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The effects of seston lipids on zooplankton fatty acid composition in Lake Washington, Washington, USA

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Abstract. We collected suspended particulate matter (seston) and zooplankton samples from Lake Washington in Seattle, Washington, USA, over a 10-month period to investigate the effects of food availability on zooplankton fatty acid (FA) composition. The percentage of nutritionally critical ω3 polyunsaturated fatty acids (PUFA) in the seston varied from 8% of the FA pool in midsummer to 30% during the spring diatom bloom. Zooplankton accumulated much higher percentages ω3 PUFA than was available in the seston. In particular, cladocerans preferentially accumulated eicosapentaenoic acid (EPA, 20:5ω3), copepods accumulated docosahexaenoic acid (DHA, 22:6ω3), and both copepods and cladocerans accumulated 18 carbon chain ω3 PUFAs (C_{18} ω3). By comparison, the FA of zooplanktivorous juvenile sockeye salmon (*Oncorhynchus nerka*) were strongly dominated by EPA (12.5% ± 2.1%) and DHA (28.2% ± 8.7%). The saturated fatty acid and the arachidonic acid (ARA, 20:4ω6) composition of *Diaptomus ashlandi* was strongly ($r^2 = 0.76$) and moderately ($r^2 = 0.54$) correlated with the prevalence of these FAs in the seston. Furthermore, the DHA content of *Diaptomus* was moderately correlated with the seston's DHA content ($r^2 = 0.45$) and very strongly correlated with seston EPA ($r^2 = 0.89$). Since EPA was the most prevalent PUFA in the seston and DHA was the most prevalent PUFA in *Diaptomus*, these results suggest that *Diaptomus* may synthesize DHA from the EPA in their food. In general, zooplankton species in Lake Washington were strongly enriched with those FA molecules that are most physiologically important for fish nutrition (i.e., ARA, EPA, and DHA), indicating a clear mechanism by which changes in seston composition influence fisheries ecology.

Key words: fatty acids; fish; food quality; freshwater food web; Lake Washington, Seattle, Washington, USA; seston; zooplankton.

Introduction

Fatty acids (FAs) are nutritionally critical molecules that have been used as biomarkers to analyze food-web interactions in a variety of terrestrial and aquatic ecosystems. Fatty acids are useful as biomarkers because they are chemically diverse (i.e., 20–30 FAs per sample); fatty acid composition varies among dietary organisms (e.g., terrestrial vegetation, bacteria, cyanobacteria, algae), and consumers often incorporate FAs unmodified into somatic tissues (Dalsgaard et al. 2003). Soil ecologists have used FA biomarkers to define feeding strategies and dietary composition (i.e., bacteria, fungi, animals, and plant litter) of decomposer invertebrates (Ruess et al. 2005), whereas marine scientists have used FA biomarkers to infer the relative contributions of forest litter, marine macroalgae, and phytoplankton to hagfish and clam production in nearshore environments (McLeod and Wing 2007). Similarly, stream ecologists have used FAs to assess the relative importance of terrestrial organic inputs and autochthonous production (mainly algae) to stream invertebrates (Torres-Ruiz et al. 2007). Fatty acids have also been used to infer the diets of marine vertebrates such polar bears (Thiemann et al. 2007), sea birds (Dahl et al. 2003), and most commonly seals (Iverson et al. 1997). However, the large majority of studies employing fatty acids as trophic markers have been conducted in marine planktonic systems to examine the flow of lipids across the phytoplankton–zooplankton interface (Graeve et al. 1994, Dalsgaard et al. 2003). To realize the full potential of the FA biomarker approach, it is critical to know the extent to which consumer FA composition is influenced by and is different from their known diets; however, for many organisms this is difficult to ascertain.

Certain ω3 and ω6 polyunsaturated fatty acids (PUFAs) are nutritionally essential because all vertebrates and most invertebrates lack the enzymes necessary to desaturate oleic acid (18:1ω9) to the analogous 18 carbon chain (C_{18}) ω3 and ω6 fatty acids, i.e., α-linolenic acid (α-LA, 18:3ω3) and linoleic acid (LIN, 18:2ω6; Tocher et al. 1998). The physiologically active essential fatty acids (EFAs) in animals are the ω3 FAs eicosapentaenoic acid (EPA, 20:5ω3) and docosahexae-

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noic acid (DHA, 22:6ω3), as well as the ω6 FA arachidonic acid (ARA, 20:4ω6; Sargent et al. 1999). EPA and DHA are almost exclusively synthesized by algae (Brett and Müller-Navarra 1997), although many animals can synthesize these molecules from the α-LA found in algae and higher plants. Long-chain EFAs regulate cell membrane properties (in particular fluidity) due to their extremely low melting points (approximately −50°C; Farkas 1970), while ARA and EPA are also precursors for the eicosanoid class of hormones, which are locally active signaling molecules that control inflammation, pain, immunity, vascular permeability, blood pressure, blood clotting, and reproductive processes (Simopoulos 1999, Funk 2001). ARA and especially DHA are also major structural components of nervous system tissues in vertebrates (Simopoulos 1999).

Essential fatty acids have important effects on the growth, reproduction, and lipid composition of zooplankton (Müller-Navarra et al. 2000, Ravet et al. 2003, Brett et al. 2006) and fish (Sargent et al. 1999). The FA composition of phytoplankton varies considerably among taxa (Brett and Müller-Navarra 1997), with cryptophytes and diatoms high food quality, green algae intermediate quality, and cyanobacteria poor food quality based on the somatic growth rates of zooplankton that consume them (Brett et al. 2000). In fact, Danielsdottir et al. (2007) recently suggested high food quality phytoplankton at the base of the food web should intensify trophic interactions between phytoplankton, herbivorous zooplankton, and fish. However, to date there have been few direct tests of the effects of EFAs on aquatic food-web linkages.

It has been shown that the polyunsaturated fatty acid (PUFA) content of zooplankton increases with the size class and trophic position of taxa (Kainz et al. 2004, Persson and Vrede 2006) due to phylogenetic position and life history characteristics (Persson and Vrede 2006). For example, cladocerans preferentially accumulate EPA and have very little DHA relative to available food, whereas copepods mostly accumulate DHA (Ballantyne et al. 2003, Persson and Vrede 2006). Although some field studies suggest zooplankton fatty acid composition is independent of seston composition (Persson and Vrede 2006, Smyntek et al. 2008), laboratory experiments showed that the EPA and ARA content of *Daphnia* can be strongly influenced by the FA composition of phytoplankton (Brett et al. 2006). Resolution of the effects of diet on zooplankton FA content is important because the main phytoplankton groups in seston differ greatly in their FA composition (Brett et al. 2009) and because the FAs of zooplankton (EPA, DHA, and ARA) play important roles in the nutrition and development of fish (Rainuzzo et al. 1997, Sargent et al. 1999, Bell and Sargent 2003). Thus, preferential uptake or modification of EFAs by zooplankton may influence fisheries ecology, as well as trophic cascades in lakes.

