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Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels

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Abstract

We measured concentrations of essential fatty acids (EFAs) in four size categories of planktonic organisms—seston (10–64 μm), microzooplankton (100–200 μm), mesozooplankton (200–500 μm), and macrozooplankton (>500 μm)—and in rainbow trout (*Oncorhynchus mykiss*) in coastal lakes. Size-dependent patterns in concentrations of specific fatty acids (FAs) are important for ecosystem function, because planktivorous fish and some invertebrates are size-selective predators. We demonstrate that the retention of individual FAs differs among the four size categories of planktonic organisms in our study systems. Changes in individual EFA concentrations within the planktonic food web were similar in all coastal lakes sampled, which indicates the generality of our findings. Although concentrations of arachidonic acid, eicosapentaenoic acid (EPA), and linoleic acid increased steadily with plankton size, the concentration of α-linolenic acid decreased slightly in larger size fractions of zooplankton. Concentrations of another EFA, docosahexaenoic acid (DHA), declined sharply from mesozooplankton to the cladoceran-dominated macrozooplankton size class. Our results indicate that the retention of EFAs, as a function of plankton size, is related, in part, to the taxonomic composition of planktonic food webs. We suggest that, in general, zooplankton exhibit an EPA-retentive metabolism with increasing body size, whereas different taxonomic groups within the planktonic food web retain DHA differently. Finally, we conclude that EPA is highly retained in zooplankton, whereas in rainbow trout DHA is highly retained.

The assimilation and retention of key nutrients in consumers is fundamental to the optimal physiological performance of animals in aquatic food webs. Their beneficial effects are quickly realized in the form of enhanced somatic growth (Elser et al. 2000; Wacker and Von Elert 2001; Pazzia et al. 2002) and reproductive rates (Williamson et al. 1996; Montel and Lair 1997). Phosphorous (Sterner and Elser 2002) and essential fatty acids (EFAs; Arts and Wainman 1999) have been identified as two key nutrients that influence food quality and, subsequently, affect the somatic growth and reproduction of zooplankton in freshwater ecosystems.

Physiological processes in zooplankton are tightly linked to the dietary uptake of nutrients. In *Daphnia*, for example, the greatest somatic growth rates were measured at dietary C : P (molar) ratios <200 (Wacker and Von Elert 2001), with P becoming the limiting nutrient for growth at C : P ratios >300 (Sterner 1993). In addition to P as a growth-enhancing element, it has been suggested that certain polyunsaturated fatty acids (PUFAs) are essential for optimal physiological performance. EFAs cannot be synthesized by organisms at rates sufficient to meet their basic biochemical requirements and, thus, must be obtained largely through the diet. EFAs are required for maintaining cell membrane fluidity (Pruitt 1990) and for regulating hormonal processes (Bell et al. 1991). In the present article, we designate the following PUFAs as EFAs: arachidonic acid (ARA; C20:4ω6), eicosapentaenoic acid (EPA; C20:5ω3), and docosahexaenoic acid (DHA; C22:6ω3). It has previously been demonstrated that linoleic acid (LIN; C18:2ω6) can be converted into ARA (Stanley-Samuelson 1994) and α-linolenic acid (ALA; C18:3ω3) into EPA (Von Elert 2002). Thus, we include LIN and ALA as EFAs because they may become essential when the longer-chain EFAs are in short supply.

Results from laboratory tests have suggested that the presence of dietary EFAs enhances the somatic growth of *Daphnia*. For example, Müller-Navarra et al. (2000) found correlative evidence that dietary EPA increased the somatic growth and egg production of *Daphnia magna* and proposed that EPA may be of general importance for trophic transfer efficiency in aquatic food webs. Wacker and von Elert (2001) demonstrated that ALA, rather than EPA, was significantly correlated with the somatic growth of *D. galeata*

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Table 1. Water chemistry measured in natural lakes and drinking-water reservoirs of southern Vancouver Island, British Columbia. T: temperature; DO: dissolved oxygen; DOC: dissolved organic carbon; Chl *a*: chlorophyll *a*. Data are mean values of epi-, meta-, and hypolimnetic Chl *a* values ± SD.

Sta.	Sta. depth (m)	Secchi depth (m)	T* (°C)	pH	DO* (mg L^{-1})	Chl *a* mean ± SD (μg L^{-1})	DOC* (mg L^{-1})
Natural lakes							
SHL-A	24.0	4.5	20.8	7.1	7.9	1.7±0.5	3.7
SHL-B	49.0	6.0	21.3	7.1	7.6	1.3±1.2	3.7
COL	20.0	7.0	20.6	6.7	7.3	1.0±0.3	2.5
ELL†	13.0	4.5	22.7	7.5	9.1	3.2±0.6	6.0
Reservoirs							
SOL-A	15.5	6.5	21.5	7.1	7.5	0.5±0.2	2.9
SOL-B	67.0	7.0	19.3	7.1	7.6	0.7±0.3	3.1
GOL	28.0	7.5	20.8	6.7	8.5	0.4±0.2	2.4
BUL	41.0	8.0	20.9	6.9	6.7	0.5±0.4	2.4

* Epilimnetic values.
† ELL turned anoxic below 9 m water depth.

in the laboratory and proposed that those two PUFAs should both, depending on the circumstances, be considered essential for *Daphnia*. Although PUFAs are required for optimal physiological performance, it has been demonstrated for daphnids that de novo FA synthesis rates are generally <2% (Goulden and Place 1990), which has led to the assumption that most FAs in daphnids are largely dietary in origin.

Food quality is also important for the development and survival of fish. It has been reported that EFAs in marine larvae promote fish growth (Izquierdo et al. 2000). For example, yellowtail flounder (*Limanda ferruginea*) larvae grew significantly larger and had higher survival rates when fed EFA-enriched (mainly DHA) rotifers than on diets without EPA, DHA, or ALA (Copeman et al. 2002).

