic lakes (Salen, Östersjön and Grunnen) was collected in late spring (first week of June) and late summer (first week of September) for two laboratory biotests with *D. magna*. The lakes are situated in southern Sweden, and most are relatively shallow and small (Table 1). The lakes were selected to comprise two categories (clear water versus humic) with distinct contribution of terrestrial organic matter to the dissolved organic matter pool, but with similar total phosphorus and Chl *a* concentrations. Dissolved organic carbon (DOC) was used to normalise absorbance (Abs) at 260 nm (Abs/DOC) to provide a semi-quantitative measure of the terrestrial contribution to the dissolved organic matter pool (Fukushima *et al*., 1996), as this ratio has been suggested to correlate with the contribution of aromatic compounds (lignin, tannin degradation products) in dissolved organic matter (Imai *et al*., 2001).

For each sampling date a laboratory biotest was performed with *Daphnia* feeding on natural seston at ambient concentrations. Lake water used in the experiment was collected from the lake surface (0–1 m) and was stored cold (4 °C) and dark. The experiments started on the same day that the water was collected. The *Daphnia* clone used in the experiments was originally from a pond located at the university campus in Lund, Sweden, and had been kept for a couple of years on a diet of phosphorus-sufficient *Scenedesmus obliquus* (Turpin) Kützing at non-limiting concentrations (>1 mg C L^{-1}). *Daphnia* used in the biotests were born within 24 h and were fed P-sufficient *S. obliquus* daily at non-limiting concentrations (>1 mg C L^{-1}) until they were 3 days old. The 3-day old organisms were rinsed three times with filtered (GF/F, Whatman, Maidstone, U.K.; 0.7-μm nominal pore size) lake water before being transferred to the experimental microcosms, which consisted of three 2.5-L plastic aquaria per lake. Fifteen 3-day old *Daphnia* were placed in each aquarium containing 2 L

Table 1 Characteristic of the lakes used in the experiments. Mean (±SD) ($n = 3$) of some variables measured in spring and summer in the two categories of lakes are shown.

	Location	Lake area (km^2)	Max. depth (m)	Mean depth (m)	Colour†	pH*	DOC*	Abs/ DOC*	TP	Chl *a*	POC	PON	POP	C : P	C : N*	N : P
Clear water lakes																
Spring																
Havgård	55°28′N 13°21′W	0.57	5.7	2.9	-	8.5	11	17	46	14	2071	259	29	189	9.3	20
Kranke	55°41′N 13°28′W	3.4	3	0.7	20	8.4	14	19	32	6	1154	166	17	181	8.1	22
Lyng	55°36′N 13°22′W	0.05	6	-	-	8.6	14	20	26	10	1356	207	18	207	7.6	27
Mean (±SD)						8.5 (0.1)	13 (2)	19 (2)	35 (10)	10 (4)	1527 (482)	211 (47)	21 (7)	192 (13)	8.4 (0.9)	23 (4)
Summer																
Havgår						8.8	21	6	47	17	2331	344	33	185	7.9	23
Kranke						8.5	19	14	24	4	884	132	16	148	7.8	19
Lyng						8.5	14	21	30	12	1791	264	18	265	7.9	33
Mean (±SD)						8.6 (0.2)	18 (3)	14 (7)	34 (12)	11 (7)	1668 (731)	247 (107)	22 (9)	200 (60)	7.9 (0.1)	25 (7)
Humic lakes																
Spring																
Öster	56°28′N 15°18′W	0.7	2.2	1.6	98	6.6	27	32	60	19	1907	147	59	114	8.3	7
Salen	56°51′N 14°98′W	18.3	5.6	2.3	99	6.9	28	30	60	10	3366	336	37	234	11.7	20
Grunnen	57°09′N 14°25′W	0.5	3.0	1.0	175	7.0	21	30	13	5	643	74	7	237	10.0	24
Mean (±SD)						6.8 (0.2)	25 (4)	31 (1)	44 (27)	11 (7)	1972 (1363)	186 (135)	34 (26)	195 (70)	10 (1.7)	17 (9)
Summer																
Öster						7.0	17	44	31	12	946	121	21	117	9.1	13
Salen						6.7	22	50	103	7	2030	220	73	76	10.7	7
Grunnen						6.6	24	61	20	8	1481	130	13	293	13.2	22
Mean (±SD)						6.7 (0.2)	21 (4)	52 (8)	51 (45)	9 (3)	1481 (542)	157 (54)	36 (33)	162 (116)	11 (2.1)	14 (8)

DOC: dissolved organic carbon (mg L^{-1}); TP: total phosphorus (μg L^{-1}); POC, PON and POP: particulate organic carbon, nitrogen and phosphorus (μg L^{-1}). Elemental ratios expressed as atoms : atoms.

*Significant difference at the 5% level for lake category (two-way ANOVA).