The objective of this study was to determine whether the FA composition of phytoplankton in seston, the main component of herbivorous zooplankton diets, affects the FA composition of zooplankton. This study also examined whether zooplankton preferentially accumulate the most physiologically active FAs and whether zooplankton modify primary-producer lipids (by, for example, converting α-LA or EPA to DHA), as these molecules are transferred to zooplanktivorous fish. These objectives were addressed by comparing the fatty acid composition of seston to that of the five most abundant macrozooplankton taxa in Lake Washington, Seattle, Washington, USA, over a 10-month period, as well as previous research on FAs in juvenile sockeye salmon (*Oncorhynchus nerka*), an important zooplanktivore at this site. Sampling was initiated prior to the spring phytoplankton bloom and continued through to the following winter turnover to examine how the FA composition of zooplankton varies seasonally in response to changes in seston lipid composition and abundance. Using these data, we examined the relative importance of seston lipids and zooplankton taxonomic affiliation on zooplankton fatty acid composition.

Methods

Study site

Lake Washington is located in Seattle, Washington, USA, and has a surface area of 87.6 km^2, a mean depth of 32.9 m (maximum depth = 65.2 m), and a hydraulic retention time of ~2.4 yr (Edmondson 1994). During 1995–2000, the mean annual concentrations of total phosphorus and total nitrogen in the lake were 19 and 360 μg/L, respectively, and the mean annual Secchi transparency was ~3.9 m (Arhonditsis et al. 2003). During our study, the mean chlorophyll *a* concentration in the upper 10 m ranged from 2.8 ± 1.3 μg/L (mean ± SD) during the summer stratified period to 11.9 ± 2.9 μg/L during the spring bloom. The phytoplankton assemblage in Lake Washington was dominated by cyanobacteria between 1955 and 1973, but wastewater diversion resulted in a dramatic decline in cyanobacteria (Edmondson 1994). Currently, the seasonal phytoplankton cycle is characterized by a large spring bloom dominated by diatoms (e.g., *Aulacoseira*, *Asterionella*, *Fragilaria,* and *Stephanodiscus*; see Plate 1), and an assemblage that is fairly evenly distributed among chlorophytes, cyanobacteria, diatoms, and cryptophytes during thermal stratification (Arhonditsis et al. 2003).

Lake Washington sampling protocol

We sampled Lake Washington in the vicinity of King County Metro station 852, aka Edmondson's Madison Park station. Water samples for the analyses of seston composition were collected during the morning at 0.5, 5, and 10 m depth using a 10-L Van Dorn sampler, and then pooled to form an epilimnetic composite prior to laboratory analyses. Water was filtered through a 50-μm mesh to remove zooplankton and placed into acid-washed

polypropylene bottles. Samples were stored in coolers for ~3 h prior to being processed in the laboratory. Zooplankton was collected using a 120-μm mesh plankton net using two 10-m vertical tows. Each tow was stored separately and kept cool until processed (generally within 2 h).

In the laboratory we filtered 200–500 mL of screened, pooled epilimnetic water onto Whatman GF/F filters and analyzed for chl *a* content, corrected for phaeophytin (Marker 1977). Seston samples collected at 10 m depth were subsequently used for the determination of ash-free dry mass (Widbom 1984). The filters were dried at 85°C for 24 h, cooled in a desiccator for 10 min, weighed, combusted at 550°C for 2 h, and then cooled and reweighed. Seston fatty acid samples were collected by filtering 0.5–2.5 L of screened, pooled epilimnetic water onto 47-mm diameter precombusted glass fiber filters (Whatman GF/F) and transferring these filters into 1-mL Eppendorf centrifuge tubes. Zooplankton were identified to genus using a dissecting microscope and collected while alive using a Pasteur pipette. Generally 40–500 individuals from each zooplankton taxon were collected for fatty acid analyses. Five zooplankton groups occurred in sufficient abundance to be analyzed individually: *Daphnia* spp. (i.e., *Daphnia pulicaria* and *D. thorata*), *Bosmina longirostris*, *Diacyclops thomasi*, *Diaptomus ashlandi* (see Plate 1), and *Epischura nevadensis*. Because zooplankton were isolated using a dissecting microscope, it was not possible to distinguish between adults and subadults (i.e., copepodites). Nauplii (larva) were not used for these analyses. All fatty acid samples were stored at −80°C prior to extraction.

Fatty acid analyses

Fatty acids were extracted from freeze-dried seston and zooplankton samples and methylated according to Kattner and Fricke (1986). Fatty acid methyl esters were analyzed using a gas chromatograph (HP 6890; Agilent, Foster City, California, USA) equipped with a programmable temperature vaporizer injector, a fused silica capillary column (DB-WAX, 30 m × 0.32 mm with 0.25-μm film thickness; J and W Scientific, Folsom, California, USA), and a flame ionization detector. We injected 5 μL of sample and used helium as the carrier gas. The temperature program applied was as follows: 40°C held for 5 min, then heated up at 10°C per minute to 150°C, held for 5 min, then heated up at 1°C per minute to 220°C where it was kept for 20 min. Individual fatty acids were identified based on the retention times of fatty acid methyl ester standards (37-mix Supelco; Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in n-hexane. Quantification was performed with an internal standard (21:0) and quantitative mixes (Alltech, Deerfield, Illinois, USA) to calculate response factors for each fatty acid analyzed.

Statistical analyses

All data used for statistical analyses were arcsine (square root)-transformed, except for the ω3:ω6 ratio, which was $\log_{10}$-transformed. Throughout this paper, the less common FA congeners were combined with the main fatty acids in each FA category, e.g., eicosapentaenoic acid (EPA; EPA and 20:3ω3), docosahexaenoic acid (DHA; DHA, 22:3ω3, and 22:5ω3), and so on. The relative percentage of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), C_{18} ω6 (linoleic acid and γ-linolenic acid), C_{18} ω3 (α-linolenic acid and stearidonic acid), arachidonic acid (ARA), EPA, docosadienoic acid (DDA, 22:2ω6), DHA, and the ω3:ω6 ratio were used in a discriminant function analysis (DFA) to assess whether the fatty acid composition differed when the zooplankton taxa and seston were used as grouping variables. The DFA classifications were cross-validated using a leave-one-out scheme in which all observations were used to generate the classification except for the sample being classified. We also used paired *t* tests to compare the FA composition of the seston and zooplankton (*Diaptomus*, *Diacyclops*, *Epischura*, and *Daphnia*) for dates when both samples were available. Linear regression of the seston and *Diaptomus* FA composition was used to determine which FA had the greatest potential as diet biomarkers. We also calculated a series of regressions between the DHA content of *Diaptomus* and the C_{18} ω3, EPA, and DHA content of seston to infer potential sources of DHA in *Diaptomus*.