These observations encouraged us to gather more information about the EFA composition of planktonic food webs. There is a need to better understand (1) their value as nutritional sources for higher trophic levels, (2) how the distribution of the various EFA in different species and size fractions within planktonic food webs affect the transfer and retention of these compounds within and among trophic levels and in lakes of different trophic status, (3) which components of the planktonic food web will contribute most to the sedimentation of EFAs to benthic communities, and (4) which size fractions, from a conservation perspective, merit the most attention/protection. The last point draws attention to the concept of ''cornerstone'' aquatic species, because some species (common in certain size fractions) will be disproportionately important with respect to their EFA concentrations.

Various EFAs have been tested for their effect on growth and reproduction of laboratory-raised *Daphnia* in an effort to shed light on the role of these compounds in the transfer of materials across the plant-animal interface. For example, it has been shown that EFAs added to unialgal food sources improve growth and reproduction of *Daphnia* (Sundbom and Vrede 1997). However, in natural systems, algal and zooplankton species diversity is higher, and communities are correspondingly more complex than in controlled laboratory experiments. Our knowledge about the concentrations of PUFAs sequestered in different size fractions of planktonic food webs and their rates of movement among compartments is currently very limited. In addition, we know little about the critical link between PUFA concentrations of the planktonic food web and their ecological role for fish (c.f., Ballantyne et al. 2003). Thus, we conducted a field study to examine size-dependent patterns of PUFA concentrations in plankton ranging in size from large seston particles to macrozooplankton. Our null hypothesis was that concentrations of essential PUFAs would not increase with increasing particle size in the three zooplankton size fractions. To test this, we investigated the relationship between retention patterns of individual PUFA concentrations in different size classes of planktonic organisms in six coastal lakes. Our underlying assumption was that EFAs do not generally serve as biochemical precursors that are rapidly transformed to other compounds. In addition, we compared the available planktonic PUFA concentrations (standing crop estimates) to PUFA composition and concentration in rainbow trout (*Oncorhynchus mykiss*) from three of the lakes.

Methods

Our study was conducted in late June 2002 in six oligotrophic, monomictic coastal lake systems on southern Vancouver Island, British Columbia, Canada. Shawnigan Lake (SHL; 48°37′N, 123°38′W) and Elk Lake (ELL; 48°31′N, 123°23′W) are natural lakes that are used for recreational activities, including sport fishing. Council Lake (COL; 48°31′N, 123°40′W), Sooke Reservoir (SOL; 48°33′N, 123°41′W), Goldstream Reservoir (GOL; 48°30′N, 123°38′W), and Butchard Reservoir (BUL; 48°32′N, 123°39′W) are located in the protected Capital Regional District watershed area. SOL, GOL, and BUL are drinking-water reservoirs in which the artificial drawdown of water occurs. The morphometry of SHL and SOL is very similar; both lakes have a shallow (A) and a deep (B) basin. Selected physicochemical characteristics of these lakes are listed in Table 1.

For chlorophyll *a*, 1 liter of epi-, meta-, and hypolimnetic lake water, collected by a Van Dorn water sampler, was filtered through a Gelman glass-fiber filter (0.45 μm pore size). The samples were kept frozen until extraction. For dissolved organic carbon (DOC) samples, epilimnetic lake water was obtained with a plastic syringe and filtered (GN-6 mixed cellulose ester Gelman membranes, 0.45 μm pore size). Samples were stored in precombusted glass vials (without a head space) at 4°C until analysis.

Zooplankton was collected at the deepest stations in each lake by towing a 64-μm plankton net from 1 m above the bottom to the surface. The zooplankton were first rinsed with filtered (0.45 μm) lake water to remove adhered matter and then size fractionated using Nitex meshes with 100-, 200-, and 500-μm openings. For seston, lake water was collected using an integrated sampling tube (10 m length), filtered through a 64-μm mesh and, retained on a 10 μm mesh filter cup. Therefore, the ''seston fraction'' excluded nanoplanktonic algae and exceeded what is generally considered to be the most edible size fraction ($<$30 μm; Burns 1968) in the diet of cladocerans. Size-fractionated zooplankton and seston were transferred in polypropylene vials and immediately put on dry ice. All samples were kept frozen at −80°C until lyophilization, and then again stored at −80°C until FA analysis.

Rainbow trout (*Oncorhynchus mykiss*) was chosen as a model fish species for PUFA analysis because it is a widespread species in Canadian lakes and rivers. We collected fish by gillnets, but only dorsal muscle samples of fish of similar length (25.6 ± 2.0 cm) and weight (182 ± 37 g wet weight) from SHL-A (n = 1), SHL-B (n = 3), ELL (n = 3), SOL-A (n = 2), and SOL-B (n = 3) were used for lipid analysis, so that our results would not be biased by size or weight. Results from gut analysis showed that the rainbow trout from these lakes were planktivores (R. McMackin pers. comm.). We define the ''accumulation'' of EFA as the increase in the concentrations of particular EFA from smaller to larger plankton sizes and ''retention,'' in a particular size class, as the ability of organisms in that size class to regulate and control ingested EFAs.

Analyses

Zooplankton classification, Chl a, *and DOC*—Zooplankton were transferred to a zooplankton counting wheel under a microscope for identification, enumeration, measurement, and subsequent biomass estimation using Z-Counts software (version 2.3; Voila Data). For Chl *a*, samples were extracted with 95% ethanol, followed by spectrophotometer measurements. For DOC analysis, triplicate 8-ml filtered lake water samples were acidified to 2 N with HCl before analysis in a Shimadzu TOC-5000A analyzer (Shimadzu). Dissolved oxygen (DO) and temperature profiles were measured using an YSI Model 3800 multisampler (YSI).