†Colour (μg Pt L^{-1}) was not measured in this study.

lake water, which was prescreened with a 100-μm mesh sized net to remove large zooplankton. It is usual to screen the water with a 30-μm mesh size net, as this represents the edible fraction for small and juvenile *Daphnia* (Burns, 1968). However, we believe that a larger mesh size would reflect more closely the natural conditions in a lake, where a mix of large and small particles is available for zooplankton. All lake water in the aquaria was exchanged daily and the experiment lasted for 5 days. Food was re-suspended twice a day using a plastic Pasteur pipette. The aquaria were brushed daily to avoid growth of organisms on the walls. The experiment was performed in a temperature-controlled room at 20 °C with a light : dark cycle of 12 : 12 h. Cool White lamps (Lumilux®, Osram, Munich, Germany) were used and the light intensity just above the water surface was 25 μmol m^{-2} s^{-1}. Immediately prior to each experiment, 15 3-day old *Daphnia* were dried at 60 °C for 24 h and weighed on an electronic microbalance to the nearest 0.01 μg to obtain the initial dry weight. At the end of the experiment, the number of live organisms and the number of eggs per individual (clutch size) were counted. Organisms were then dried and weighed as above. Growth rate per day was calculated as:

$$g = (\ln \mathrm{Wt} - \ln \mathrm{W0})/t$$

where Wt is the dry weight of the organisms after t = 5 days; and W0 is the initial dry weight. The proportion of gravid females was calculated as the number of organisms carrying eggs divided by the number of organisms found alive at the end of the experiment. No eggs hatched during the experiment. Survival was calculated as the proportion of the initial number of organisms found alive at the end of the experiment.

Samples for all analyses were collected at the beginning of the experiment. Additionally, we collected water for fatty acid, particulate carbon, particulate nitrogen and particulate phosphorus analyses at the end of the experiment. We then averaged the initial and the final values of those parameters to reflect the overall food concentration (particulate C concentration) and food biochemical (fatty acids) and elemental (carbon to phosphorus and carbon to nitrogen ratios) conditions during the 5 days of experiment.

Samples for fatty acid analysis were collected on GF/F filters (precombusted at 450 °C for 6 h and rinsed with hexane) and stored frozen (−80 °C) under a nitrogen atmosphere until extraction. Total lipids were extracted according to Bligh & Dyer (1959) on lyophilised samples. All solvents and chemicals used were of analytical grade. The lipids were transesterified to fatty acid methyl esters (FAMES) by sulfuric acid catalyzed methanolysis (1% H_2SO_4 in methanol) (Wesén *et al.*, 1992). C19 : 0 was used as internal standard and was added before methanolysis. Fatty acids were analyzed by gas chromatography, which was carried out on a Hewlett-Packard 5890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA, U.S.A.) equipped with flame ionisation detector. A 50-m HP5 capillary column (phenylmethyl silicone; Hewlett-Packard Co.) was used and hydrogen was the carrier gas. Injections were made in a splitless mode. The oven was programmed as follows: 80 °C for 1 min, increasing at 20 °C min^{-1} to 160 °C and then increasing at 5 °C min^{-1} to the final temperature of 270 °C, which was kept for 5 min. Relative retention times of supposed FAMES were compared with those of standards. Standards were obtained from Larodan Fine Chemicals (Malmö, Sweden).

Organisms >5 μm and <100 μm in size, hereafter referred to as microplankton, were counted and measured using a inverted microscope. Colonies of Cyanophyceae, Chrysophyceae, Diatomatophyceae and Chlorophyceae were counted as one unit. At least 100 of the most common organisms and all the ciliates were counted. Biovolumes were converted to biomass assuming that the density of the organisms equals that of water (1 mm^3 L^{-1} = 1 mg L^{-1}). Biomasses were converted to carbon by assuming percentages of wet weight suggested in Wetzel & Likens (2000): thecate dinoflagellates 13%; diatoms 11%; chlorophytes 16%; all other phytoplankton species and nanoflagellates 11%; protozooplankton 15%. Chlorophyll *a* was extracted in 10 mL of ethanol overnight from samples collected on GF/F filters (Jespersen & Christoffersen, 1987). Absorbances at 665 nm, for Chl *a*, and at 750 nm, for correction of background turbidity, were measured on a Beckman DU 650 Spectrophotometer (Beckman Coulter, Inc., Fullerton CA, U.S.A.). DOC concentration was analyzed using a Shimadzu TOC-5000 total carbon analyzer (Shimadzu Corporation, Kyoto, Japan) equipped with an ASI-5000 auto sampler. Absorbance at 260 nm was measured on a Beckman DU 650 Spectrophotometer. Samples for particulate carbon and nitrogen analyses were collected on precombusted (450 °C for 6 h) GF/F filters and dried overnight at 65 °C. Carbon and nitrogen content were measured

with a CHN elemental analyzer (FISONS Instruments, NA 1500 NC, Saddle Brook, NJ, U.S.A.). Total P and soluble reactive P (SRP) were determined by Alcontrol Laboratories (Malmö, Sweden) (ISO 1561/SS028127). Particulate phosphorus was obtained by subtracting SRP from the total P.

