

Essential fatty acid concentrations of different seston sizes and zooplankton: a field study of monomictic coastal lakes

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Plankton particle size is a central issue for aquatic ecologists. In this field study, we examined essential fatty acids (EFAs) in different plankton particle sizes and obtained initial estimates of how EFA concentrations, and thus high dietary lipid quality for consumers, vary with seston size. We measured EFA concentrations in three seston size classes (i.e. micro = 0.7–35 µm; meso = 35–64 µm and macroseston = 64–100 µm) and in cladocerans and calanoid copepods of monomictic, coastal lakes. Algal pigment analysis identified a mixture of Chlorophyta, Cryptophyta, diatoms and Cyanophyta in all seston size classes. Total EFA concentrations did not vary with increasing seston size. There was no difference in linoleic (LIN), eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid concentrations among the three seston size classes; however, α-linolenic (ALA) and arachidonic (ARA) acid concentrations were significantly higher in microseston. For herbivorous zooplankton, concentrations of LIN, ALA and EPA did not differ significantly between cladocerans and calanoid copepods; however, ARA concentrations were significantly higher in cladocerans and DHA concentrations were significantly higher in calanoid copepods. Our results indicate that microseston represents the most nutritious dietary component, per unit biomass, with respect to ALA and ARA and that seston size alone does not predict EFA concentrations, i.e. dietary access to larger seston particle sizes is not necessarily associated with access to particles with higher EFA concentrations.

INTRODUCTION

Pelagic primary consumers are faced with a spectrum of dietary particle sizes (Sheldon *et al.*, 1972; Hall *et al.*, 1976). The biochemical composition of these particles is a key determinant of growth and reproductive rates in zooplankton, thereby affecting population size as well as predator–prey interactions within and among trophic levels (Peters, 1983). In their classical essay, Sieburth *et al.* (Sieburth *et al.*, 1978) subdivided different taxonomic-trophic compartments of plankton in a spectrum of size

fractions, but also noted that one particle size-class (2–200 µm) was occupied by both phyto- and protozooplankton. They differentiated between phytoplankton and animals by visual inspection and by the presence of chlorophyll *a*. Moreover, it has been recognized that, from a consumer’s perspective, energy content is not necessarily a simple function of particle size. For example, Hall *et al.* (Hall *et al.*, 1976) argued that consumers specializing on very fine particles may encounter a greater proportion of low energy-yielding particles.

Lipids are energy-yielding substrates, some of which are considered essential dietary constituents. In particular, algal-derived polyunsaturated fatty acids (PUFA) are required for somatic growth and other key physiological processes of aquatic organisms. For zooplankton, it has recently been demonstrated that concentrations of such essential fatty acids (EFAs), i.e. arachidonic (ARA, 20:4 n – 6) and eicosapentaenoic acid (EPA, 20:5 n – 3), accumulate with increasing plankton size and independently of taxonomic composition (Kainz *et al.*, 2004). In fact, EPA and docosahexaenoic acid (DHA, 22:6 n – 3) (Müller-Navarra *et al.*, 2000; Becker and Boersma, 2003; von Elert, 2004), as well as α -linolenic acid (ALA, 18:3 n – 3; von Elert, 2002) have been identified as dietary constituents that enhance somatic growth and reproduction in laboratory-raised *Daphnia*. For marine zooplankton, it was demonstrated that egg production of the calanoid copepod *Temora longicornis* was significantly related to dietary DHA (Jonasdottir *et al.*, 1995; Evjemo *et al.*, 2008). Although the physiological role of the n – 6 PUFA, linoleic acid (LIN, 18:2 n – 6), is not clearly understood for lacustrine zooplankton, it is known to be a precursor for ARA (Cook and McMaster, 2004).

Polyunsaturated fatty acids contained in seston are essential for herbivorous consumers because of their low ability to biosynthesize them *de novo* (for *Daphnia*, see Goulden and Place, 1990). However, the physiological reason as to why some FA, DHA in particular, is retained differently in cladocerans and copepods remains an open question. Recent studies have shown that concentrations of EPA, but not DHA, are highly accumulated in cladocerans (e.g. Ballantyne *et al.*, 2003; Kainz *et al.*, 2004), whereas copepods are enriched in both, EPA and DHA (Ballantyne *et al.*, 2003; Evjemo *et al.*, 2003; Persson and Vrede, 2006). On the basis of the relatively high DHA concentrations in algae such as *Cryptomonas pyrenoidifera* (Weers *et al.*, 1997) and *Stephanodiscus hantzschii* (von Elert, 2002), which are ingested by daphnids, it appears that daphnids may not retain dietary DHA as efficiently as copepods. The n – 6 PUFA ARA is the most highly retained n – 6 PUFA in both, lake cladocerans and copepods (Kainz *et al.*, 2004), as well as in fasting *Daphnia pulex* (Schlechtriem *et al.*, 2006).