Lipid and FA analysis—Total lipids and FAs from homogenized, freeze-dried zooplankton samples (5–10 mg) and dorsal muscle samples of rainbow trout (25–35 mg) were analyzed as described by Parrish (1999). In brief, the samples were sonicated and vortexed four times in a 4 : 2 : 1 chloroform methanol water mixture, and the organic layers were removed and pooled. Total lipid concentrations were determined gravimetrically by removing and weighing a 2-ml subsample of the lipid extract, which had been evaporated.

FAs were analyzed as methyl esters using a gas chromatograph (GC; Varian CP-3800; Varian) equipped with a flame ionization detector (FID). The methyl esters were prepared by trans-esterifying the lipid extract in BF_3-CH_3OH at 85°C for 1 h (for details on lipid extraction and fatty acid methyl esters [FAME] formation, *see* Kainz et al. 2002). The FAMEs were analyzed on a Supelco 2560 Capillary Column (100 m, 0.25 mm inner diameter, and 0.2 μm film thickness). Helium was used as the carrier gas (1 ml min^{-1} flow rate). The following temperature ramp was used: 65°C for 0.5 min, holding at 195°C for 15 min after ramping at 40°C min^{-1}, and holding at 240°C for 10 min after ramping at 2°C min^{-1}. Helium (make-up gas) and air (combustion) had flow rates of 30 and 300 ml min^{-1}, respectively. The FID was isothermal at 260°C, whereas the injector was programmed to increase to 250°C at a rate of 200°C min^{-1} after holding at 150°C for 0.5 min. FAMEs were identified by comparison of their retention times with known standards (37-component FAME mix, Supelco 47885-U) and quantified with reference to seven-point calibration curves derived from 2.5, 50, 100, 250, 500, 1,000, and 2,000 ng μl^{-1} solutions of the FAME standard.

Data analysis—We used paired *t*-test analysis to determine the effect of hydrographical differences between lakes and reservoirs (water body effect) on plankton size and to compare the means of PUFA concentrations in planktonic organisms and *O. mykiss* between the sampled lakes and reservoirs. Size difference factors were calculated by comparing the mean plankton sizes among the different size fractions of the planktonic food web. To examine the effect of zooplankton taxonomy on differences in EFA concentrations among the sampled lakes, we performed nearest neighbor, hierarchical cluster analysis. This numerical test grouped zooplankton of different taxa into classes so that similar ones formed clusters. The level of similarity of clusters was expressed by distance (taxonomy effect). Using analysis of covariance (ANCOVA) to correct for the effect of sampling station, we applied linear and quadratic regression models to examine the relationships between size of planktonic organisms and their EFA concentrations (plankton size effect); plankton size was the dependent variable, and EFA concentrations were the independent variables. We used analysis of variance with subsequent post hoc tests to examine the relationship between total lipid concentrations and size classes of planktonic organisms.

Results

Lake characteristics—The mean (±SD) epilimnetic water temperature was 21°C (±1), and all lakes and reservoirs were thermally stratified (thermocline started, on average, at 6 m depth). The water columns were generally well oxygenated ($>$2 mg DO L^{-1}), with the exception of ELL, which turned anoxic below 9 m. The lake and reservoir waters had pH values of ~7. Mean Chl *a* levels in reservoirs were $<$1

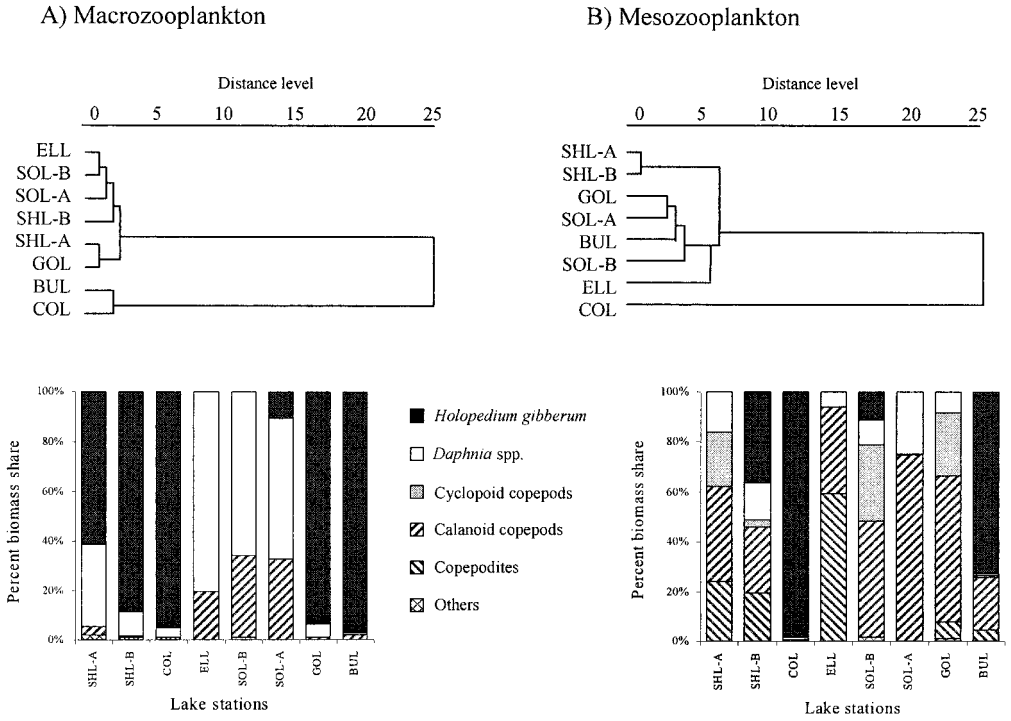

Fig. 1. Dendrograms from cluster analysis and percentages of biomass shares (cumulative bars) of (A) macrozooplankton (>500 μm mesh size) and (B) mesozooplankton (200–500 μm mesh size). Results from nearest neighbor, hierarchic cluster analysis are based on counts of individuals and include zooplankton genera from Elk Lake (ELL); Sooke Lake, Sta. A and B (SOL-A and SOL-B), Shawnigan Lake, Sta. A and B (SHL-A and SHL-B), Goldstream Lake (GOL), Butchard Lake (BUL), and Council Lake (COL). Macrozooplankton taxa from BUL and COL and mesozooplankton taxa from COL form a cluster by themselves.