Data were explored using the software SPSS 11.0 (Chicago, IL, U.S.A.) for Mac OS X. Two-way ANOVA with lake category (humic and clear water) and season (spring and summer) as factors was used to compare the lake categories with respect to several dependent variables taking into account the season. Data were log transformed (log 10 (x + 1) to meet assumptions of normality and equality of variances (Dytham, 2003). Percentage data, such as the proportion of gravid females and survival, were arcsine square root-transformed (Dytham, 2003). To test whether there were differences in food quality (fatty acids and elements) and food quantity (particulate organic carbon and Chl *a*) between lake categories a principal component analysis (PCA) was performed. Both the fatty acid per unit carbon and the absolute concentration were included in the PCA analysis, resulting in a total of 17 variables. A correlation matrix with varimax rotation was used. Axes with standardised eigenvalues >1 were retained (Dillon & Goldstein, 1984), and variables with an absolute loading of 0.6 (1% significance level, n = 17) (Watt, 1993; McGarigal, Cushman & Stafford, 2000) were considered to be important. PCA scores were compared between clear and humic lakes with independent sample t-test. Pearson product-moment correlation was used to investigate the relation between growth, clutch size and proportion of gravid females with the PCA scores. Two fatty acids (20:4ω6 and 20:3ω6) were not included in the PCA analysis as they did not fulfil the assumption of normality even after transformation. An independent sample t-test was used to compare the PCA scores between clear water and humic lakes. Additionally, we performed correlations for the lake categories in separate to explore the relationship between growth and reproductive parameters with the variables explaining most of the variance in the PCA axis that correlated with growth and reproductive parameters.

Results

The lake categories were very similar with respect to several of the measured variables (Table 1). The ratio Abs/DOC was high in the humic category, reflecting the high contribution of terrestrial carbon to the dissolved organic matter pool in these lakes. The pH values were comparatively low in the humic lakes. The particulate organic carbon (POC) concentrations in the study lakes were higher than, or very close to, 1000 μg C L^{-1} (with the exception of Lake Grunnen in spring). Seston of the clear water category was relatively rich in nitrogen as shown by the comparatively low C : N ratios (two-way ANOVA, $P < 0.05$). There were no major differences in seston content of fatty acids relative to particulate carbon as well as their absolute concentration between lake categories (two-way ANOVA, $P > 0.05$) (Table 2).

The microplankton biomasses in the clear water and humic lake categories were similar in spring, but very different in summer (Fig. 1). The microplankton biomass to POC ratio in the clear water and humic lakes was, respectively, 0.22 and 0.24 in spring and 0.86 and 0.26 in summer. Cryptophyceae (mainly *Rhodomonas* sp. Karsten and *Cryptomonas* sp. Ehrenberg) and Cyanophyceae (mainly *Microcystis* sp. Kützing) con-

Table 2 Mean values (±SD) (n = 3) of polyunsaturated fatty acid in seston in the experiments performed in spring and summer

	Clear spring	Clear summer	Humic spring	Humic summer
18:2ω6	3.3 (1.0)	4.1 (1.5)	4.1 (1.6)	3.0 (1.1)
	4.8 (1.3)	7.3 (5.5)	5.3 (2.0)	3.2 (1.3)
18:3ω6	0.4 (0.1)	0.8 (0.4)	0.7 (0.2)	0.7 (0.3)
	0.7 (0.3)	1.5 (1.2)	1.0 (0.5)	1.0 (0.4)
18:3ω3	4.5 (1.6)	3.4 (2.2)	3.9 (2.9)	3.6 (3.2)
	6.5 (2.1)	5.5 (4.7)	6.4 (4.6)	6.1 (3.3)
18:4ω3	2.5 (1.6)	2.2 (1.4)	2.6 (2.1)	2.3 (1.8)
	3.5 (1.9)	3.4 (2.8)	3.2 (2.1)	2.6 (1.4)
20:3ω6	0.08 (0.06)	0.07 (0.06)	ND	0.06 (0.02)
	0.09 0.05	0.13 0.14	ND	0.02 (0.03)
20:4ω6	0.2 (0.2)	0.4 (0.3)	0.04 (0.07)	0.7 (0.4)
	0.27 (0.3)	0.53 (0.5)	0.03 (0.05)	0.5 (0.3)
20:5ω3	1.8 (0.6)	1.3 (0.6)	1.9 (2.0)	2.0 (1.3)
	2.8 (0.8)	1.8 (0.7)	2.2 (2.1)	1.4 (1.0)
22:6ω3	0.4 (0.3)	0.5 (0.2)	0.06 (0.1)	0.7 (0.6)
	0.6 (0.4)	0.8 (0.2)	0.1 (0.07)	0.6 (0.4)
Sum ω3	9.3	7.5	8.7	8.7
	13.4	11.5	12.5	11.0
Sum ω6	4.0	5.6	4.8	4.5
	5.9	9.5	6.3	4.7
ω3:ω6	2.2	1.3	1.9	2.0

Upper value: fatty acid content (μg mg C^{-1}). Lower value: fatty acid absolute concentration (μg L^{-1}).
ND, not detected.