It is known that the dietary size spectrum can be broader for herbivorous copepods (occasionally including particles >100 μm ; e.g. Vanderploeg, 1990) than for cladocerans (typically <30 μm ; Burns, 1968); however, little is known about EFA concentrations in different seston size classes. In addition to algae, larger seston size classes also contain small animals (e.g. protozoans, nauplii, small rotifers), which typically have higher PUFA concentrations than algae (for protozoans, see Bec *et al.*, 2003). For example, Støttrup *et al.*

(Støttrup *et al.*, 1999) reported high concentrations of n – 3 PUFA in *Acartia tonsa* nauplii; moreover, Boëchat and Adrian (Boëchat and Adrian, 2006) found positive correlative evidence for rotifer eggs and n – 3 PUFA concentrations. It is thus reasonable to expect that concentrations of n – 3 PUFA, and perhaps EFA in general, should increase with the presence of animals in seston. However, dietary access to EFA will be restricted to the consumers' ability to ingest different particle sizes. Motivated by previous ecological studies which demonstrated the importance of particle size-specific relationships at the base of the aquatic food webs, we designed a field study to investigate how EFA concentrations vary with seston size classes and, subsequently, how EFA retained in zooplankton vary compared with the spectrum of different seston size classes.

We hypothesized that: (a) EFA concentrations would increase with increasing seston size and (b) the preferred, yet small (<30 μm), dietary particles for daphnids would contain lower EFA concentrations than larger seston particles because these larger particles also contained some animals. To test these hypotheses, we assessed algal taxonomic composition of different seston size classes, investigated EFA concentrations of different seston sizes and examined EFA retention patterns between cladocerans and calanoid copepods.

METHOD

This field study was conducted in July 2005 in six coastal, monomictic lakes on Vancouver Island, Canada: Shawnigan Lake (SHL; N48°37', W123°38'; 24 m, SHL-S, shallow station; and 49 m, SHL-D, deep station) and Elk Lake (ELL; N48°31', W123°23'; 13 m) are natural lakes, and Council Lake (COL; N48°31', W123°40'; 20 m), Sooke Reservoir (SOL; N48°33', W123°41'; 22 m, -S; and 70 m, -D), Goldstream Reservoir (GOL; N48°30', W123°38'; 28 m) and Butchard Reservoir (BUL; N48°32', W123°39'; 41 m) are man-made reservoirs that are not open to public access. The morphometry of SHL and SOL is very similar; both lakes have a shallow (-S) and a deep (-D) basin. Zooplankton were collected by vertically hauling a plankton net (64- μm Nitex mesh size) from \sim 1 m off the bottom to the surface in the deepest basin(s) in each lake. The zooplankton was then retained on a 200- μm mesh size filter cup. Cladocerans were separated from copepods by the addition of carbonated water, which caused cladocerans to float to the surface from where they could be immediately removed. All zooplankton were subsequently rinsed with filtered (0.45 μm) lake water. Cladocerans and calanoid copepods, separated

with the aid of a compound microscope, were cryogenically frozen for latter analyses.

Lake seston (<100 μm) was collected at the Chl *a* maximum (detected by a submersible spectrofluorometer equipped with automatic chlorophyll analysis; *bbe* MoldaenkeTM, Kiel-Kronshagen, Germany) of each sampling station using a Van Dorn water sampler because this often is the preferred zone for herbivores (Lampert and Grey, 2003; Reichwaldt and Abrusan, 2007). Subsequently, seston was filtered using Nitex meshes with different pore sizes to create three size classes: (a) “microseston” (0.7–35 μm , including most pico- and nanoplankton; Sieburth *et al.*, 1978), and seston >35 μm which was further subdivided into (b) “mesoseston” (35–64 μm) and (c) “macroseston” (64–100 μm). Microseston was filtered onto combusted (450°C) Gelman[®] GF/F filters (0.7 μm pore size) whereas meso- and macroseston were filtered through a 100 μm mesh and subsequently retained in filter cups with 35 and 64 μm openings, respectively. To verify the composition of different seston sized, visual inspection of macroseston revealed the presence of algae and, occasionally, also rotifers and copepod nauplii. These size fractions were chosen, (a) to examine the distribution of the potential dietary EFA pool among these seston size fractions and (b) to evaluate the nutritional role of microseston, representing the most edible diet size spectrum for cladocerans (Burns, 1968), in comparison with larger particles that are accessible to calanoid copepods (Vanderploeg, 1990), even nauplii (Paffenhöfer and Lewis, 1989), and occasionally by daphnids (reviewed by Lampert, 1987). Zooplankton and seston samples were immediately placed on dry ice to limit lipolytic activity and degradation of organic matter and were subsequently stored in a cryogenic freezer (–80°C) until lyophilization following which they were again stored at –80°C until FA analysis. The body length of cladocerans and copepods was measured on replicate samples using Z-Counts software (version 2.3, Voila Data).

Lipid and fatty acid analysis

Total lipids and fatty acids from freeze-dried and then homogenized zooplankton (3–5 mg DW) and seston (1–3 mg DW) were analyzed as described by Parrish (Parrish, 1999). Briefly, the samples were sonicated and vortexed 4X in a 4:2:1 chloroform–methanol–water mixture and the organic layers were removed and pooled.

