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Essential versus potentially toxic dietary substances: A seasonal comparison of essential fatty acids and methyl mercury concentrations in the planktonic food web

Martin Kainz [a,*], Michael T. Arts [b], Asit Mazumder [a]

[a] *Water and Aquatic Sciences Research Program, University of Victoria, Department of Biology, P.O. Box 3020, Stn. CSC, Victoria, BC V8W 3N5, Canada*
[b] *Aquatic Ecosystem Management Research Division, National Water Research Institute, Environment Canada, 867 Lakeshore Road, P.O. Box 505, Burlington, ON L7R 4A6, Canada*

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The structure of planktonic food webs largely regulates the composition and dietary supply of essential fatty acids, while MeHg bioaccumulates with zooplankton size.

Abstract

We investigated seasonal variability of essential fatty acids (EFA) and methyl mercury (MeHg) concentrations in four size categories of planktonic organisms in two coastal lakes. MeHg concentrations increased significantly with increasing plankton size and were independent of plankton taxonomy. However, total EFA increased from seston to mesozooplankton, but decreased in the cladoceran-dominated macrozooplankton size-class. Analysis of EFA patterns revealed that linoleic, alpha-linolenic, arachidonic, and eicosapentaenoic acids increased with increasing zooplankton size, but docosahexaenoic acid (DHA) in the cladoceran-dominated macrozooplankton was generally lower than in seston. This consistent pattern demonstrates that cladocerans, although bioaccumulating MeHg, convey less DHA than similar-sized copepods to their consumers. It is thus evident that fish consuming cladocerans have restricted access to DHA, yet unrestricted dietary access to MeHg. Thus, the structure of planktonic food webs clearly affects the composition of EFA and regulates dietary supply of these essential nutrients, while MeHg bioaccumulates with increasing zooplankton size.

Keywords: Essential fatty acids; Methyl mercury; Zooplankton; Planktonic food webs

1. Introduction

The plant–animal interface plays a crucial ecological as well as ecotoxicological role in aquatic ecosystems because this is where both dietary nutrients and contaminants are transferred into the animal part of the food chain. Primary consumers assimilate organic and inorganic compounds from their diet for their own biosynthesis reactions, somatic growth, reproduction and repair, but they also, inadvertently, incorporate contaminants that do not serve any beneficial physiological purpose.

With respect to essential dietary compounds, a suite of mostly algal-derived polyunsaturated fatty acids (PUFA) have been shown to have significant positive effects on the biochemical quality of the diet for consumers as evidenced by enhanced somatic growth and reproduction of the cornerstone freshwater herbivore *Daphnia galeata* (Müller-Navarra, 1995; Wacker and von Elert, 2001). In contrast to storage lipids that represent the primary source of metabolic energy in zooplankton during periods of low ambient food (Lee et al., 2006), some PUFA are essential constituents of

* Corresponding author at present address: WasserCluster Lunz—Biologische Station, Dr. Carl Kupelwieser Promenade 5, A-3293 Lunz am See, Austria.
E-mail address: martin.kainz@donau-uni.ac.at (M. Kainz).

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eukaryotic membranes (for humans see Holub, 2002) and as such, play a critical role in maintaining the optimal physiological performance of organisms. For example, some dietary omega-3 (ω3)-PUFA compounds increased somatic growth (e.g., eicosapentaenoic acid, EPA: Müller-Navarra et al., 2000; α-linolenic acid, ALA: von Elert, 2002) and clutch sizes of daphnids (Ravet et al., 2003). For fish, it has been demonstrated that ω3-PUFA improve egg quality and larval viability (Bell et al., 1997) as well as reproductive success (Izquierdo et al., 2001; Tocher, 2003). Dietary docosahexaenoic acid (DHA) is the most favorable growth-enhancing dietary nutrient for larval yellowtail flounder (*Limanda ferruginea*; Copeman et al., 2002) and is, generally, the most highly retained PUFA in many freshwater fish (Ahlgren et al., 1994; Kainz et al., 2004). Arachidonic acid (ARA) and possibly its precursor, linoleic acid (LIN), are also important fish nutrients, as dietary ARA affects positively somatic growth, survival, and egg production (Bell and Sargent, 2003). Such evidence suggests that these PUFA must be considered essential FA (EFA) that therefore ought to be highly retained in aquatic organisms.