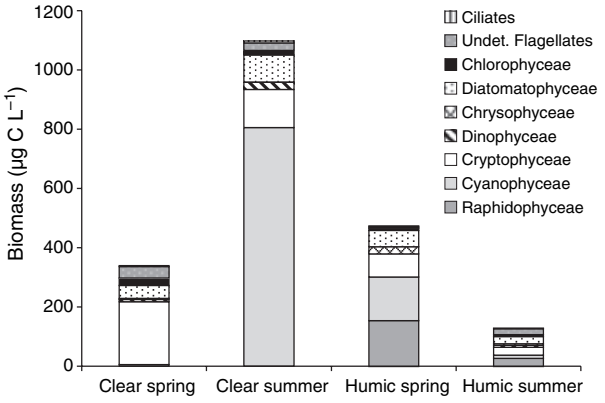

Fig. 1 Mean ($n = 3$) microplankton (organisms >5 µm and <100 µm) biomass and taxonomic composition in the clear water and humic lakes in spring and summer.

tributed to more than 50% of the total microplankton biomass in the clear water category in spring and summer, respectively (Fig. 1 and Table 3). In the humic category, Cyanophyceae (mainly *Anabaena* sp. Bory) and Raphidophyceae [*Gonyostomum semen* (Ehrenberg) Diesing] were important in one of the lakes (Östersjön) during spring, whereas in the other two lakes, Diatomatophyceae (mainly *Cyclotella* Kützing) and Cryptophyceae (mainly *Cryptomonas* sp.) were important (Fig. 1 and Table 3). During summer, several phytoplankton groups were important in the humic lakes.

Daphnia growth, clutch size and proportion of gravid females were higher in the clear water than in the humic lake category (two-way ANOVA, $P < 0.05$ for lake category) with no differences between spring and summer. Survival was not different between lake categories or season, and was always higher than 90% (Table 4).

Five principal component axes (eigenvalues >1) explained 90% of the variation in food quality and quantity aspects in our lakes (Table 5). *Daphnia* somatic growth, clutch size and proportion of gravid females were correlated with the third axis (Pearson's correlation, $r^2 = 0.47$; $P = 0.01$ for growth; $r^2 = 0.70$; $P = 0.001$ for clutch size; $r^2 = 0.70$; $P = 0.001$ for proportion of gravid females), which explained about 14% of the variance in the data. The content (per carbon) and the absolute concentration (per litre) of DHA, and the C : N ratio were the variables with important loadings (>0.6) in the third axis. The scores of the third axis differed between lake categories (*t*-test, $P < 0.05$) (Table 5), suggesting differences in overall quality of the seston between lake categories.

Looking at the correlations for each lake category separately, in the clear water lakes, growth, clutch size and % of gravid females were correlated with DHA

Table 3 Most important phytoplankton species in terms of carbon biomass in the lakes used in the experiment

	Clear water lakes			Humic lakes		
	Havgård	Kranke	Lyng	Salen	Öster	Grunnen
Achantoceras sp. Honigman	Su+					
Anabaena sp.					Spr+++	
Aphanothece sp. Nägeli			Su+	Su+		
Asterionella sp. Hassal			Spr+			
Coelastrum sp. Nägeli	Spr+					
Cryptomonas sp.	Su+	Spr++		Spr++	Su+	Spr+; Su++
Cyclotella sp.	Spr+		Su+	Spr++	Su++	Spr+++
Dinobryon sp. Ehrenberg						Spr+
Gonyostomum semen					Spr+	Su++
Merismopedia sp. Meyen			Su+			
Microcystis sp.	Su++	Su+++	Su+			
Monoraphidium sp. Komárkova-Legenerová				Spr+		
Mougeotia sp. Agardh				Su+	Su+	
Oocystis sp. Nägeli	Spr+					
Rhodomonas sp.	Spr+	Spr+++; Su+	Spr+++			
Undet. flagellates			Spr+	Su++		

Spr, spring; Su, summer.
Biomass contribution relative to total phytoplankton biomass: +, >10–30%; ++, >30–50%; +++, >50%.

	Growth (per day)	Clutch size (eggs per female)	% gravid females	Survival
Clear water				
Spring	0.25 (0.09)	7.3 (6.3)	0.52 (0.46)	0.97 (0.01)
Summer	0.21 (0.06)	6.8 (0.42)	0.65 (0.23)	0.98 (0.01)
Humic				
Spring	0.14 (0.06)	0	0	0.93 (0.07)
Summer	0.16 (0.05)	2.3 (1.8)	0.1 (0.1)	0.96 (0.03)
Season (*P* value)	0.78	0.89	0.50	0.37
Lake category (*P* value)	**0.001**	**0.003**	**0.002**	0.23
Season × lake category (*P* value)	0.18	0.59	0.97	0.72

Table 4 Mean values (±SD) ($n = 9$) of *Daphnia magna* growth, clutch size, proportion of gravid females and survival in the clear water and humic lake categories in the experiments performed in spring and summer. Results (*P* values) from two-way ANOVA for growth, survival, clutch size and proportion of gravid females as dependent variables with season and lake category as fixed factors are also shown. Bold values denote significance at the 5% level.