μg L^{-1} and lower than those of natural lakes (2.1 $\pm$ 1.1 μg L^{-1}).

Size fraction and taxonomic composition—The average size of plankton did not differ significantly between natural lakes and reservoirs ($p > 0.05$) in any of the size fractions. For macrozooplankton, this measurement was 1,144 μm $\pm$ 172 and 1,102 μm $\pm$ 228 for the natural lakes and reservoirs, respectively. Although meso- and microzooplankton were collected using mesh sizes between 200 and 500 and 100 and 200 μm, respectively, mean plankton sizes were larger (i.e., 633 $\pm$ 104 and 205 $\pm$ 10 μm for natural lakes; 623 $\pm$ 142 and 237 $\pm$ 21 μm for reservoirs), probably because some of the larger zooplankton passed head-first through the mesh. The size of seston (10–64 μm) was not verified microscopically. Plankton size increased between (1) seston (largest particle size, 64 μm) and microzooplankton (3.5× $\pm$ 0.4), (2) micro- and mesozooplankton (2.9× $\pm$ 0.3), (3) meso- and macrozooplankton (1.8× $\pm$ 0.3) and, (4) seston and macrozooplankton (17.8× $\pm$ 1.9).

Macrozooplankton was mainly composed of calanoid copepods, *Daphnia* spp., and *Holopedium gibberum* (Fig. 1A). The mesozooplankton size fraction consisted mostly of calanoid and cyclopoid (missing in ELL) copepods, *Daphnia* spp., *H. gibberum*, and copepodites (Fig. 1B). The biomass of macrozooplankton was largely composed of large cladocerans (*H. gibberum* and *Daphnia* spp.; Fig. 1). Biomass shares of the mesozooplankton size fraction changed considerably among the lakes; however, *H. gibberum* dominated the biomass in COL. The microzooplankton size class was composed of both zooplankton (copepod nauplii and *Keratella* spp.) and phytoplankton (e.g., *Asterionella formosa, Tabellaria fenestrate, T. flocculosa, Cyclotella* spp., *Ceratium hirundinella,* and *Dinobryon divergens*). The sestonic size fraction of all lakes was mainly comprised of *A. formosa, T. fenestrata,* and *Chrysosphaerella longispina.* Taxa of this size-fraction (10–64 μm) were probably grazing resistant and thus did not represent the entire ingestible FA diet pool for cladocerans. Results from cluster analyses showed that the taxonomic composition of macrozooplankton was similar among ELL, SHL, SOL, and GOL, because they shared clusters; however, the zooplankton communities of BUL and COL formed a cluster by themselves. Communities of mesozooplankton from ELL, SHL, SOL, GOL, and BUL shared clusters, and the community structure of COL formed its own cluster.

Lipids and FAME in zooplankton—Total lipid concentrations differed between size fractions. Post hoc tests using linear contrasts showed that concentrations of total lipids increased linearly from seston (120 $\pm$ 36 mg g^{-1}) to microzooplankton (144 $\pm$ 35 mg g^{-1}) to mesozooplankton (228 $\pm$ 95 mg g^{-1}; $p < 0.05$) but decreased in macrozooplankton (183 $\pm$ 41 mg g^{-1}; $p < 0.05$). The relative amount of PUFAs in the total lipid mass of the planktonic food web increased from seston (8.9% $\pm$ 3.8) to microzooplankton (13.9% $\pm$ 5.2) and mesozooplankton (15.4% $\pm$ 7.1) and decreased in macrozooplankton (13.7% $\pm$ 2.3). Accordingly, total PUFA

Table 2. Mean concentrations (mg g dry weight^{-1}) of polyunsaturated fatty acids (PUFA, ± SD) and essential fatty acids (EFA, ±SD; including arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid) in seston, micro-, meso-, and macrozooplankton of all lakes/reservoirs.

Size fraction	PUFA	EFA
Macrozooplankton	24.3 (±4.0)	17.2 (±3.2)
Mesozooplankton	29.7 (±5.6)	21.6 (±5.0)
Microzooplankton	19.1 (±6.2)	13.9 (±4.7)
Seston	9.5 (±1.7)	5.8 (±1.6)

concentrations increased from seston to mesozooplankton and decreased in macrozooplankton (Table 2).

Mean concentrations of individual EFAs generally increased from seston to macrozooplankton: for ARA from 0.4 to 4.3 mg g^{-1} (10.8×) and for EPA from 2.6 to 10.8 mg g^{-1} (4.2×), respectively. However, although the mean concentration of DHA increased from seston (2.8 mg g^{-1}) to mesozooplankton (10.2 mg g^{-1}), it sharply decreased to 2.2 mg g^{-1} in the macrozooplankton size fraction. LIN also increased on average by 2.3× from seston (1.5 ± 0.8 mg g^{-1}) to macrozooplankton (3.4 ± 0.7 mg g^{-1}; Fig. 2A); however, as was observed for DHA, ALA increased from seston (2.1 ± 1.9 mg g^{-1}) to mesozooplankton (5.0 ± 2.4 mg g^{-1}) but then decreased in macrozooplankton (3.6 ± 0.9 mg g^{-1}; Fig. 2B).