Fatty acid methyl esters (FAMEs) were formed using hexane and $\text{BF}_3\text{-CH}_3\text{OH}$ (10% w/w) and analyzed using a gas-chromatograph (GC; Varian CP-3800, Varian Inc., Palo Alto, CA, USA) equipped with a flame ionization detector. FAME were identified by

comparison of retention times with known standards (37-component FAME mix, SupelcoTM). Quantification of individual FAME components was calculated on the basis of known amounts of injected standard dilutions (2000, 1000, 500, 250 and 1.25 ng μL^{-1}). Detailed methodologies for FAME formation and detection are described elsewhere (Kainz *et al.*, 2002).

Plankton pigment analysis

To assess the taxonomic composition of natural phytoplankton populations within the different seston size classes of the study lakes, we measured pigments that are unique to certain algal taxa. For identification of major divisions and classes of freshwater algae, including Chlorophyta (Berggreen *et al.*, 1988), Cryptophyta, Bacillariophyta (diatoms) and Cyanophyta (cyanobacteria), the algal pigments lutein, alloxanthin, fucoxanthin and zeaxanthin, respectively (Mantoura and Llewellyn, 1983), were analyzed as described by Leavitt *et al.* (Leavitt *et al.*, 1989). Briefly, pigments were extracted from freeze-dried samples using 90% acetone, sonicated, evaporated and subsequently dissolved under low-intensity, indirect, lighting with an ion-pairing solution and Sudan II[®] (Sigma-Aldrich Inc., St. Louis, MO, USA), as an internal standard, for analysis by high-performance liquid chromatography equipped with UV-Vis photo-diode array (PDA) detection. The separation of pigments was performed on a Radial-Pak C18 column (0.8 \times 15 cm, 5 μm particle size). The flow rate was 1.5 mL min^{-1} and absorbance was read at 435 nm. Peak areas were converted to concentrations by the external standard calibration method using commercially available pigment standards (Dionex Canada Ltd., ON, Canada). We excluded pigments that were common in more than one algal group, such as diadinoxanthin, which is found in dinoflagellates, and also in diatoms.

Data analysis

Body size difference between cladocerans and copepods was examined using independent-sample *t*-tests. To analyze concentration differences of total EFA and their compounds among the three seston size classes, we employed one-way analysis of variance (ANOVA), using size class as factor. Similarly, we used ANOVA to determine the effect of different zooplankton taxa (cladocerans versus calanoid copepods) on concentration differences of total and individual EFA (LIN, ARA, and ALA, EPA and DHA). Using linear regression analysis we examined the relationships between concentrations of Chl *a* and EFA for each seston size class.

Table I: Temperature (T), dissolved oxygen (DO) and chlorophyll a (Chl a) in coastal monomictic lakes in southern Vancouver Island measured at seston sampling depths

Lake	Sampling depth ^a (m)	T (°C)	DO (mg L ⁻¹)	Chl a (µg L ⁻¹)
Shawnigan Lake (S ^p)	7.2	13.9	10.7	4.0
Shawnigan Lake (D ^o)	7.0	14.5	10.9	3.6
Council Lake	13.5	5.8	N/A	5.8
Elk Lake	7.2	15.6	N/A	42.4
Sooke Reservoir (S ^p)	8.0	16.7	9.2	2.7
Sooke Reservoir (D ^o)	11.0	12.0	8.7	2.7
Goldstream Reservoir	16.3	5.9	8.1	2.4
Butchard Reservoir	16.2	4.6	10.5	2.9

^aLake depth for seston sampling at highest Chl a concentrations.

^bShallow sampling station.

^cDeep sampling station.

The relationships between the relative abundance of algal-derived pigments (used as a surrogate for taxonomic algal composition) and EFA compositions of the size-classed seston were related to each other by canonical correlation analysis (CCA). CCA identified optimal linearly coupled patterns in the two data sets. The independent data comprised the percent composition of each analyzed pigment for the different seston size classes at the different lake stations (4 pigments × 21 POM samples). The dependent data comprised the composition of the different EFA (percent of total identified FA) for the corresponding seston size classes at the corresponding lake stations (5 EFA × 21 POM samples). Data were arcsin (square-root) transformed and normality was checked (Kolmogorov–Smirnov test). Variability shared by the two data sets was estimated from the canonical correlation coefficient on the two first pairs of canonical variables. Statistical analyses were performed on R (CRAN-Project).

RESULTS

Lake characteristics

Epilimnetic water temperature ranged between 20 and 22°C in all lakes. All the lakes were circum-neutral (pH ~7), thermally stratified, and had well-oxygenated (>2 mg DO L⁻¹) water columns. Epilimnetic Chl a concentrations were <1 µg L⁻¹, except at ELL (1.85 µg L⁻¹), and <6 µg L⁻¹ at depths of deep Chl a maxima (42.4 µg L⁻¹ at ELL; Table I).

Seston and zooplankton

Algal pigment analysis identified a mixture of *Chlorophyta*, *Cryptophyta*, diatoms and *Cyanophyta* in all

seston size classes (Fig. 1). Pigments of diatoms dominated in the micro- and mesoseston size classes, whereas *Cyanophyta* pigments prevailed in these size classes at mesotrophic ELL. Pigments in the macroseston size class were mainly related to *Chlorophyta* and diatoms, however, due to the small mass of sample material obtained, pigment analysis could not be performed for COL, GOL and SOL-D for this size class.