	PC1	PC2	PC3	PC4	PC5
Variance explained (%)	32.8	25.7	14.4	10.1	7.4
Eigenvalues	5.6	4.4	2.4	1.7	1.2
POC (μg L^{-1})	**0.870**	−0.366	−0.254	−0.049	−0.005
18:3ω3 (μg L^{-1})	**0.839**	0.357	−0.082	−0.010	−0.043
18:2ω6 (μg L^{-1})	**0.824**	0.182	0.054	0.420	0.229
Chlorophyll *a* (μg L^{-1})	**0.782**	0.350	0.135	−0.189	−0.244
18:3ω6 (μg L^{-1})	**0.759**	−0.006	−0.050	0.031	0.440
18:4ω3 (μg mg C^{-1})	0.066	**0.902**	0.330	−0.024	−0.117
18:3ω3 (μg mg C^{-1})	0.344	**0.832**	0.071	−0.083	−0.025
18:2ω6 (μg mg C^{-1})	0.007	**0.790**	0.150	0.384	0.221
18:4ω3 (μg L^{-1})	0.435	**0.670**	0.268	0.057	−0.162
22:6ω3 (μg mg C^{-1})	0.104	0.120	**0.952**	−0.053	−0.033
22:6ω3 (μg L^{-1})	−0.162	0.156	**0.882**	−0.141	−0.066
C : N (atomic ratio)	0.019	−0.186	**−0.876**	−0.099	0.231
N : P (atomic ratio)	−0.012	−0.004	0.137	**0.975**	0.037
C : P (atomic ratio)	0.054	0.076	−0.386	**0.885**	0.056
20:5ω3 (μg L^{-1})	0.216	0.212	0.447	−0.255	**−0.729**
18:3ω6 (μg mg C^{-1})	0.019	0.467	−0.040	−0.135	**0.716**
20:5ω3 (μg mg C^{-1})	−0.203	0.484	0.486	−0.311	−0.562
t-test (*P* value)	0.732	0.699	**0.017**	0.116	0.884

Table 5 Component loadings of food quality (fatty acid content per unit carbon, fatty acid concentration per litre, and elemental ratios) and food quantity (particulate organic carbon and Chl *a*) parameters of seston in the studied lakes in spring and summer, and percentage of variance explained by the five retained principal component axes. Important loadings (>0.6) are bold. *P* values refer to independent-sample *t*-tests ($n = 6$), testing the differences in scores between clear water and humic lakes.

content (per carbon) (Fig. 2 and Table 6) but not with DHA concentration (per litre) (Pearson's correlation, $r^2 = 0.47$; $P = 0.34$ for growth; $r^2 = 0.80$; $P = 0.051$ for clutch size; $r^2 = 0.75$; $P = 0.08$ for proportion of gravid females). In the humic lakes, clutch size and % of gravid females were correlated both with DHA content (Fig. 2 and Table 6) and with DHA concentration (Pearson's correlation, $r^2 = 0.84$; $P = 0.03$ for clutch size; $r^2 = 0.85$; $P = 0.03$ for proportion of gravid females). No significant correlations were found between growth and reproduction with the content (per carbon) or the concentration of EPA (per litre) in the clear water or in the humic lakes (Pearson correlation, $P > 0.05$). Even though the correlations between growth and reproduction with EPA were not significant, EPA patterns were very similar to DHA patterns for each lake category (Fig. 2). C : N ratios correlated with reproductive parameters in the clear water lakes. The slopes of the relationships for the reproductive parameters with DHA content (per carbon) were greater than those for growth (Figs 2 & 3 and Table 6). The slopes of the relationships between reproductive parameters and DHA were greater in the clear water than in the humic lakes (Fig. 2 and Table 6).

Discussion

We have shown that the fatty acid quality of the seston, both as absolute concentration and as carbon units, was very similar between lake categories. However, growth and reproduction of *Daphnia* was higher in the clear water lakes. These differences can be explained by a combination of high fatty acid (especially DHA) quality and higher elemental (N) content of seston in the clear water lakes. Addition-