Results of linear correlations showed that the following FAs increased significantly ($p < 0.05$) with plankton size: ARA ($r^2 = 0.86$; Fig. 2C), EPA ($r^2 = 0.75$; Fig. 2D), and LIN ($r^2 = 0.73$). Because concentrations of ALA and DHA (Fig. 2E) decreased in macrozooplankton, second-order polynomial regression analysis was used to calculate the maximum body size at which ALA and DHA started to decrease. The sampling-station–corrected polynomial fit was significant for both EFA and resulted in the following equations:

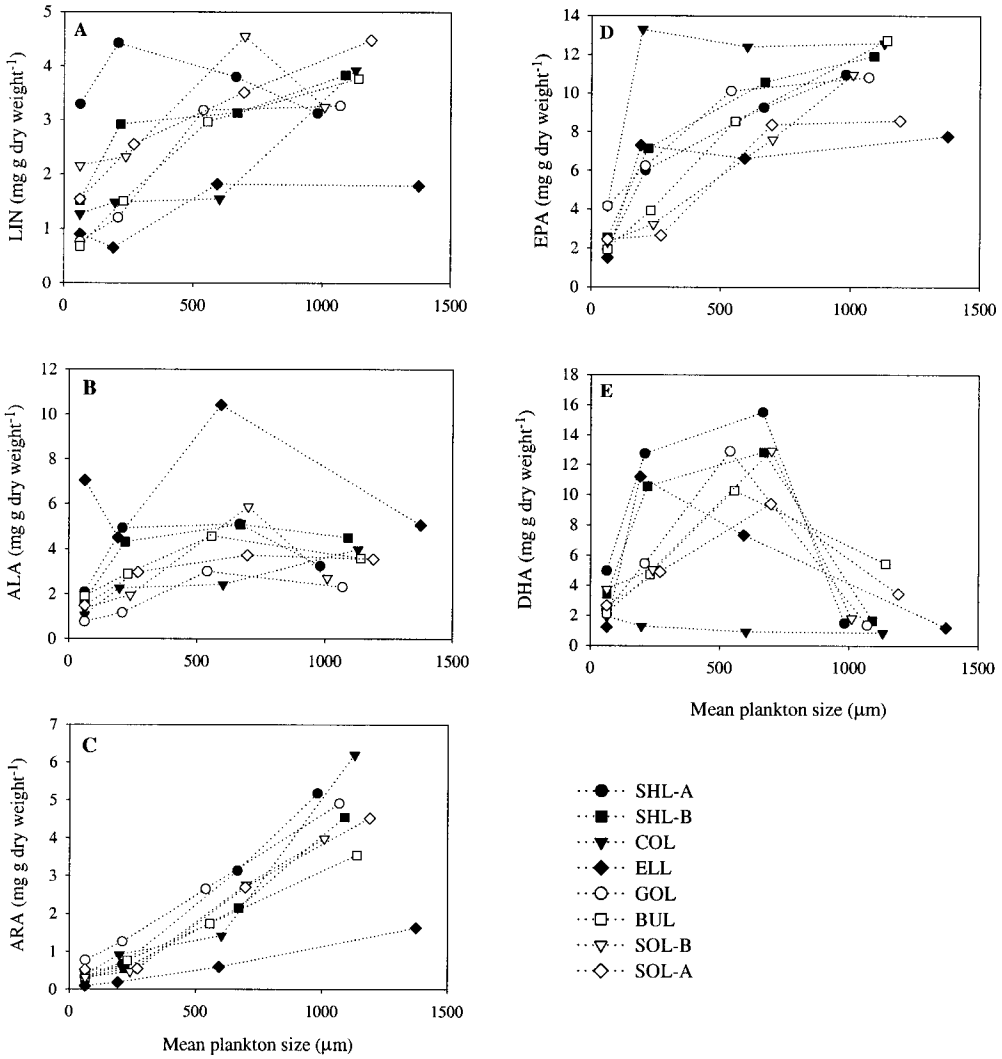

Fig. 2. Polyunsaturated fatty acid concentrations in seston (10–64 µm), microzooplankton (100–200 µm), mesozooplankton (200–500 µm), and macrozooplankton (>500 µm) from Council Lake (COL), Elk Lake (ELL), Shawnigan Lake (SHL-A and SHL-B), Goldstream Reservoir (GOL), Butchard Reservoir (BUL), and Sooke Reservoir (SOL-A and SOL-B): (A) LIN (18:2ω6), (B) ALA (18:3ω3), (C) ARA (20:4ω6), (D) EPA (20:5ω3), and (E) DHA (22:6ω3).

Table 3. Accumulation factors of PUFA compounds (mean ± SD of all lakes/reservoirs) within the planktonic food web, from seston (10–64 μm), to microplankton (micro; 100–200 μm), to mesoplankton (meso; 200–500 μm), to macrozooplankton (macro; >500 μm). LIN: linoleic acid, ALA: α-linolenic acid, ARA: arachidonic acid, EPA: eicosapentaenoic acid, and DHA: docosahexaenoic acid.

PUFA	Seston–micro	Micro–meso	Meso–macro	Seston–macro
LIN	1.5 (±0.5)	1.7 (±0.7)	1.2 (±0.6)	2.9 (±1.5)
ALA	1.8 (±0.6)	1.7 (±0.8)	0.8 (±0.4)	2.3 (±1.0)
ARA	1.9 (±0.5)	3.6 (±1.6)	2.3 (±0.4)	13.2 (±4.3)
EPA	2.5 (±1.2)	1.8 (±0.8)	1.2 (±0.2)	4.5 (±1.4)
DHA	2.9 (±2.6)	1.6 (±0.7)	0.3 (±0.3)	0.9 (±0.7)
Total	2.1 (±0.7)	2.1 (±0.6)	1.2 (±0.4)	4.7 (±0.8)

$$y = 1{,}825.4 + 7.12x - 0.0046x^2; \qquad r^2 = 0.20 \text{ for ALA}$$

and

$$y = 2{,}046.6 + 24.26x - 0.0205x^2; \qquad r^2 = 0.40 \text{ for DHA}$$

These two equations provided estimates for maximum concentrations of ALA and DHA at zooplankton sizes of 774 and 592 μm, respectively. EPA : ARA ratios decreased significantly with increasing plankton size ($r^2 = 0.63$, $p < 0.05$; ANCOVA) and did not reach values <1. PUFA concentrations (in seston and macrozooplankton organisms) were not significantly ($p > 0.05$) related to Chl *a* concentrations in the study lakes.