The CCA revealed significant interactions between phytoplankton group and size-specific sestonic EFA composition (coefficient of multiple correlation, $R = 0.93$ on the first and $R = 0.65$ on the second canonical components; Fig. 2). The first canonical axis was dominated by the variation in pigment composition of diatoms

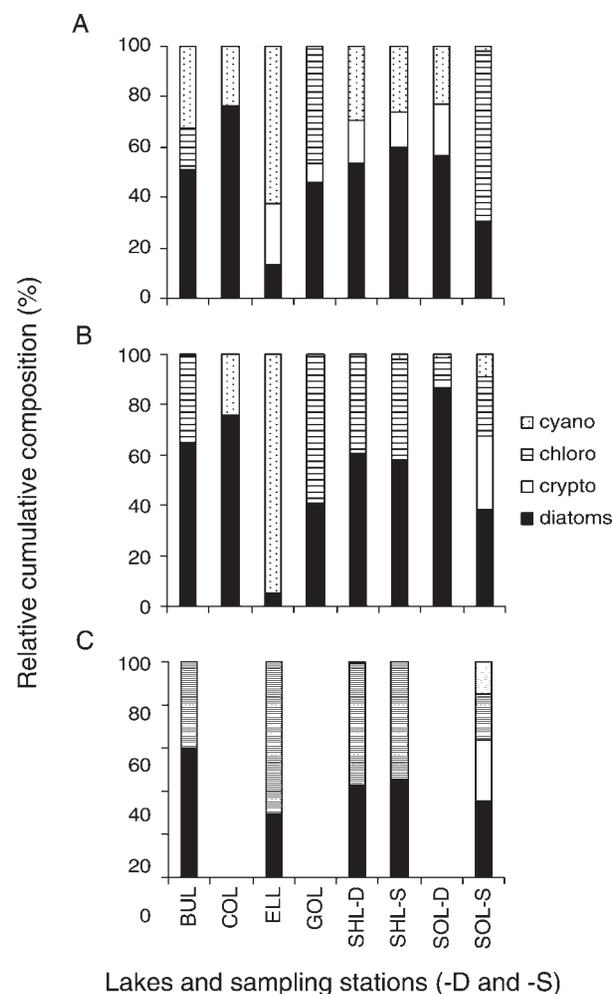


Fig. 1. Seston composition (grouped in cyano-, chloro- and cryptophyta, and diatoms as relative cumulative composition) of (A) microseston (0.7–35 µm), (B) mesoseston (35–64 µm) and (C) macroseston (64–100 µm) size classes. BUL, Butchard Reservoir; COL, Council Lake; ELL, Elk Lake; GOL, Goldstream Reservoir; SHL, Shawnigan Lake; SOL, Sooke Reservoir; -S, shallow; -D, deep sampling stations at SHL and SOL.

(positively correlated) versus cyanobacteria (negatively correlated) within the seston (Fig. 2A). The variation in the diatom contribution co-varied with ARA and EPA contents in seston, whereas the cyanobacteria contribution co-varied with seston DHA content (Fig. 2B). The latter correlation seems spurious as cyanobacteria, in general, lack DHA (Brett *et al.*, 2006). We suggest that this is a spurious correlation that is likely due to the presence of similar-sized DHA-containing heterotrophic microorganisms, as was shown to be the case for heterotrophic nanoflagellates (Bec *et al.*, 2006).

The second canonical axis was dominated by variation in the pigment contributions of chlorophytes and diatoms (negatively correlated) versus cryptophytes and cyanobacteria (positively correlated) to the seston (Fig. 2C). Variation in the chlorophyte and diatom contribution co-varied with LIN contents in seston, whereas the cyanobacteria and cryptophyte contributions co-varied with seston ARA contents (Fig. 2D).

Seston size classes from the different lake stations were represented by the two first canonical axes of the CCA (Fig. 3). Except for ELL macro- and mesoseston, seston samples were not particularly grouped on the canonical representation according to their size-class or lake station, suggesting that phytoplankton taxonomic composition and relative EFA contents of seston were not strongly related to seston size or lake station.

FA concentrations were analyzed for large cladocerans (*Daphnia* spp. and *Holopedium gibberum*) and calanoid copepods (*Hesperodiaptomus franciscanus* and *Leptodiaptomus tyrelli*) from all stations with the exception of ELL where

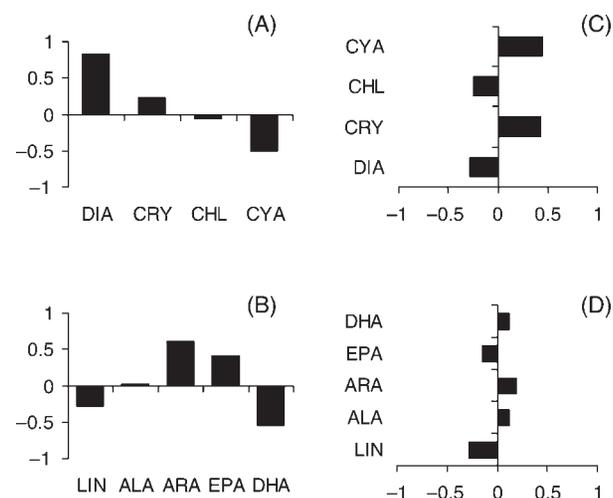


Fig. 2. First and second canonical patterns. Correlations of (A) phytoplankton taxonomic composition, estimated from pigment composition and (B) EFA compositions of sized-seston with the first canonical component. Correlations of (C) phytoplankton taxonomic composition, estimated from pigment composition and (D) EFA composition of sized-seston with the second canonical component.