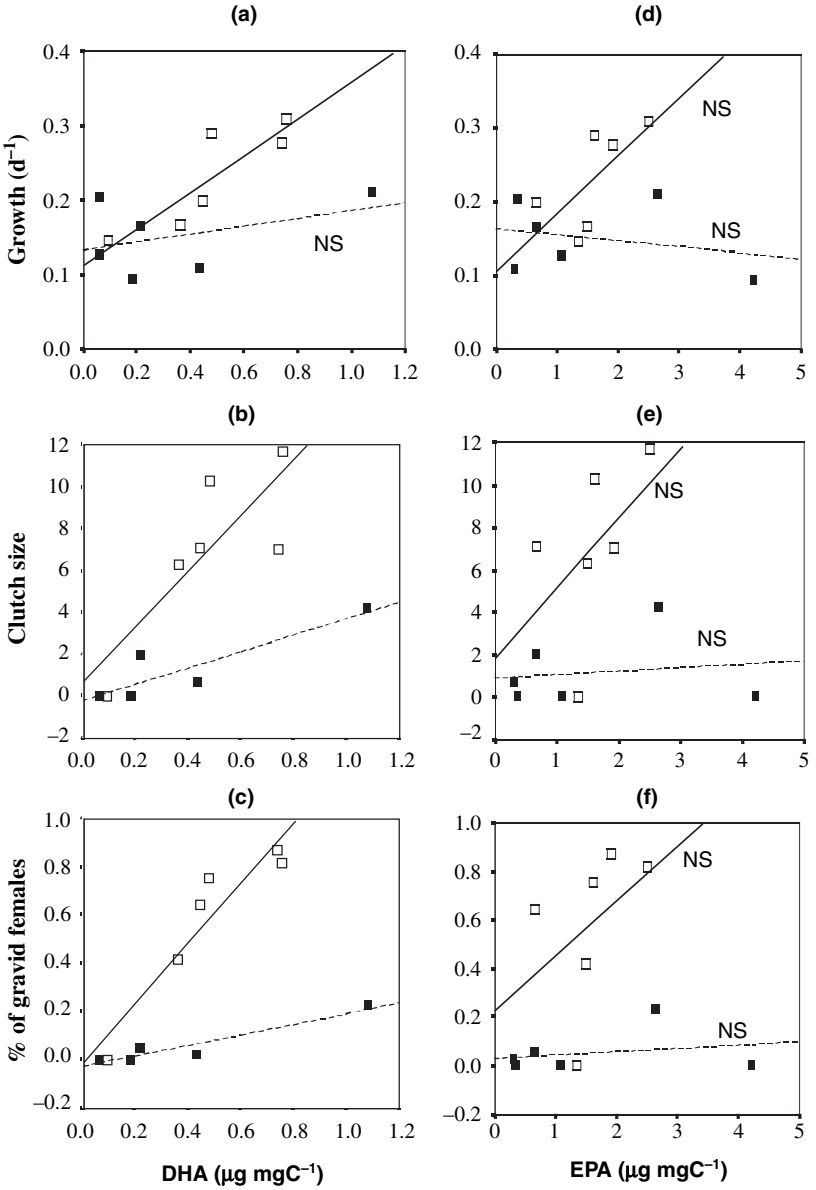

Fig. 2 Relation between growth, clutch size and proportion of gravid females with the seston content of DHA (a–c) and EPA (d–f) (as µg mg C^{-1}) in clear water (open symbols and filled line) and humic (filled symbols and dashed line) lakes. NS: non-significant at the 0.05% level from Pearson correlation.

ally, differences in food digestibility and pH likely contributed to the results.

Our results agree well with several studies that have reported the importance of fatty acids to *Daphnia* growth and egg production (Müller-Navarra, 1995; Brett & Müller-Navarra, 1997; Sundbom & Vrede, 1997; Weers & Gulati, 1997; Müller-Navarra *et al*., 2000; Park *et al*., 2002, 2003; Becker & Boersma, 2003, 2005; Ravet, Brett & Müller-Navarra, 2003). Patterns observed for growth and reproduction with DHA were very similar to those for EPA (Fig. 2). However, and contrary to what is usually reported, in our study the correlation of *Daphnia* growth and reproductive parameters with DHA was much stronger than that with EPA, probably because of the larger variation in EPA data. DHA is believed to be less important than EPA for *Daphnia* growth and reproduction (Ravet *et al*., 2003), and daphnids are poor in DHA but rich in EPA (Weers, Siewersen & Gulati, 1997; Persson & Vrede, 2006). However, as DHA can be converted into EPA (Von Elert, 2002), DHA may still play an important role in *Daphnia* nutrition.

Together with DHA, nitrogen explained most of the variation in food quality and quantity aspects in our lakes. Several studies have shown that *Daphnia* fed algae with high C : N or C : P ratios had reduced

Table 6 Relationships between growth and reproduction with seston DHA and EPA (as μg mg C^{-1}) content and C : N and C : P ratios (atomic ratios) in the clear water and humic lakes. Bold values denote significance at the 0.05 level (from Pearson's correlation, $n = 6$).

	Growth (per day)	Clutch	% of gravid females
Clear water			
DHA	**$y = 0.24x + 0.11$**	**$y = 13.19x + 0.76$**	**$y = 1.24x - 0.006$**
	$r^2 = 0.75$	**$r^2 = 0.73$**	**$r^2 = 0.90$**
EPA	$y = 0.08x + 0.11$	$y = 3.27x + 1.84$	$y = 0.22x + 0.23$
	$r^2 = 0.46$	$r^2 = 0.24$	$r^2 = 0.17$
C : N	$y = -0.07x + 0.81$	**$y = -5.543x + 52.01$**	**$y = -0.46x + 4.36$**
	$r^2 = 0.40$	**$r^2 = 0.73$**	**$r^2 = 0.78$**
C : P	$y = -0.0008x + 0.38$	$y = -0.0002x + 7.1$	$y = -0.003x + 1.15$
	$r^2 = 0.18$	$r^2 = 0.00$	$r^2 = 0.12$
Humic			
DHA	$y = 0.05x + 0.13$	**$y = 3.89x - 0.17$**	**$y = 0.22x - 0.02$**
	$r^2 = 0.27$	**$r^2 = 0.72$**	**$r^2 = 0.78$**
EPA	$y = -0.008x + 0.16$	$y = 0.16x + 0.88$	$y = 0.01x + 0.03$
	$r^2 = 0.06$	$r^2 = 0.02$	$r^2 = 0.06$
C : N	$y = 0.0002x + 0.15$	$y = -0.24x + 3.65$	$y = -0.01x + 0.21$
	$r^2 = 0.00$	$r^2 = 0.06$	$r^2 = 0.09$
C : P	$y = -0.0001x + 0.17$	$y = -0.01x + 2.9517$	$y = -0.0004x + 0.13$
	$r^2 = 0.05$	$r^2 = 0.28$	$r^2 = 0.18$