Accumulation factors for the various EFAs were calculated as the quotients of EFA concentrations between different (larger and smaller) plankton size classes. EFA concentrations increased from seston to macrozooplankton by an average of 4.7×. For individual EFA, accumulation factors were found to be greatest for ARA (13.2× ± 4.3), followed by EPA (4.5× ± 1.4) and ALA (2.3× ± 1.0). No significant increase of DHA from seston to macrozooplankton was detected. LIN concentrations increased by 2.9× ± 1.5 from seston to macrozooplankton (Table 3).

FAME in fish—Mean concentrations of PUFA (mg g dry weight^{-1}; ±SD) were 18.7 (±1.5), 18.0 (±3.0), and 21.4 (±1.8) in dorsal muscle samples from rainbow trout collected from SOL, SHL, and ELL, respectively. The relative amounts of EFA (i.e., ARA, EPA, and DHA) were 73%, 89%, and 84% for SOL, SHL, and ELL, respectively. Total PUFA concentrations in muscle tissues of SOL and SHL rainbow trout were not significantly different from each other ($p > 0.05$), whereas fish from ELL had significantly higher PUFA concentrations in their muscles than fish from the other lakes ($p < 0.05$). The highest concentrations of individual EFAs in rainbow trout muscle were measured in DHA, followed by EPA, ARA, LIN, and ALA (Table 4).

Discussion

PUFA in the planktonic food web—EFAs are crucial for aquatic organisms because they affect metabolic activity, individual and population growth rates, and reproduction. Some studies have analyzed PUFA patterns in larger zooplankton, mostly using species of *Daphnia* (e.g., Demott and Müller-Navarra 1997; Müller-Navarra et al. 2000; Wacker and Von Elert 2001). Here we examine concentrations of total and of specific PUFAs (EFAs) in different size fractions of the planktonic food web rather than in individual species. Concentrations of PUFA in macrozooplankton in our study lakes were similar to those of larger (>300 μm) zooplankton collected during summer in Lake Erken, Sweden (Ahlgren et al. 1997). However, analyses of PUFA concentrations from different plankton sizes over a range of lakes have not, to our knowledge, been investigated.

Although the taxonomic composition of mesozooplankton of COL and macrozooplankton of BUL and COL was different from that of other lakes, total PUFA concentrations are not significantly different from those of other study lakes, which suggests that total PUFA concentrations within these two size classes of planktonic food web do not strongly depend on the taxonomic composition of the zooplankton. We demonstrate that the accumulation of PUFA is highest between seston and mesozooplankton and decreases toward macrozooplankton. Although some PUFAs continued to increase in the macrozooplankton size fraction, decreasing concentrations of total PUFAs from the meso- to the macrozooplankton size fraction may be related to different PUFA metabolism or to loss of PUFA-enriched eggs in the largest

Table 4. Mean concentrations (±SD; mg g dry weight^{-1}) of PUFA compounds (mean ± SD) in rainbow trout (*Oncorhynchus mykiss*) of SHL-A ($n = 1$), SHL-B ($n = 3$), ELL ($n = 3$), SOL-a ($n = 2$), and SOL-B ($n = 3$). LIN: linoleic acid, ALA: α-linolenic acid, ARA: arachidonic acid, EPA: eicosapentaenoic acid, and DHA: docosahexaenoic acid.

PUFA	SHL-A	SHL-B	ELL	SOL-A	SOL-B
LIN	2.2	0.9 (±0.5)	1.8 (±0.7)	3.3 (±1.0)	0.9 (±0.4)
ALA	1.1	0.6 (±0.1)	1.6 (±0.4)	2.0 (±0.7)	1.9 (±0.5)
ARA	2.5	2.2 (±0.4)	2.1 (±0.2)	2.8 (±0.5)	2.7 (±0.3)
EPA	2.0	2.8 (±0.9)	4.1 (±0.3)	3.9 (±0.6)	4.1 (±0.2)
DHA	14.7	10.1 (±0.9)	11.9 (±1.2)	7.7 (±1.3)	6.5 (±0.6)

zooplankton size fraction. In addition, such changes in PUFA concentrations may be associated with zooplankton taxa that vary in their PUFA retention abilities. Therefore, we examined size and taxa-dependent differences of each PUFA compound within the planktonic food web.

EFAs in the planktonic food web—In the planktonic food web, the accumulation factor of EFAs is higher between seston and macrozooplankton (3.0×) than that of total reported PUFAs (2.6×), including LIN and ALA. This indicates that some PUFA, are transferred, metabolized, and/or possibly retained differently through the planktonic food web.

EPA in the planktonic food web—Within the planktonic food webs studied here, EPA concentrations increased significantly with plankton size, which indicates that EPA is increasingly accumulated and, by implication, required by planktonic organisms. However, the increase in EPA concentrations was not always linear among the different size classes. EPA is most highly accumulated between seston and microzooplankton, and, although absolute EPA concentrations continued to increase, the accumulation factors associated with this compound decreased as plankton size increased. This might indicate that the contribution of larger phytoplankton to the microzooplankton fractions results in higher EPA concentrations in this size fraction. The continuous increase in EPA concentrations in larger zooplankton also suggests that EPA is retained throughout the life span of zooplankton and is perhaps essential for high reproductive success (Becker and Boersma 2003).