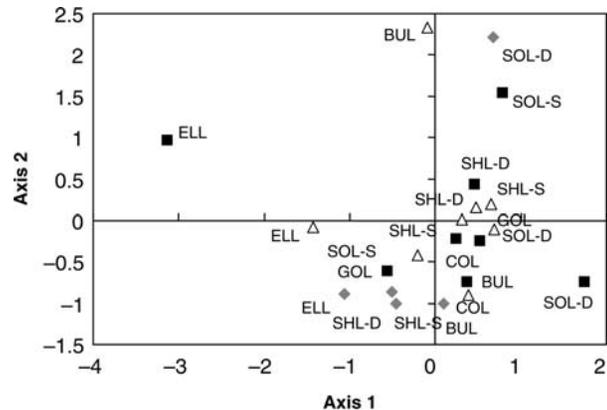


Fig. 3. Representation of different seston sizes at different lake stations on the first two canonical axes of the CCA (see text for acronyms of lake stations; open triangles: micro-, 0.7–35 μm ; full squares: meso-, 35–64 μm ; grey diamonds: macroseston, 64–100 μm).

no copepods were found. Cladocerans were significantly larger ($1317 \pm 300 \mu\text{m}$) than calanoid copepods ($993 \pm 169 \mu\text{m}$; $P < 0.001$); however, there was no significant body size difference between cladocerans and calanoid copepods at GOL ($P = 0.16$; Table II).

Essential fatty acids in seston and zooplankton

Total EFA concentrations did not vary significantly among the three seston size classes (one-way ANOVA; Table III); microseston contained 34%, mesoseston 21% and macroseston 45% of total sestonic EFA concentrations. Although the mean concentration of total EFA was twice as low in the mesoseston as in the macroseston size fraction, this difference was not significant; in this respect, it is important to note that the macroseston size class is generally considered to be less ingestible for herbivorous zooplankton than particles from smaller size classes.

Concentrations (per unit biomass) of ALA, the essential precursor for longer chain $n - 3$ PUFA, and ARA were significantly higher in microseston than in the larger seston size classes ($P < 0.001$; Holm-Sidak pairwise multiple comparisons). Microseston had significantly higher concentrations of LIN ($P = 0.02$) and EPA ($P = 0.03$), but not DHA ($P = 0.9$) than mesoseston. However, there were no significant differences for LIN, EPA and DHA concentrations among the three seston size classes (Table III) as EFA concentrations increased again from meso- to macroseston.

In zooplankton, EFA concentrations were higher than in any of the seston size classes (with the exception of DHA in cladocerans, Fig. 4). Concentrations of LIN, ALA and EPA did not vary significantly between cladocerans and

Table II: Mean (\pm SD) zooplankton length (mm) of cladocerans and calanoid copepods

Lakes	Cladocerans	<i>n</i>	Calanoid copepods	<i>n</i>	<i>P</i> -value
Shawnigan Lake (shallow station)	1.18 (\pm 0.2) ^a	37	1.01 (\pm 0.2) ^c	32	0.001
Shawnigan Lake (deep station)	1.48 (\pm 0.2) ^a	26	0.98 (\pm 0.2) ^c	32	0.001
Council Lake	1.17 (\pm 0.2) ^b	34	1.06 (\pm 0.1) ^d	32	0.026
Elk Lake	1.61 (\pm 0.3) ^a	33	n.p.		
Sooke Reservoir (shallow station)	1.09 (\pm 0.2) ^a	38	1.00 (\pm 0.2) ^d	15	0.16
Sooke Reservoir (deep station)	1.42 (\pm 0.4) ^a	28	0.86 (\pm 0.1) ^d	33	0.001
Goldstream Reservoir	1.32 (\pm 0.2) ^a	24	1.00 (\pm 0.2) ^d	36	0.001
Butchard Reservoir	1.33 (\pm 0.2) ^a	29	1.03 (\pm 0.2) ^d	40	0.001

n.p., not present.

^a*Daphnia* spp.

^b*Holopedium gibberum*.

^c*Hesperodiaptomus franciscanus*.

^d*Leptodiaptomus tyrelli*.