growth and reproduction (Sterner, 1993; Sterner *et al*., 1993; Urabe & Sterner, 2001). Acharya, Kyle & Elser (2004) showed that *Daphnia* growth rate was highest when the C : N ratio of the diet was 6, which is the mean herbivore C : N ratio of herbivorous zooplankton (Elser *et al*., 2000). Additionally, growth rates decreased markedly as the C : N ratio increased, especially from 9 to 15. The mean seston C : N ratio in our clear lakes was around 8 (range: 7.6–9.3), while for humic lakes the mean C : N ratio was 10.5 (range: 8.3–13.2), indicating that seston from both lake categories had an excess of C relative to N when compared with reported *Daphnia* C : N ratios. The mean seston C : N ratio was thus higher in the humic than in the clear water lakes, and in most of the humic lakes it was above the value demonstrated by Acharya *et al*. (2004) to cause a decrease in *Daphnia* growth. As *Daphnia* growth and reproduction were significantly lower in the humic lakes compared with the clear water lakes, our results suggest that dietary N may have been more limiting in the humic lakes.

The slopes of the relationships for the reproductive parameters with DHA content were greater than those for growth (Fig. 2 and Table 6), suggesting a clearer effect of DHA content on reproduction. This is in line with the idea that fatty acids are more important for reproduction than for growth (Becker & Boersma, 2003). Additionally, when comparing the slopes of the relationships of DHA and reproduction between lake categories, it seems there was a greater effect of DHA on reproduction in the clear water, where C : N ratios were lower. Apparently, at C : N ratios lower than approximately 8, the fatty acid content of the food led to larger differences in reproduction (Figs 2b,c & 3b,c). It is important to point out that *Daphnia* were probably not limited by P in our study, as the C : P ratios were below the commonly accepted element threshold of 300 (Sterner & Schulz, 1998; Brett *et al*., 2000). Our results suggest that not only P (Sundbom & Vrede, 1997; Sterner & Schulz, 1998; Becker & Boersma, 2003), but also N requirements need to be met first in order for fatty acids to have a substantial effect on *Daphnia* reproduction. Even though some studies reported *Daphnia* egg production (Hessen, 1989) and growth (Müller-Navarra, 1995) to be correlated to particulate nitrogen, most studies show positive correlations of *Daphnia* growth and reproduction with algal P content (Gulati & Demott, 1997; Sterner & Elser, 2002). However, Acharya *et al*. (2004) found that the tight coupling between *Daphnia* growth, P and RNA content was broken under N limiting but P-sufficient conditions, indicating the importance of other growth limiting factors, like N (proteins). The role of N in determining food quality for freshwater zooplankton has received considerably less attention than the role of P, partly because production in freshwaters is thought more likely to be P limited (Elser, Marzolf & Goldman, 1990). Our results suggest that N may play a role in freshwater food webs when P is not limiting and therefore