Phytoplankton and salmonid fish fatty acid composition

To more clearly elucidate how zooplankton may influence the trophic transfer of essential fatty acids in aquatic foods, we reviewed previous literature to compare how the fatty acid composition varied for the major phytoplankton groups, salmonid eggs, and juvenile *Oncorhynchus nerka*.

Results

Zooplankton assemblage

The macrozooplankton community of Lake Washington, Seattle, Washington, USA, shifted from being dominated (>2 adults/L) by *Diacyclops thomasi* and *Diaptomus ashlandi* preceding and during the spring diatom bloom (Chl *a* > 10 μg/L) to *Daphnia* spp. (mainly *Daphnia pulicaria*, but also *Daphnia thorata*) in the summer, and to *Bosmina longirostris* and *Diaptomus* in the fall (Fig. 1).

Seston fatty acid, Chl a*, and particulate organic carbon concentrations*

The mean percentages of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) in the surface layer (0–10 m) seston were ~45%, 22%, and 32%, respectively; however, these percentages varied considerably during the 10-month sampling period as evidenced by the high standard deviations about the means (Table 1, Fig. 1). For example, eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) constituted 20% ± 1% (all data are

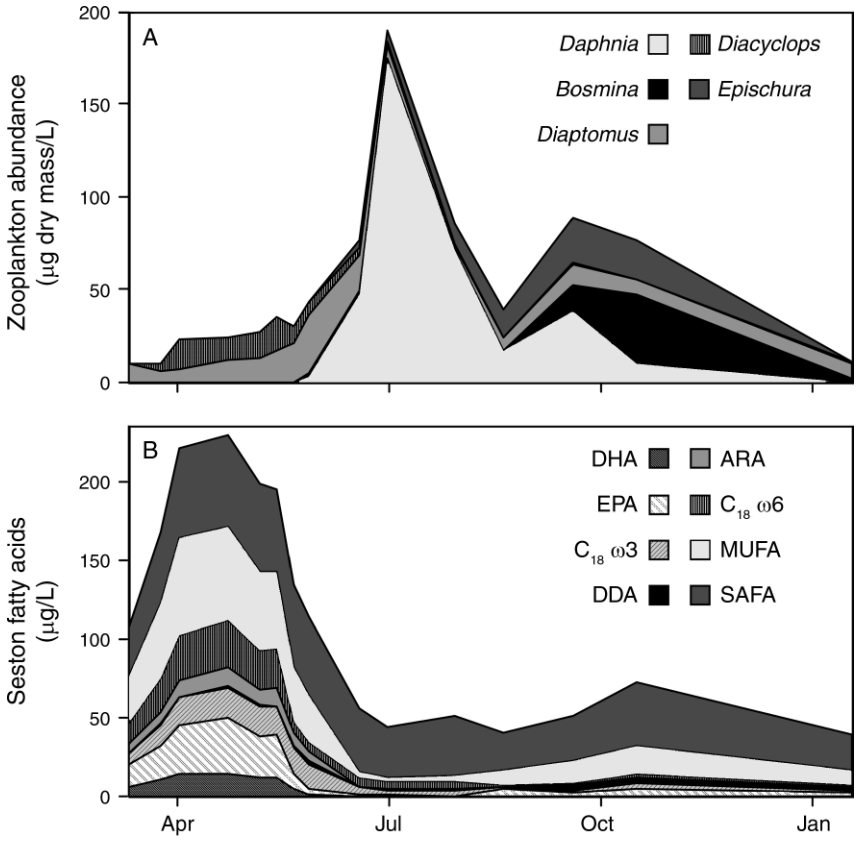

FIG. 1. (A) Seasonal abundances of the most prevalent zooplankton taxa in Lake Washington, Seattle, Washington, USA, during this field study. These data were based on vertical tows through the upper 10 m of the water column. (B) Seasonal concentrations of seston fatty acids. These data were for a pooled epilimnetic sample (0.5, 5, and 10 m depths). Total fatty acid biomass for the last four sampling dates (September–January) was estimated based on ash-free dry mass and Chl *a* concentrations.

mean ± SD) of total fatty acids (FAs) during the spring bloom period, while these PUFAs only accounted for 6% ± 4% of total FAs during the summer (Fig. 1). The mass-specific concentrations of EPA and DHA in the seston averaged 7.6 ± 4.9 μg/mg C and 3.1 ± 2.2 μg/mg C, respectively, but reached annual minima (~1 μg/mg C) during the summer months for EPA and during the summer and fall for DHA. Chlorophyll *a* concentrations ranged from 2.8 ± 1.3 μg/L (mean ± SD for all data shown) during the stratified period to 11.9 ± 2.9 μg/L during the spring bloom. There was a small increase of Chl *a* between mid summer and late fall (from 2 to 4 μg/L). Total fatty acid concentrations averaged 115 ± 72 μg/L, with a pronounced peak during the spring phytoplankton bloom (Fig. 1). Total FAs were moderately correlated with Chl *a* concentrations ($r^2 = 0.64$, $P = 0.001$) and strongly correlated with seston particulate organic carbon concentrations ($r^2 = 0.97$, $P = 0.001$).

Zooplankton fatty acids

Paired *t* test comparisons revealed *Diaptomus ashlandi* had significantly ($P < 0.05$) smaller percentages MUFA and C_{18} ω6 in their tissues than was present in seston, but significantly larger percentages C_{18} ω3, arachidonic acid (ARA), and especially DHA (Table 1). *Diaptomus* also had a significantly higher ω3:ω6 ratios than the seston. *Diacyclops* had significantly less C_{18} ω6, significantly more C_{18} ω3 and DHA, and higher ω3:ω6 ratios than the available seston. *Epischura* had significantly less SAFA, and more C_{18} ω3, and especially more DHA, than the seston. Overall EPA + DHA constituted 32.2% of *Epischura* fatty acids compared to 8.5% for seston collected at the same time. In general, the three copepod species were all substantially enriched with C_{18} ω3 and DHA relative to seston, whereas *Daphnia* had less SAFA and more C_{18} ω3 and ARA than the seston. Zooplankton lacked docosadienoic acid (DDA) (22:2ω6), whereas this FA constituted 1.2% ± 1.2% of the seston FAs.