Such clearly favorable effects of EFA are opposite to the effects of contaminants in aquatic food webs. One of the potentially toxic and ubiquitous contaminants is the neurotoxin methyl mercury (MeHg), which is bioavailable for aquatic organisms and can cause severe health problems including neurological (Bernard et al., 2001; Castoldi et al., 2001) and cardiovascular damage (Yoshizawa et al., 2002) in humans. MeHg, in contrast to non-reactive elemental Hg, biomagnifies (Wiener et al., 2003) along the food chain. To enter the aquatic food chain, MeHg must be first transported across the lipid membrane of unicellular organisms (Morel et al., 1998), which is in contrast to EFA that are mostly synthesized by algae. Understanding MeHg bioaccumulation in organisms at the aquatic plant–animal interface is ecotoxicologically fundamental as MeHg concentrations in zooplankton can increase manifold with respect to MeHg concentrations in their algal diet (Kainz and Mazumder, 2005; Tremblay, 1999). Although there is as yet no evidence that MeHg, at concentrations found in aquatic ecosystems, is toxic to aquatic invertebrates, the planktonic food web plays a key role for MeHg bioaccumulation in organisms at higher trophic levels (Cabana and Rasmussen, 1994; Garcia and Carignan, 2000; Watras et al., 1998), and eventually humans.

In natural aquatic systems, both EFA as mostly structural parts of membrane lipids and typically cytoplasmic MeHg (Pickhardt and Fisher, 2007; Morel et al., 1998) are supplied to consumers through their diet. So far, however, field studies only examined the trophic transfer of EFA and MeHg separately, or at a single sampling time (Kainz and Mazumder, 2005). Thus, there is a need to investigate how EFA and MeHg are conveyed concurrently along the planktonic food web for a longer period of time as the amount of dietary EFA as well as MeHg in zooplankton might be expected to vary, presumably independently from each other, with time of the year, plankton size, and taxonomic composition of the planktonic food web. Examining the role of such ecological factors is fundamental for understanding MeHg accumulation in relation to the type of energy/diet transfer along aquatic food webs.

To address these issues, we designed a field study to test the hypothesis that concentrations of essential dietary nutrients (i.e., EFA) and the potentially toxic substance MeHg increase with increasing plankton size throughout a 6-month sampling period, and that these trends would be independent of the taxonomic composition of the planktonic food web. We grouped lake plankton in different size classes to determine how concentrations of EFA as well as MeHg are affected by increasing plankton size from spring to early fall. The results of this field study: (a) provide detailed information on how essential dietary nutrients (EFA) and the potentially toxic MeHg are associated with increasing body size of zooplankton and its taxonomic composition within the planktonic food web from spring to early fall, and (b) assess the quantity of these dietary substances that are concurrently passed on to planktivorous consumers at higher trophic levels.

2. Materials and methods

Sampling was carried out from spring to early fall (April, June, August, and September 2002) in two monomictic coastal lakes on southern Vancouver Island, British Columbia, Canada. Shawnigan Lake (SHL; 48°37′ N, 123°38′W; mean depth = 14.3 m) is a natural lake used for recreational activities including sport fishing. Sooke Lake (SOL; 48°33′ N, 123°41′ W; mean depth = 23.2 m) is a drinking-water reservoir that undergoes annual water level variations (average of 6 m). The two lakes are morphometrically similar, both have a shallow (Station A) and deep (Station B) basin; their water residence times are 2.0 (SHL) and 1.4 (SOL) years.