Table III: Fatty acid concentrations (mg g^{-1} dry weight \pm standard error) and ANOVA results of fatty acids comparisons among different seston size classes (micro-, meso- and macroseston) and between cladocerans and calanoid copepods

	Microseston	Mesoseston	Macroseston	<i>F</i>	<i>P</i> -value
Seston size classes					
EFA	7.7 \pm 0.8	4.9 \pm 0.7	10.4 \pm 2.5	2.91	0.076
LIN	1.6 \pm 0.3	0.5 \pm 0.1	1.4 \pm 0.6	2.23	0.13
ALA	1.1 \pm 0.2	0.4 \pm 0.1	0.6 \pm 0.1	7.41	0.0036
ARA	0.3 \pm 0.0	0.1 \pm 0.0	0.2 \pm 0.0	9.86	0.0009
EPA	2.0 \pm 0.3	1.2 \pm 0.1	2.3 \pm 0.8	1.31	0.29
DHA	2.8 \pm 0.6	2.7 \pm 0.6	5.8 \pm 1.5	3.26	0.0584
<i>n</i> - 3: <i>n</i> - 6	3.4 \pm 0.5	6.3 \pm 1.1	8.0 \pm 2.9	1.52	0.24
	Cladocerans	Copepods	<i>F</i>	<i>P</i> -value	
EFA	18.3 \pm 2.0	32.4 \pm 2.4	20.4	0.00057	
LIN	3.0 \pm 0.3	3.3 \pm 0.5	0.24	0.62	
ALA	3.1 \pm 0.5	3.6 \pm 0.5	0.51	0.48	
ARA	2.6 \pm 0.2	1.3 \pm 0.2	20.85	0.00053	
EPA	8.2 \pm 1.0	8.0 \pm 0.8	0.02	0.89	
DHA	1.4 \pm 0.3	16.1 \pm 1.0	232.86	0.00001	
<i>n</i> - 3: <i>n</i> - 6	2.3 \pm 0.2	6.5 \pm 0.6	20.44	0.00001	

EFA, essential fatty acids; LIN, linoleic acid; ALA, α -linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; *n* - 3:*n* - 6, omega-3:omega-6 ratio.

copepods (Table III). However, cladocerans had significantly higher ARA concentrations than copepods, whereas DHA concentrations were significantly lower in cladocerans than in copepods. Concentration differences of individual EFA compounds between zooplankton and their documented preferred diet, microseston, revealed that ARA was the mostly accumulated EFA in cladocerans (9.3 \times), whereas, in calanoid copepods, it was DHA (7.6 \times).

Although the mean *n* - 3:*n* - 6 ratio increased with increasing seston size, there was no significant difference among the seston size classes due to the large variation among *n* - 3:*n* - 6 ratio estimates within a given size class (Table III). However, cladocerans had a significantly lower *n* - 3:*n* - 6 ratio than copepods (*P* <

0.001; Table III). The *n* - 3:*n* - 6 ratio of cladocerans was significantly lower (*P* < 0.05) than any of the ratios within the seston size classes. In contrast, *n* - 3:*n* - 6 ratios of copepods were significantly higher (*P* = 0.006) than those of microseston, but not significantly different from meso- or macroseston (*P* > 0.05).

DISCUSSION

Essential fatty acids are pivotal compounds for aquatic and terrestrial organisms alike because they enhance somatic development and reproduction and therefore form an important functional constituent of food web stability. We examined how EFA concentrations varied