The SAFA composition of *Diaptomus* was strongly correlated with that of the seston ($r^2 = 0.76$, $P = 0.001$; Fig. 2). This figure shows that during the spring, seston SAFA content was low and *Diaptomus* SAFA content was higher. In contrast, during the summer, the seston's proportional SAFA content was high, but *Diaptomus* had considerably less SAFA than the seston. Furthermore, the ARA and DHA content and

TABLE 1. The composition of major fatty acid (FA) functional groups (as a percentage of total FAs, mean ± SD) for seston (suspended particulate matter) and zooplankton in Lake Washington, Seattle, Washington, USA.

Organism and fatty acid	Fatty acid, as percentage of total sample					
	Zooplankton	Seston	t	P	r^2	P
Diaptomus ashlandi						
SAFA	41.9 ± 7.7	45.7 ± 18.5	−1.12	0.280	0.76	0.001
MUFA	13.2 ± 1.8	22.0 ± 8.2	−3.34	0.005	0.13	0.183
C_{18} ω6	4.6 ± 1.3	8.5 ± 4.3	−2.90	0.012	0.05	0.441
C_{18} ω3	14.3 ± 3.4	7.5 ± 3.0	5.57	0.001	0.00	0.808
ARA	4.9 ± 1.3	3.2 ± 2.4	3.48	0.004	0.54	0.002
EPA	6.5 ± 1.0	8.4 ± 5.2	−0.74	0.471	0.01	0.725
DDA	0 ± 0	1.3 ± 1.2	−7.32	0.001		
DHA	14.7 ± 4.8	3.5 ± 2.6	13.26	0.001	0.45	0.006
EPA + DHA	21.2 ± 5.0	11.8 ± 7.6	7.13	0.001	0.78	0.001
ω3:ω6 ratio	3.7 ± 0.8	1.7 ± 1.2	9.47	0.001	0.37	0.017
Diacyclops thomasi						
SAFA	43.1 ± 9.0	38.1 ± 19.0	1.09	0.337	0.95	0.005
MUFA	14.1 ± 0.8	22.2 ± 8.3	−2.04	0.111	0.74	0.057
C_{18} ω6	3.4 ± 0.5	10.7 ± 3.3	−5.20	0.007	0.00	0.923
C_{18} ω3	17.6 ± 6.0	9.2 ± 2.3	3.54	0.024	0.35	0.296
ARA	5.6 ± 1.6	4.2 ± 2.4	1.39	0.236	0.54	0.159
EPA	5.6 ± 0.3	9.9 ± 5.6	−1.30	0.262	0.06	0.696
DDA	0 ± 0	0.8 ± 0.5	−6.98	0.002		
DHA	10.7 ± 1.7	5.0 ± 2.1	8.73	0.001	0.91	0.012
EPA + DHA	16.3 ± 1.7	14.9 ± 7.7	0.70	0.521	0.82	0.033
ω3:ω6 ratio	3.8 ± 0.8	1.5 ± 0.4	16.48	0.001	0.87	0.021
Epischura nevadensis						
SAFA	38.8 ± 4.2	62.8 ± 8.9	−4.47	0.011	0.29	0.346
MUFA	13.1 ± 3.0	17.6 ± 9.8	−0.82	0.457	0.06	0.701
C_{18} ω6	4.3 ± 0.4	5.7 ± 3.7	−0.58	0.592	0.07	0.656
C_{18} ω3	11.6 ± 0.9	5.5 ± 1.5	7.47	0.002	0.02	0.824
ARA	4.9 ± 2.8	0.8 ± 1.2	2.73	0.053	0.38	0.276
EPA	9.2 ± 2.3	4.8 ± 3.8	2.25	0.088	0.00	0.937
DDA	0.1 ± 0.1	2.0 ± 1.5	−2.42	0.072	0.53	0.168
DHA	18.0 ± 1.0	0.9 ± 0.7	17.01	0.001	0.23	0.407
EPA + DHA	27.1 ± 3.0	5.7 ± 4.1	7.16	0.002	0.02	0.830
ω3:ω6 ratio	4.5 ± 1.1	2.0 ± 2.1	2.58	0.061	0.25	0.387
Daphnia spp.						
SAFA	48.0 ± 5.3	64.7 ± 9.1	−5.53	0.012	0.64	0.200
MUFA	21.1 ± 3.4	16.0 ± 10.6	1.43	0.249	0.89	0.058
C_{18} ω6	5.4 ± 3.0	6.3 ± 4.0	−0.44	0.689	0.27	0.477
C_{18} ω3	11.3 ± 1.8	5.7 ± 1.6	3.20	0.049	0.99	0.006
ARA	4.9 ± 0.5	0.5 ± 1.0	5.23	0.014	0.01	0.891
EPA	8.5 ± 1.7	4.4 ± 4.3	2.69	0.075	0.91	0.046
DDA	0 ± 0	1.6 ± 1.4	−2.78	0.069		
DHA	0.7 ± 0.4	0.8 ± 0.8	0.15	0.894	0.63	0.206
EPA + DHA	9.2 ± 1.6	5.2 ± 4.5	2.54	0.085	0.95	0.028
ω3:ω6 ratio	2.2 ± 1.1	2.1 ± 2.4	0.68	0.545	0.00	0.955

Notes: Sample sizes are as follows: seston ($n = 15$ samples), *Diaptomus ashlandi* ($n = 15$), *Diacyclops thomasi* ($n = 5$), *Epischura nevadensis* ($n = 5$), and *Daphnia* spp. ($n = 4$). The seston values compared to *Diaptomus* represent all seston samples collected, whereas the seston values reported for the other zooplankton represent the paired seston samples collected on the same dates as those zooplankton samples. The percentage values reported in this table are for the raw data; the data used for the statistical analyses were transformed by taking the arcsine(square-root) percentage. The ω3:ω6 ratio was $\log_{10}$-transformed. The t tests for systematic differences between zooplankton and seston FA composition and the r^2 values characterize the relationship between seston and *Diaptomus* FA composition. In each case, the r^2 values represent the statistical associations between the zooplankton fatty acid composition and that of the seston collected at the same time as the zooplankton samples. Abbreviations are as follows: SAFA (saturated fatty acids), MUFA (monounsaturated fatty acids), C_{18} ω6 (linoleic acid and γ-linolenic acid), C_{18} ω3 (α-linolenic acid and stearidonic acid), ARA (arachidonic acid), EPA (eicosapentaenoic acid), DDA (docosadienoic acid), DHA (docosahexaenoic acid), and ω3:ω6 ratio ([C_{18} ω3 + EPA + DHA]/[C_{18} ω6 + ARA + DDA]). Empty cells indicate that no data are available.