Although EPA can serve as a growth-enhancing nutrient for juvenile zooplankton, EPA concentrations in the macrozooplankton size class of ELL were not only lower than in any of the other lakes but were also lower than EPA concentrations in the mesozooplankton size class in some of the other lakes (Fig. 2). This suggests that EPA should not be used as a proxy for zooplankton size and that additional factors that are known to affect somatic growth (e.g., phosphorus availability) must also be considered. For example, *Daphnia* fed a P-enriched diet in the laboratory grew bigger than those fed a diet low in P (Urabe and Sterner 2001). Although C : P ratios of zooplankton diet have not been measured in these study lakes, the molar C : P ratio from unfiltered lake water of mesotrophic ELL was lower than C : P ratios of all other study lakes (A.M. unpubl. data). Thus, it is possible that the low C : P ratio in mesotrophic ELL (relative to our other study lakes) created elemental nutrient conditions that were more conducive (relatively higher P) to improved postjuvenile somatic growth in zooplankton. In conclusion, the accumulation of EPA along the planktonic food web strongly suggests that this FA is an essential compound and is perhaps selectively retained during all life stages of zooplankton.

DHA in the planktonic food web—DHA is found in phospholipids of cell membranes and is essential to biochemical processes (Spector 1999). Dietary DHA also improves the somatic growth of *Daphnia,* although possibly to a lesser extent than EPA (Müller-Navarra 1995; Wacker and Von Elert 2001). DHA patterns within the planktonic food web were distinctly different from those of EPA. Although accumulation factors between seston and mesozooplankton size fractions were similar to those of EPA, DHA concentrations decreased sharply in macrozooplankton. This led to significant differences in EPA versus DHA retention patterns from meso- to macrozooplankton. The decreasing DHA concentrations between meso- and macrozooplankton demonstrate an allometric relationship between body size and this EFA, which suggests that DHA may be less essential for the species that make up the macrozooplankton size class. These results suggest that the requirement of DHA for organisms of the planktonic food web differs from that of EPA.

Results from polynomial regression analysis showed that DHA concentrations start to decrease in zooplankton >592 μm. However, because the rate of DHA retention already begins to level off in smaller organisms in COL, we suggest that the retention of DHA may be related to the taxonomic composition of zooplankton. COL was the only lake in which *H. gibberum* dominated, which suggests that *H. gibberum* contains low DHA concentrations and reinforces the hypothesis that patterns of DHA retention will change when different zooplankton species dominate. For example, it has generally been observed that cladocerans (Weers et al. 1997; Ballantyne et al. 2003) contain less DHA than copepods (Desvilettes et al. 1997).

Dietary DHA is essential for the development and somatic growth of fish (Izquierdo et al. 2000; Copeman et al. 2002; Ballantyne et al. 2003). The planktonic pool of dietary DHA for fish is highest in the mesozooplankton size fraction and is considerably lower in macrozooplankton. As a consequence, the most efficient transfer of DHA to fish, on the basis of DHA concentration per unit mass of tissue, would be provided by the mesozooplankton size fraction. Our results suggest that nutrient accumulation within the planktonic food web is compound- and size-specific and reveal that DHA concentrations in zooplankton do not automatically increase with size. We therefore conclude that the potential for the most efficient DHA transfer from the planktonic food web to fish occurs at the mesozooplankton size fraction and is thus decoupled from maximum prey size.

ARA in the planktonic food web—ARA, like EPA, is a precursor for eicosanoid synthesis and a constituent of the membrane phospholipids involved in signal transduction (Smith and Fitzpatrick 1996). In fish, ARA is required for the formation of cortisol, a compound that allows fish to mitigate stress (Koven et al. 2001).

ARA is the most efficiently accumulated PUFA in the planktonic food webs studied here. On average, macrozooplankton contained more than 13 times as much ARA as seston, and, when compared with the other PUFA we measured, ARA had the highest accumulation factor between micro- and macrozooplankton (Table 3). Although ARA concentrations were similar among the various size classes of zooplankton from the oligotrophic lakes and reservoirs, they were considerably lower in mesotrophic ELL. The sestonic ARA concentration in ELL was 4.6 times lower than the mean ARA concentration in the other lakes. It is possible that the low sestonic quantity of this compound constrains ARA concentrations in zooplankton from ELL. However,

ARA is most efficiently accumulated from seston to macrozooplankton in ELL (accumulation factor, 18.3), which indicates that ARA accumulation may not necessarily be solely related to the sestonic quantity of ARA. Therefore, high accumulation factors between seston and macrozooplankton do not necessarily result in high ARA concentrations in macrozooplankton. Finally, the taxonomic composition of zooplankton in ELL does not account for differences in quantity and accumulation of ARA, because the taxonomic composition of zooplankton genera of ELL is similar to those of other lakes with higher ARA concentrations.

Our knowledge about the relationship between ARA and the physiological processes in zooplankton is still limited. Because ARA is rapidly accumulated as a function of plankton size in these lakes, it is tempting to speculate that ARA, like EPA, may be essential for the somatic growth of zooplankton. However, the results of laboratory experiments have revealed that ARA has positive, but nonsignificant, effects on the somatic growth of *D. galeata* (von Elert 2002). Therefore, the role of ARA in physiological processes in planktonic food webs may differ from the demonstrated growth-enhancing role of EPA and DHA in daphnids. Further investigations are needed to identify specific physiological roles of ARA in plankton organisms. We conclude that predation on macrozooplankton results in the most efficient transfer of ARA to higher trophic levels.