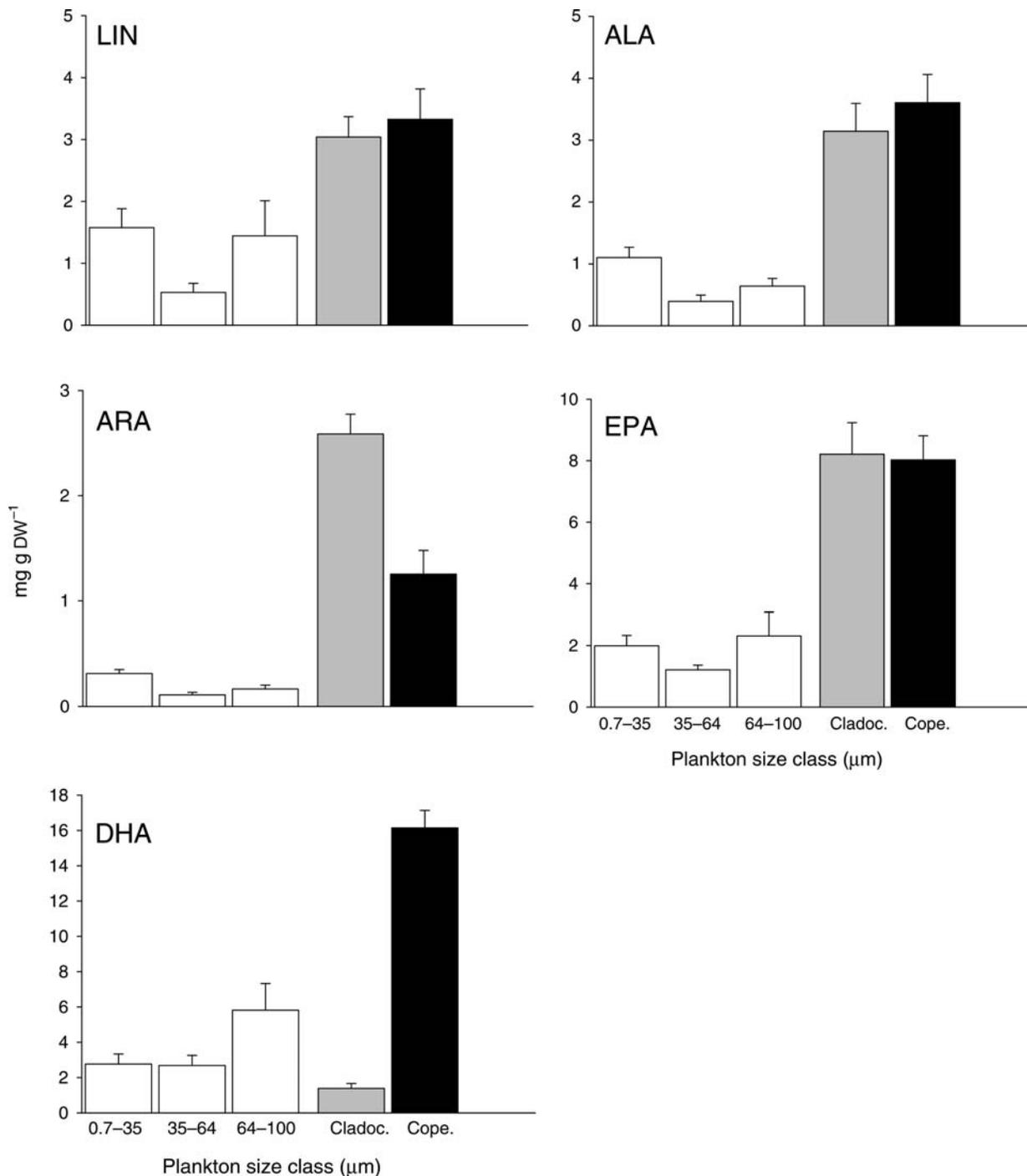


Fig. 4. Mean (\pm SE) concentrations (mg g^{-1}) of essential fatty acid compounds in plankton size classes: microseston (0.7–35 μm), mesoseston (35–64 μm) and macroseston (64–100 μm) (white bars), and in >200 μm cladocerans (grey bars) and copepods (dark bars). LIN, linoleic acid; ALA, α -linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

with seston size and found that individual EFA concentrations in microseston, with the exception of DHA, were significantly higher than in mesoseston. However, the finding that total EFA concentrations of seston did not significantly increase with increasing particle size

demonstrates that patterns in EFA concentrations, as a function of seston size, are different from previously reported patterns in EFA concentrations with increasing zooplankton size (Kainz *et al.*, 2004). Results from this latter study showed that zooplankton individual EFA

concentrations increased, with the exception of DHA, with increasing body size regardless of taxonomic composition.

The nutritional contribution that different sized seston particles make to a zooplankters diet is largely determined by their size-specific biochemical composition. Microseston generally represent the most important diet size class for cladocerans and most copepods in lakes. The relative EFA composition of seston was related, in part, to algal taxonomic composition: diatom-rich seston provided high amounts of EPA and ARA to the overall FA contents, whereas chlorophyte-rich seston was enriched by LIN. However, as diatoms, chlorophytes and also cyanobacteria contributed to all seston size-fractions, we suggest that differences in EFA contents among the different size-fractions cannot solely be explained by size-specific taxonomic differences. Similarly, Müller-Navarra *et al.* (Müller-Navarra *et al.*, 2004) argued that factors other than taxonomic algal composition, such as physiological status of phytoplankton and/or the contribution of detritus and heterotrophic organisms to seston biomass, interact to determine the ultimate FA composition of seston. For example, in a recent marine study, Dutz and Peters (Dutz and Peters, 2008) found that larger seston size ($>48 \mu\text{m}$) consisted mostly of PUFA-rich ciliates that served as an excellent dietary source for the reproduction of *Acartia clausi*, indicating that larger seston sizes may be ingested by such selectively feeding zooplankton.

Microseston contributed significantly higher concentrations of ALA and ARA than other seston size classes, identifying this smallest size fraction as the most beneficial dietary particle size for transferring these EFA to consumers. Despite the fact that, of all EFA, concentrations of ARA are lowest in seston, this EFA is still highly synthesized and/or retained, especially in cladocerans. In contrast to ALA, concentrations of LIN did not vary significantly among the seston size classes indicating that these two C_{18} PUFAs are synthesized and/or retained differently within the seston. The relatively high ALA and ARA concentration of microseston has dietary implications for some cladocerans, and perhaps zooplankton in general. For example, it has been shown that algal-derived ALA enhanced somatic growth of *D. galeata* (von Elert, 2002). Although the physiological properties of ARA for zooplankton have not been clearly defined yet, ARA concentrations increase with increasing body size of pelagic zooplankton in these study lakes (Kainz *et al.*, 2004).

Mesoseston had significantly lower concentrations of LIN, ALA, ARA and EPA than microseston, and thus provides less EFA (per unit biomass) to its consumers

than microseston. This has important implications at times when microseston is in short supply, as is often the case under eutrophic conditions (e.g. Havens, 1998). As demonstrated in the laboratory, copepods shifted their diet selection toward larger particles when smaller diet sizes were scarce (DeMott, 1995). In such cases, feeding on larger-sized lake seston that contain lower EFA concentrations per unit biomass may lower somatic growth rates and/or reproduction success (e.g. DeMott and Müller-Navarra, 1997), with possible negative effects on energy and nutrient transfer to higher consumers.