ω3:ω6 ratio of *Diaptomus* were also moderately correlated with seston lipids ($r^2 = 0.54$, $P = 0.002$; $r^2 = 0.45$, $P = 0.01$; and $r^2 = 0.37$, $P = 0.02$, respectively). Sestonic C_{18} ω3 content was uncorrelated with the DHA content of *Diaptomus* ($r^2 = 0.00$, $P = 0.99$). The seston's DHA content was moderately positively associated with DHA in *Diaptomus* ($r^2 = 0.45$, $P = 0.01$). In contrast, we found a strong association between the seston's EPA content and the DHA content of *Diaptomus* ($r^2 = 0.89$, $P = 0.001$; Fig. 3).

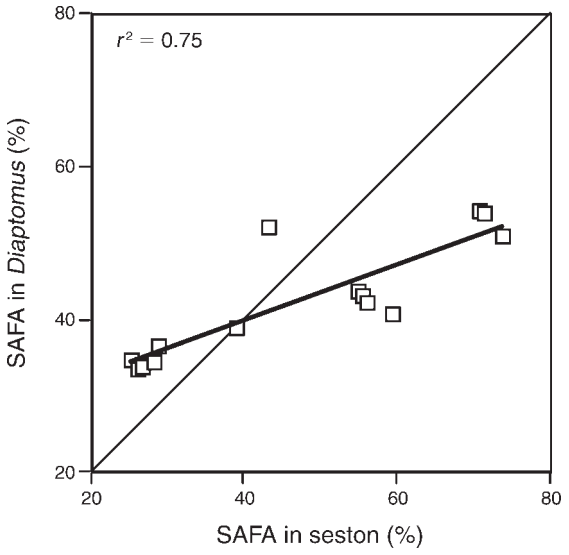

FIG. 2. Variation in the relative proportions of saturated fatty acids (as a percentage of total FAs) in the seston and *Diaptomus ashlandi*. When the SAFA was less prevalent in the seston in the spring and fall, it was in relative terms more prevalent in *Diaptomus*. Conversely, when SAFA was more prevalent in the seston during the summer, it was relatively less prevalent in *Diaptomus*. The SAFA composition of *Diaptomus* was also significantly less variable than that of the seston, i.e., 42% ± 8% and 46% ± 19% in *Diaptomus* and the seston, respectively.

Discriminant function analysis

Discriminant function analysis (DFA) showed that the fatty acid composition of the seston and individual zooplankton taxa were clearly distinguishable (Fig. 4), despite substantial seasonal variation. The first discriminant function explained 63% of the overall variability and was negatively correlated with the percentage of DDA ($r^2 = 0.67$, $P = 0.001$), and positively correlated with the ω3:ω6 ratio ($r^2 = 0.60$, $P = 0.001$), as well as C_{18} ω3 ($r^2 = 0.50$, $P = 0.001$) and DHA ($r^2 = 0.45$, $P = 0.001$) composition. The second discriminant function explained 35% of FA variability and was positively correlated with DHA ($r^2 = 0.40$, $P = 0.001$). The DFA correctly predicted zooplankton order (i.e., copepods or cladocerans) as well as seston identity in 98% of the leave-one-out cross-validation test cases. In contrast, DFA cross-validation tests correctly identified copepods and cladocerans to the genus level 85% of the time.

Phytoplankton and sockeye salmon fatty acid composition

Fatty acid composition differed among cyanobacteria, chlorophytes, diatoms, and cryptophytes (Table 2), the main phytoplankton groups in Lake Washington (Arhonditsis et al. 2003). On average, cyanobacteria had moderate percentages of C_{18} ω6 and ω3 PUFAs, and very little EPA or DHA. In contrast, green algae had high percentages of C_{18} ω6 and ω3 PUFAs, and very low percentages of ARA, EPA, and DHA. Compared to the other phytoplankton, diatoms had little C_{18} ω6 and ω3 PUFAs, and high levels of ARA, EPA, and DHA. Cryptophytes had very high percentages of C_{18} ω3 PUFAs, very little C_{18} ω6 PUFAs and ARA, and moderately high percentages of EPA and DHA. Overall, cyanobacteria had particularly high percentages of SAFA, and cryptophyes had particularly low percentages of MUFA (Table 2). Compared to phytoplankton, juvenile sockeye salmon collected from Lake Washington by Ballantyne et al. (2003) and salmonid fish eggs in general have high percentages of ARA (~2–6% of totals FAs), quite high EPA levels (9–15%), and extremely high DHA content (20–33%; Table 2).

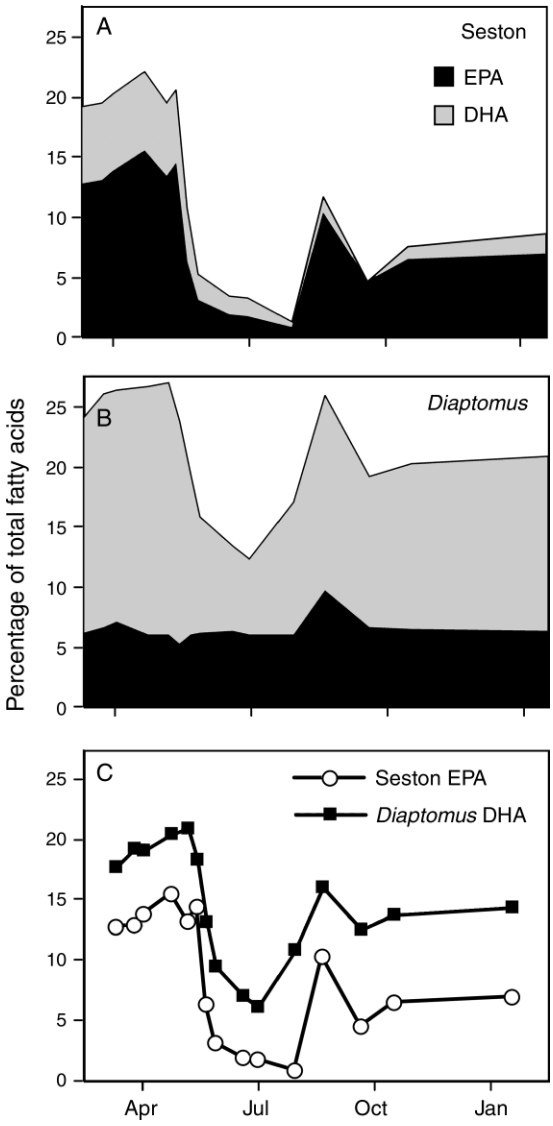

FIG. 3. Seasonal variation in the relative percentages of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) in (A) seston and (B) *Diaptomus ashlandi*; panel (C) shows seston EPA vs. *Diaptomus* DHA content.