LIN and ALA in the planktonic food web—In general, LIN and ALA increase from seston to macrozooplankton, and patterns of both C18-PUFAs are similar along the planktonic food web. However, compared with EPA and ARA, both LIN and ALA are less efficiently retained along the planktonic food web, which suggests that (1) physiological requirements differ with increasing plankton size between C18- and C20-PUFA, and/or (2) ALA and LIN are converted, more efficiently than previously believed, to EPA and ARA, respectively. Although it has been reported that LIN can be converted into ARA (Stanley-Samuelson 1994), it is unlikely that the efficiency of this conversion is high, because concentrations of LIN and ARA along the planktonic food web do not differ significantly from each other (*t*-test, $p < 0.05$). As was the case with DHA, ALA is generally lower in macro- than in mesozooplankton. Von Elert (2002) recently reported that both DHA and ALA can be converted into EPA in cultured *D. galeata.* Although our data set cannot be used to unequivocally demonstrate such conversions, which should be dependent on the scarcity of required dietary EPA, the relatively lower accumulation factors in the microzooplankton–mesozooplankton and mesozooplankton–macrozooplankton comparisons for ALA and DHA, compared with EPA (Table 3), lend support to von Elert's findings.

Results from polynomial regression analysis show that ALA in zooplankton decreases when they are >774 μm in size. Declining concentrations of ALA with increasing plankton size suggest that retaining dietary ALA is less essential than EPA for the species in the largest zooplankton size class. Whether different accumulation factors for ALA and EPA in planktonic organisms are related to different assimilation or conversion rates remains a subject for further investigation.

Role of planktonic PUFA composition for higher trophic levels—In addition to the physiological requirements of PUFAs for planktonic animals, certain PUFAs are also essential for aquatic animals at higher trophic levels. For fish, it has been suggested that dietary EFAs improve somatic growth (Ballantyne et al. 2003), reproduction, and optimal pigmentation and reduce the probability that they will exhibit various pathologies (e.g., Watanabe 1982). Because PUFAs from the planktonic food web are an important component of the PUFAs transferred to higher trophic levels such as fish, we analyzed the PUFA levels of rainbow trout in three of the lakes, to determine the ecological importance of the dietary PUFA pool of the planktonic food web.

The nutritional pool of PUFAs, especially of EFAs, from the planktonic food web varies with the size of planktonic organisms. The body size of prey has important implications for the transfer of nutrients to higher trophic levels. Macrozooplankton are the preferred prey size for planktivorous fish because of the generally accepted size-selective feeding concept for planktivores, by which large-bodied zooplankton are favored over smaller ones (Brooks and Dodson 1965).

The PUFA composition in rainbow trout shows that DHA is, by far, the most abundant EFA. This result agrees with the results of Ahlgren et al. (1994), which also demonstrated that DHA was the dominant FA in freeze-dried dorsal muscle tissue samples from freshwater fish, including roach (*Rutilus rutilus*), perch (*Perca fluviatilis*), pike (*Esox lucius*), and grayling (*Thymallus thymallus*). Moreover, DHA concentrations in the rainbow trout from the present study were higher than those of any other EFA in juvenile brook trout (*Salvelinus fontinalis*; A.M. et al. unpubl. data). Although it has been reported that some fish can synthesize EFA de novo (Henderson 1996), dietary uptake of EFA is preferred, because less enzymatic activity (and, thus, energy) is required.

The dietary uptake of macrozooplankton as the preferred prey size, however, does not always result in the most efficient transfer of EFA to fish. Although concentrations of ARA and EPA are highest in macrozooplankton, the highest DHA concentrations are found in mesozooplankton. Therefore, the most efficient transfer of ARA and EPA to fish is achieved when macrozooplankton is consumed and assimilated, whereas predation on mesozooplankton results in the most efficient DHA uptake. The distribution of PUFAs within the planktonic food web demonstrates that PUFA transfer efficiency to higher trophic levels will likely vary with the size and taxonomic composition of planktonic organisms and perhaps with trophic status of the lake.

Finally, it is apparent that fish have different EFA requirements than zooplankton. This is evident when comparing mean concentrations of ARA, EPA, and DHA in rainbow trout (2.4, 3.3, and 10.2 mg g dry weight^{-1}, respectively) and macrozooplankton (4.3, 10.8, and 2.2 mg g dry weight^{-1}, respectively). Thus, DHA seems to be the most highly retained EFA in fish, whereas it is EPA in macrozooplankton. Because it has been suggested that DHA limits the growth of fish (e.g., Ballantyne et al. 2003) and EPA supports the somatic growth of zooplankton (e.g., Müller-Na-

varra et al. 2000), we propose that aquatic animals retain different FA compounds to optimize physiological performance, including somatic growth. Although macrozooplankton contain lower levels of DHA than fish, we found that total PUFA concentrations of the planktonic food web are generally higher than the assimilated total PUFA concentrations in fish muscle tissue. As a consequence, DHA may be the only EFA that cannot be supplied sufficiently when planktivorous fish select the largest zooplankton as their preferred diet item.

We have demonstrated that the accumulation patterns of EFA along the planktonic food web are compound specific. We interpret the steady increase of ARA, EPA, and LIN with increasing plankton size as the tendency of these planktonic organisms to selectively retain these EFA. In contrast, ALA and DHA do not increase continuously from the seston to the macrozooplankton size fraction.

Despite the fact that our study only dealt with EFA data from June, the patterns of EFA concentration as a function of size within the planktonic food web were consistent throughout the year (M.K. unpubl. data). It appears that the assimilation and retention of EFA within the planktonic food web is influenced by both taxonomic composition and the size-specific physiological requirements and abilities of the organisms in each size class. We recognize that detailed biochemical studies have yet to be performed to establish the biosynthetic potential (i.e., FA chain elongation/shortening and/or desaturation/resaturation) of different zooplankton species (e.g., for copepods). Although these and further studies are clearly needed to establish the generality of our findings (e.g., in lakes differing in trophic status), we suggest that zooplankton will exhibit an EPA-retentive metabolism, whereas DHA will be retained differently among different species of the planktonic food web.

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