Little attention has been paid to investigating the FA composition of macroseston. This large seston size class contains the highest, but not significantly different, total EFA concentrations (45%), which was most likely due to the presence of some EFA-containing animals. However, from a nutritional point of view, this EFA pool is generally not readily accessible to freshwater zooplankters that filter food particles (e.g. Cladocera) compared with zooplankters that are known to be capable of selective raptorial feeding (e.g. Copepoda). Macroseston contains significantly lower ALA and ARA concentrations than microseston; thus, if it were readily accessible it would not provide any additional nutritional benefit with respect to these EFA, per unit biomass, for herbivorous consumers. Moreover, concentrations of LIN, EPA and DHA were not significantly different among these seston size classes, demonstrating that increasing seston size is not necessarily related to improved dietary supply of these EFA for potential consumers.

EFA from lake seston to cladocerans and calanoid copepods

Many studies investigating FA pathways through the planktonic food web report FA patterns as relative values (i.e. individual FA as a percent of total identified FA). This reporting method complicates inter-study comparisons. Here we demonstrate, using absolute values, that herbivorous copepods contain significantly higher total EFA concentrations per unit biomass than cladocerans. The significantly higher $n - 3:n - 6$ ratios of copepods compared with cladocerans agree with previous findings that copepods often contain high concentrations of DHA, whereas cladocerans hardly retain this long-chain $n - 3$ FA (e.g. Ballantyne *et al.*, 2003; Becker *et al.*, 2004; Kainz *et al.*, 2004; Persson and Vrede, 2006). This observation indicates that lacustrine cladocerans retain EFA differently than copepods and, consequently, relay different concentrations of the individual dietary EFA to their consumers.

Concentrations of LIN and ALA were not significantly different between cladocerans and copepods, but they were higher in the zooplankton than in any of the seston size classes. Microseston, containing significantly higher ALA concentrations than the larger size classes, convey the greatest amounts of sestonic ALA (per unit biomass) to its consumers, whereas LIN concentrations were not significantly different among seston size classes, which implies that there is no dietary advantage for zooplankton to feed on larger seston size classes to obtain higher LIN concentration per ingested unit biomass.

In agreement with Persson and Vrede (Persson and Vrede, 2006), ARA concentrations in cladocerans were significantly higher ($2\times$) than in calanoid copepods. These results clearly suggest that predators preferentially feeding on daphnids, or cladocerans in general (e.g. Ballantyne *et al.*, 2003), ingest more ARA than predators that specialize more on copepods. Although higher ARA concentrations in cladocerans may be directly linked to higher dietary ARA supply from microseston or to differences in the rates of ARA metabolism within these organisms, it is clear that further studies are required to investigate the physiological requirement of ARA in these two zooplankton groups. However, our data on ARA concentrations in microseston and zooplankton suggest that sestonic ARA is the most efficiently retained EFA in cladocerans. There was no significant difference in EPA concentrations among the seston size classes or between cladocerans and calanoid copepods, suggesting that feeding on seston size classes that are larger than the generally preferred microseston does not yield any additional dietary EPA advantage for zooplankton or other seston consumers.

Docosahexaenoic acid concentrations clearly vary most between these two zooplankton groups as calanoid copepods contained $11.5\times$ more DHA than cladocerans, and this result agrees with other field studies (Smyntek *et al.*, 2008; Ballantyne *et al.*, 2003). For cladocerans, DHA concentrations are lower than in any seston size class, suggesting that dietary DHA supply from lake seston is not the reason why cladocerans generally contain only traces of DHA. The physiological reason as to why DHA concentrations are significantly different between cladocerans and copepods has not yet been elucidated. Although Persson and Vrede (Persson and Vrede, 2006) proposed that higher DHA in copepods than cladocerans may be linked to a higher developed nervous system in copepods, it is clear that differences of DHA between these copepods are not simply related to dietary supply of DHA.

Our study is based on standing-stock information from the summer period, but results from a different

seasonal study from oligotrophic SOL and SHL showed that there was no significant effect of season (non-random factor) on the variability of total EFA concentrations in zooplankton of these freshwater systems (Kainz *et al.*, 2008). We conclude that concentrations of EFA are not a simple function of seston particle size and suggest that microseston, the preferred diet size class for zooplankton, likely provides the most efficient conduit for EFA across the aquatic plant–animal interface in the pelagia of these study lakes and possibly also other oligotrophic systems. Although further investigation of the biochemical and more detailed size composition of microseston (i.e. pico- and nanoplankton) is necessary, we propose that our results have important ecological implications for conservation of biodiversity and habitats because human impacts on aquatic ecosystems often increase nutrient supply skewing the algal size spectrum away from optimally sized particles to biochemically low-quality large colonial cyanobacteria. Such effects may consequently decrease EFA concentrations available to consumers (*sensu* Müller-Navarra *et al.*, 2004), a phenomenon which warrants further studies including a larger range of lake trophic status. Viewed in this way, the microseston size fraction constitutes the “first line of defense” in terms of protecting the health and integrity of aquatic food webs.

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