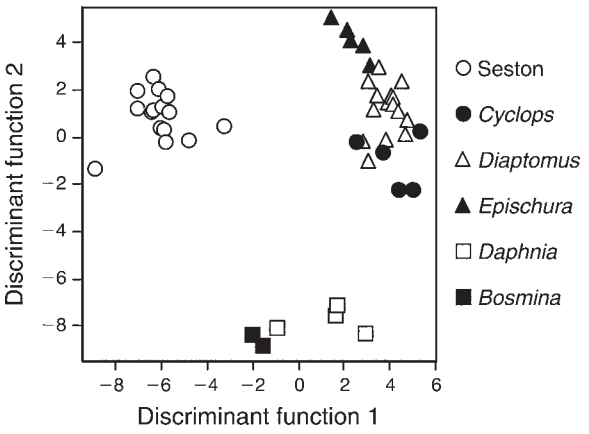

FIG. 4. A canonical ordination of a discriminant function analysis (DFA) using the percentages of the major fatty acid functional groups, SAFA, MUFA, C_{18} ω6, C_{18} ω3, ARA, EPA, DDA, DHA, and the ω3:ω6 FA ratio, in seston and zooplankton (see Table 1 for abbreviations). The sample size for this DFA was the following: seston ($n = 15$), *Diaptomus ashlandi* ($n = 15$), *Diacyclops thomasi* ($n = 5$), *Epischura nevadensis* ($n = 5$), *Daphnia* spp. ($n = 4$), and *Bosmina* ($n = 2$). The first discriminant function explained 63% of the overall variability in the zooplankton and seston fatty acid data and was negatively correlated with percentage of DDA, and positively correlated with the ω3:ω6 FA ratio, C_{18} ω3, and DHA. The second discriminant function explained 35% of the variability and was positively correlated with the percentage of DHA. The seston and zooplankton genera were correctly classified 85% of the time using a leave-one-out cross-validation scenario.

DISCUSSION

Fish production is very essential fatty acid (EFA) intensive (Sargent et al. 1999). Nearly all animals are unable to produce these molecules de novo, and in aquatic food webs, zooplankton and fish rely on algae for nearly all EFA production. Most phytoplankton have far less EFA than do fish (Table 2), in particular DHA (docosahexaenoic acid), which accounts for 28% of total fatty acids (FAs) of juvenile sockeye salmon (Table 2). Therefore, herbivorous zooplankton act as a key conduit to carry EFAs to ecologically and economically important fish. Consistent with this hypothesis, seasonal changes in the fatty acid composition of seston were clearly correlated with those of large-bodied zooplankton, the main prey of juvenile fish (Table 1, Figs. 2 and 3). Furthermore, the most common zooplankton were commonly twofold enriched with EFAs relative to seston (Table 1).

EFA bioconversions

The observation that seston eicosapentaenoic acid (EPA) seems to be the main source of DHA in *Diaptomus* is important because it indicates that freshwater copepods may convert EPA to DHA, the latter being a more important molecule for fish nutrition (Sargent et al. 1999). This inference is based on the strong correlation ($r^2 = 0.89$) between the seston's EPA content and the DHA content of *Diaptomus* (Fig. 3) and the observation that DHA only comprised 29% ± 13% (mean ± SD) of the long-chain ω3 polyunsaturated fatty acids (PUFAs) (i.e., the sum of EPA + DHA) in the seston, whereas DHA comprised 68% ± 9% of the long-chain ω3 PUFAs in *Diaptomus*. Furthermore, since DHA was four times more abundant in *Diaptomus* than in the seston, it is unlikely that direct dietary sources could meet this copepod's demands for DHA. For example, the DHA content of *Diaptomus* increased from a summer minimum of 6% to a fall mean of 14% during a period when the seston's DHA content remained around 1–2% (Fig. 3). This autumn increase in *Diaptomus* DHA corresponds to contemporaneous increases in the sestonic EPA contents. Confirmation of the hypothesis that some zooplankton can convert EPA to DHA could be obtained through direct experiments in which *Diaptomus* are fed stable-isotope-labeled EPA (e.g., Bell et al. 2007). Such experiments have shown that several marine copepods and krill larvae were unable to desaturate and elongate α-linolenic acid to EPA and DHA at ecologically meaningful rates (Bell et al. 2007), which is consistent

TABLE 2. The fatty acid composition of the main phytoplankton groups in Lake Washington (Seattle, Washington, USA) aggregated to major fatty acid functional groups (as a percentage of total fatty acids, mean ± SD).

Functional group	Cyanophytes	Chlorophytes	Diatoms	Cryptophytes	Salmonid eggs	Sockeye juveniles
SAFA	59 ± 19	33 ± 10	24 ± 11	28 ± 10	25 ± 3	28 ± 3
MUFA	25 ± 17	27 ± 13	40 ± 13	10 ± 5	32 ± 6	12 ± 3
C_{18} ω6 PUFA	7 ± 7	14 ± 6	2 ± 2	3 ± 2	3 ± 1	4 ± 3
C_{18} ω3 PUFA	7 ± 10	26 ± 10	3 ± 3	40 ± 10	3 ± 1	10 ± 3
ARA	1 ± 2	0.2 ± 0.3	2 ± 2	0.1 ± 0.2	3 ± 3	4 ± 2
EPA	1 ± 1	0.1 ± 0.2	17 ± 8	15 ± 6	11 ± 4	13 ± 2
DHA	1 ± 2	0 ± 0	3 ± 3	3 ± 2	24 ± 1	28 ± 9
ω3:ω6 ratio	1 ± 1	2 ± 1	8 ± 5	17 ± 8	10 ± 7	6 ± 2

Notes: Fatty acid data for the eggs of wild salmonid fish in general and juvenile sockeye salmon collected from Lake Washington are also summarized. The phytoplankton fatty acid data were based on results published for monocultures by various authors and were modified from values previously reported in Brett et al. (2009); the salmonid egg fatty acid composition data were obtained for the wild fish reported in Ashton et al. (1993) and Pickova et al. (1998, 1999, 2007); the Lake Washington juvenile sockeye salmon fatty acid composition data are for dorsal muscle samples and were adapted from Ballantyne et al. (2003). The numbers of samples for each group are as follows: cyanophytes, 9; chlorophytes, 11; diatoms, 6; cryptophytes, 9; salmonid eggs, 8; sockeye juveniles, 5. Abbreviations are as in Table 1.