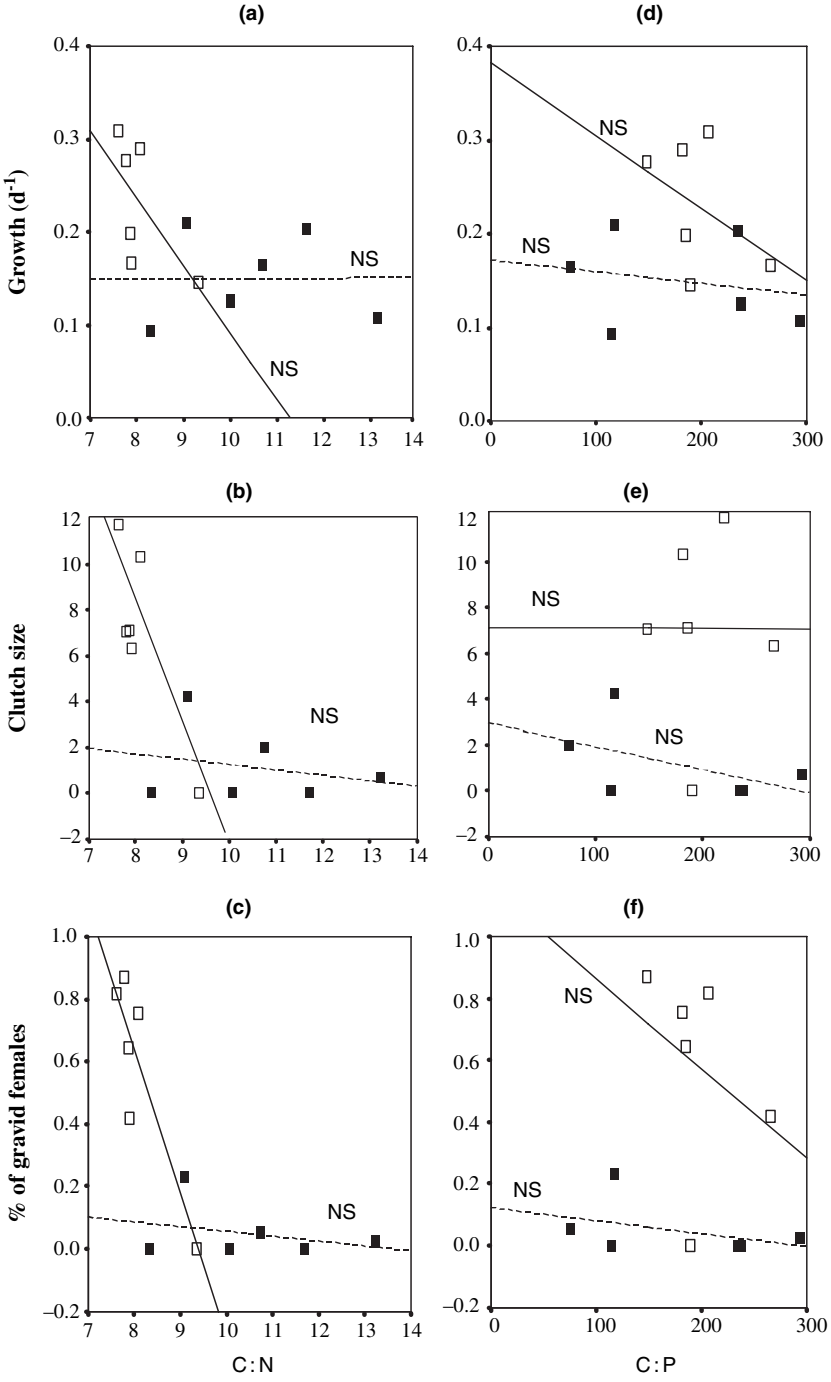

Fig. 3 Relation between growth, clutch size and proportion of gravid females with seston C : N (a–c) and C : P (d–f) ratios (atomic ratios) in clear water (open symbols and filled line) and humic (filled symbols and dashed line) lakes. NS: non-significant at the 0.05% level (from Pearson correlation).

merits more attention in studies of zooplankton nutrition.

Not only HUFA and elements but also food digestibility (DeMott & Tessier, 2002) may explain the observed differences in *Daphnia* growth and reproduction between lake categories. Edible cells, such as *Cryptomonas* sp. and *Rhodomonas* sp. (Müller-Navarra & Lampert, 1996), had comparatively high biomass in spring and summer in the clear water lakes. On the other hand, interfering algae (algae with morphological defenses; large or colony forming algae; or toxin-producing algae), such as the Cyanophyte *Anabaena* sp. Bory; the Chrysophyte *Dinobryon* sp. Ehrenberg and the Raphidophyte *G. semen*, which were important in some of the humic lakes, were unimportant or absent in the clear water lakes. Thus,

differences in algal community structure between lake categories might have influenced our results. The presence of interfering algae in the humic lakes could reflect high grazing pressure on the most palatable algal species at the time of sampling. Interfering algae are poorly digested compared with edible algae, and can lead to energy limitation of zooplankton growth (Sterner & Hessen, 1994; Van Donk *et al*., 1997; DeMott, Edington & Tessier, 2004). In spite of the high POC concentration in the humic lakes, *Daphnia* could have experienced energy limitation because of low contribution of algal carbon to the POC pool (Fig. 1). In humic lakes, the contribution of detritus and bacteria to the POC pool is usually high (Hessen, *et al*., 1990, 2003). Even though detritus and bacteria can be important food items for *Daphnia* in humic lakes (Salonen & Hammar, 1986; Hessen *et al*., 1990), they are assimilated with low efficiency compared with phytoplankton (Hessen, 1998). Thus, energy limitation in the humic lakes might have contributed to the differences in *Daphnia* growth and reproduction between clear water and humic lakes.

Differences in pH between lake categories cannot be ruled out as an additional explanation for our results. Either very low (≤5) (Geelen & Leuven, 1986; Brett, 1989) or very high (>10) (Vijverberg, Kalf & Boesma, 1996) pH can affect growth and reproduction of *Daphnia*. The optimum for growth and reproduction may differ from species to species (Geelen & Leuven, 1986). For instance, the potential for reproduction of *D. pulex* seems to be limited to pH 7.0–8.7, even though the species tolerates pH between 4.3 and 10.4 (Davis & Ozburn, 1969). *D. magna* tolerates pH as low as 4.5 (Geelen & Leuven, 1986) and Parent & Cheetham (1980) observed reproduction of *D. magna* at a pH of 5.0 or greater. Assuming that the optimum pH range for reproduction of *D. magna* is similar to that for *D. pulex*, *Daphnia* growing in the humic water was under suboptimal pH conditions for reproduction. Thus, it is possible that differences in pH between lake categories contributed to the observed differences in *Daphnia* growth and reproduction.

In conclusion, we have shown that seston from clear water and humic lakes supported different growth and reproduction of *Daphnia*. A combination of elemental and fatty acid quality of the food explained the observed differences. Differences in food digestibility, and thus energy assimilation, as well as pH might also have contributed to the results. Our results suggest that HUFA have the potential to improve herbivore growth and reproduction, and ultimately increase the efficiency of energy transfer in the food chain, if elemental (N and P) requirements are met first.

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