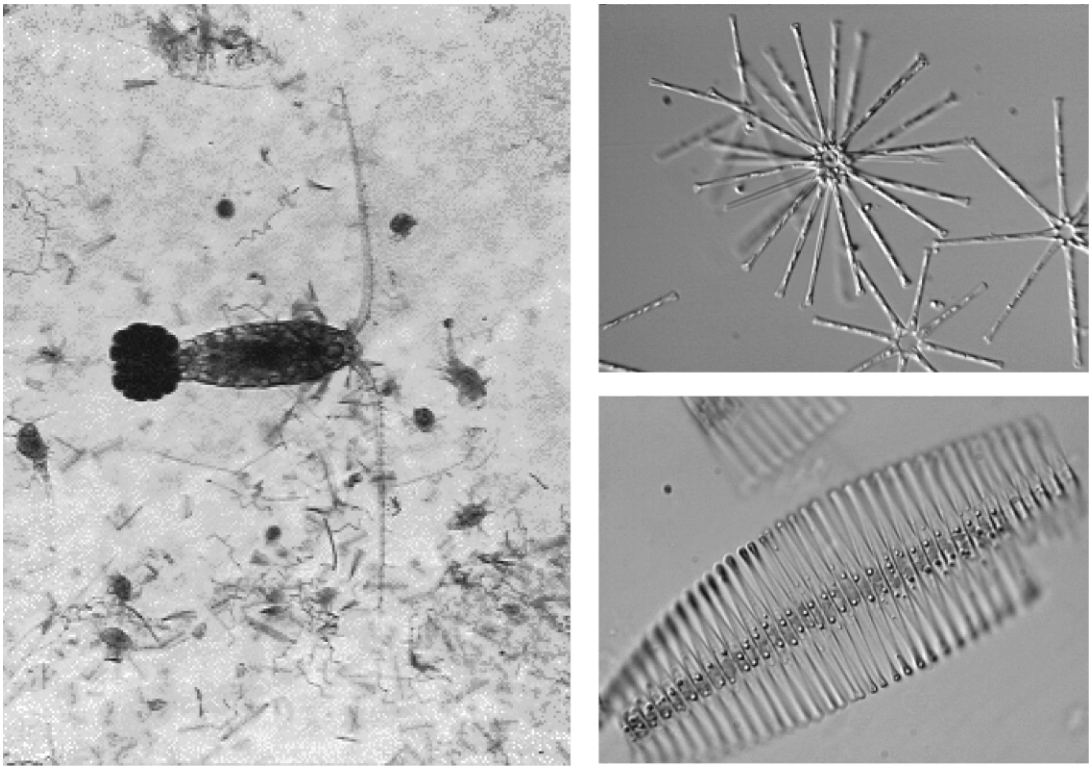

PLATE 1. Several plankton from Lake Washington, Seattle, Washington, USA, including the copepod *Diaptomus ashlandi* and the diatoms *Asterionella formosa* and *Fragilaria crotonensis*. Photo credits: *Diaptomus*, Arni H. Litt; *Asterionella* and *Fragilaria,* Monika Winder.

with our observation that C_{18} ω3 PUFA in seston was not correlated with DHA in *Diaptomus*.

Dietary fatty acid biomarkers

Fatty acids (FA) show great promise as diet biomarkers (Dalsgaard et al. 2003). To realize the full potential of this approach, it is necessary to know which FAs within a particular organism are most strongly correlated with diet. It is also necessary to know if some FAs are unrelated to diet, or alternatively systematically depleted or enriched relative to diet. In Lake Washington, the sum of saturated fatty acids (SAFAs) and the sum of EPA + DHA appear to have the greatest potential as dietary biomarkers for *Diaptomus ashlandi* because of strong correlations between seston and copepod FAs ($r^2 = 0.76$–0.78). These strong correlations between available food and *Diaptomus* lipids are noteworthy because copepods are known to be selective feeders (DeMott 1986). Our observed patterns for *Diaptomus* contrast to those of Persson and Vrede (2006) and Smyntek et al. (2008), who found no correlation between the FA composition of seston and zooplankton collected from a series of lakes. However, the results of the present study, which span a period of extensive seasonal variability in phytoplankton composition, are similar to those of Brett et al. (2006), who found a strong effect of phytoplankton FAs on the FA composition of *Daphnia*, particularly for EPA and ARA (arachidonic acid).

Environmental variability may have partially influenced the relationship between FAs in seston and zooplankton in Lake Washington. For example, Farkas (1970) found that cold-adapted *Eudiaptomus gracilis* had a higher percentage of DHA, while warm-adapted individuals had more SAFAs. Similarly, Schlechtriem et al. (2006) found that *Daphnia pulex* reared on green algae at 11°C had higher percentages of 16:1ω7 and EPA, whereas individuals raised at 22°C had more linoleic acid and α-linolenic acid. Consistent with these data, we observed lower percentages of SAFA in *Diaptomus* during the spring and a higher percentage during the summer (Fig. 2), whereas the relative abundance of DHA was lower in the summer than spring or fall (Fig. 3). Because cold tolerance in phytoplankton is a function of their unsaturated fatty acid composition (Wada et al. 1990), and phytoplankton groups with high PUFA content (e.g., diatoms) are typically dominant during cold months and phytoplankton with a high SAFA content (e.g., cyanobacteria) are more prevalent during the summer, it may not be possible to completely separate effects of food availability and environmental temperature on zooplankton FA composition in field studies.

Despite the apparent effects of seston composition on the FA composition of *Diaptomus*, the FA composition of this zooplankter also differed systematically from that of potential dietary items (Table 1, Fig. 4). In particular, *Diaptomus* had a higher ω3:ω6 ratio, less MUFA and C_{18} ω6, and more C_{18} ω3, arachidonic acid (ARA), and docosahexaenoic acid (DHA) than did the seston. Similar results were observed for *Diacyclops thomasi* with a higher ω3:ω6 ratio, less C_{18} ω6, and more C_{18} ω3 and DHA than the seston, and for *Daphnia* which exhibited less SAFA and more ARA than did seston. The observation that all zooplankton had less DDA (docosadienoic acid) than seston likely reflects a protozoan source in the seston, as this FA is uncommon in phytoplankton and macrozooplankton, but is usually encountered in natural seston samples (M. T. Brett, *unpublished data*).

The SAFA content of *Diaptomus* varied less than that of the seston (i.e., coefficient of variation [CV] = 19% and 39%, respectively; $P = 0.001$) suggesting that some invertebrates partly regulate their fatty acid content independent of diet (see Fig. 2). The EPA content of *Diaptomus* (Table 1 and Fig. 3) also varied little relative to that of seston (CV = 15% and 62%, respectively; $P = 0.001$). Similarly, EPA and the SAFA palmitic acid, the major components of structural polar phospholipids (PL), vary only within narrow bounds within marine copepods (Brett et al. 2009). Unfortunately, because we did not separately determine neutral storage FAs and polar PLs in this study, we were unable to determine whether similar structural constraints restrict variability in the fatty acid content of the freshwater zooplankton studied here.

Taxonomic differences in zooplankton FA composition

Zooplankton exhibited consistent differences in FA composition despite strong seasonal variation (Fig. 4). The most pronounced separation occurred between copepods (*Diaptomus*, *Diacyclops*, and *Epischura*), which accumulated much higher percentages of DHA, and cladocerans (*Bosmina* and *Daphnia*), which accumulated EPA (Table 1, Fig. 4). Similar differences between copepods and cladocerans have been widely reported (Farkas 1970, Ballantyne et al. 2003, Persson and Vrede 2006, Smyntek et al. 2008). Several hypotheses have been suggested to explain the high DHA content of copepods. Originally, high DHA content was proposed to be a cold-water adaptation that maintains membrane fluidity and allows copepods to overwinter in an active growth phase (Farkas 1970). DHA should have a major effect on the fluidity of the complex lipid mixtures that comprise cell membranes due its extremely low melting point, i.e., −45°C. However, copepods could alternatively use EPA or ARA to maintain fluidity, as these molecules also have extremely low melting points (i.e., approximately −50°C). For example, Schlechtriem et al. (2006) showed *Daphnia pulex* increased their percentage of EPA by a factor of four when grown at 11°C as opposed to 22°C. Persson and Vrede (2006) hypothesized that copepods require more DHA because they have a more highly developed nervous system than do cladocerans, including rapid prey attack (Kerfoot 1978) and predator avoidance strategies (Drenner et al. 1978, Lenz et al. 2000), and abundant chemoreceptors which allow them to taste food and track mates (DeMott 1986, Yen et al. 1992, Weissburg et al. 1998). Lenz et al. (2000) and Weatherby et al. (2000) noted marine calanoid copepods have thick myelin sheaths covering axons in their nervous system, which allow them to achieve exceptionally quick nerve impulse responses. Similar to nervous system tissues in vertebrates, DHA may be critical for the proper functioning of myelin and associated structures (e.g., synaptosomes and microsomes; Martinez and Vazquez 1998). Interestingly, if this nervous system hypothesis is true, the high DHA content of copepods should make them more nutritious for zooplanktivorous fish (Sargent et al. 1999), yet less vulnerable to capture by these same fish.

Persson and Vrede (2006) and Smyntek et al. (2008) suggest that cladocerans require elevated EPA to sustain their rapid growth and reproductive rates compared to copepods. However, while it is evident that EPA is essential to cladocerans (Ravet et al. 2003), it is not clear why EPA supports faster growth and reproduction than would DHA. In fact, fish physiologists have found many fish greatly enrich their eggs with DHA to provide a key substrate for rapid juvenile growth (Wiegand 1996, Sargent et al. 1999). Comparisons among zooplankton taxa suggest that the percentages of long-chained PUFA increased with increasing trophic position from herbivores to carnivores (Persson and Vrede 2006). Our findings generally agree with this observation, as the percentage of PUFA increased from herbivorous cladocerans *Daphnia* and *Bosmina*, to the omnivorous or carnivorous copepods *Diaptomus*, *Diacyclops*, and *Epischura*. However, more generally, the copepods simply had a higher percentage PUFA (i.e., 42–48% of total FAs) than did the cladocerans (i.e., 31–32% of total FAs).

Bridging the biochemical divide

Phytoplankton and zooplanktivorous fish have vastly different fatty acid compositions, suggesting the presence of a biochemical divide that is bridged by zooplankton when they transform and concentrate the physiologically important EFAs in their fatty acid pool. For example, salmonid eggs and juvenile sockeye contain a higher percentage of ARA than all phytoplankton except diatoms, a much higher percentage of EPA than cyanobacteria and chlorophytes, and a far higher percentage of DHA than any phytoplankton. It has been well established that EFA-rich diets are critical for promoting rapid growth, metamorphosis, visual acuity, disease resistance, and survival, as well as to enhance reproduction and develop normal pigmentation in a wide range of fish (Rainuzzo et al. 1997, Sargent et

al. 1999). While most marine fish have a limited capacity to convert C_{18} PUFAs to EPA and DHA, many freshwater species may have a greater capacity for these transformations (Sargent et al. 1999). However, even for fish that are known to be able to convert C_{18} PUFAs to EPA and DHA, it is unlikely bioconversion can meet the extensive nutritional demands for these FAs (Agaba et al. 2005).

Ecological consequences of enhanced EFA content in zooplankton

Laboratory studies show that EFA-rich phytoplankton increase *Daphnia* growth and reproduction rates and EFA composition (Brett et al. 2006), while field studies suggest EFA-rich cryptomonads may even affect the outcome of competitive interactions within zooplankton communities (Hampton et al. 2006). Furthermore, the model presented by Danielsdottir et al. (2007) suggested that the presence of phytoplankton with high food quality can favor inverted biomass distributions within food webs because rapidly growing zooplankton can withstand intense zooplanktivory and can be an efficient transfer of energy through the food web from primary producers to upper trophic levels. Similarly, differences in PUFA composition between zooplankton groups and within zooplankton groups due to diet could have important effects on food-web interactions by mediating the availability of those FA molecules that exert the greatest effect on the growth of fish. For example, if zooplanktivorous fish select prey based on EFA content, the present data suggest copepods with high DHA content should be the preferred prey for fish relative to cladocerans. Ballantyne et al. (2003) suggested that the growth of juvenile sockeye salmon (*Oncorhynchus nerka*) in Lake Washington could be limited by low dietary concentrations of DHA when they preferentially feed on *Daphnia*. However, Ballantyne et al. (2003) also pointed out that because sockeye preferentially fed on *Daphnia*, this suggests the energetic costs of converting EPA to DHA are smaller than the foraging cost difference between *Daphnia* and copepods. Agaba et al. (2005) also showed salmonid fish readily convert EPA to DHA.

Conclusions

Comparison of the fatty acid composition of seston and five zooplankton taxa revealed that changes in phytoplankton composition had strong effects on freshwater zooplankton fatty acid composition and that several FAs showed promise as dietary FA biomarkers of copepod feeding. Despite seston control of invertebrate fatty acid content, cladocerans and copepods also preferentially accumulated EPA and DHA, respectively, while the percentage of PUFA in zooplankton increased with trophic level. Our analyses also suggest that diaptomid copepods may upgrade EPA to DHA. Together these results demonstrate that biochemical interactions at lower trophic levels (i.e., phytoplankton, zooplankton) have the potential to affect ecological processes at higher trophic levels (i.e., fish production) as well as food-web ecology. In particular, the bottom-up influences of algal lipid composition on the production of zooplanktivorous fish may, in turn, modify their effect on intermediate trophic levels (e.g., herbivorous zooplankton). The nature of interactions in such tritrophic food webs is probably more a function of biochemistry than classic mathematical relations. We suggest phytoplankton-synthesized long-chain EFAs are keystone molecules which positively affect the nutritional physiology of zooplankton and fish, as well as lead to more favorable biomass distributions in aquatic food webs.

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