To quantify changes in trophic lake status, algal biomass (chlorophyll *a* = Chl-*a*) was measured once a month from April to September. One liter of epi-, meta-, and hypolimnetic lake water was filtered through a Gelman GF/F filter (0.45 μm pore size). The samples were kept frozen (−4 °C) until extraction. Epilimnetic water samples for dissolved organic carbon (DOC) were collected using an acid-rinsed, plastic, syringe equipped with a 0.45 μm pre-filter. These samples were kept at 4 °C in pre-combusted glass vials, without a headspace, until analysis.

We collected zooplankton by towing a conical plankton net (64-μm mesh size) vertically, from just above the bottom to the surface, at each sampling station. The zooplankters were then rinsed with filtered (0.45 μm) lake water to reduce the occurrence of adhered materials including algae and bacteria. Zooplankton were size-fractionated using 100-μm, 200-μm, and 500-μm mesh filter cups for collecting micro- (100–200 μm), meso- (200–500 μm), and macrozooplankton (>500 μm), respectively. These organisms were not further sorted into different taxonomic groups to limit thermal-induced degradation of lipids. Seston was filtered from lake water using an integrated sampling tube (10 m length), filtered through a 64-μm mesh and collected in a 10-μm filter cup. This method was chosen to ensure that all plankton samples could be immediately stored at cryogenic temperatures (−80 °C) and subsequently analyzed under the same conditions. This seston size-fraction (10–64 μm) excluded pico- and smaller nano-plankton and exceeded what is generally accepted to be the most ingestible particle size range (<30 μm) for cladocerans (Burns, 1968). Although plankton <10 μm represent a nutritious fraction of the total available particle size spectrum for herbivorous zooplankton, studies with copepods, feeding on a wide range of particle sizes, reported that the preferred particle size is around 20 μm (Bern, 1994; Vanderploeg et al., 1984). Algal wet biomass of this size-fraction was calculated based on cell counts of each algal class and expressed as a proportion of total cells counted.

Zooplankton were identified, counted, measured and their biomass (per liter body weight of each size class) estimated in a plankton counting wheel using Z-Counts software (Version 2.3, Voila Data Inc., Gloucester, Ontario).

Chl-*a* was extracted from filters using 95% ethanol, followed by spectrophotometer measurements. The pH of epilimnetic water was determined with a VWR Scientific Products model 2000 pH meter. For DOC analysis, triplicate water samples (8 ml) were acidified with HCl (2 N) before measurements in a Shimadzu TOC-5000A analyzer (Shimadzu Corp., Kyoto, Japan). Dissolved oxygen (DO) and temperature profiles of lake water were measured using an YSI model 3800 multisampler (YSI Yellow Springs, OH).

2.1. Lipid and fatty acid analysis

All plankton samples were immediately stored on dry-ice to minimize the risk of lipolytic degradation and kept at −80 °C until lyophilization, lipid extraction and fatty acid methyl ester (FAME) analysis. Lipids from plankton samples (5–10 mg dry weight) were extracted as described elsewhere (Parrish, 1999). Briefly, the samples were sonicated, vortexed and centrifuged four times in a 4:2:1 chloroform/methanol/water mixture, and the organic layers were removed and pooled. Fatty acids (FA) were derivatized to FAME and analyzed by a gas-chromatograph (GC; Varian CP-3800, Varian Inc., Palo Alto, CA) equipped with a flame-ionization detector (FID). FAME were identified by comparison of their retention times with known standards; see Kainz et al. (2004) for further details on FAME formation and analysis.

2.2. Methyl and total mercury analysis

For MeHg (mono-MeHg) analysis, freeze-dried zooplankton (0.5–1 mg) were homogenized and digested in 0.5 ml of a KOH/MeOH (1 g · 4 ml^{-1}) solution for 8 h at 68 °C. Details of this method are given by Pichet et al. (1999). Briefly, MeHg was separated by GC and subsequently quantified using atomic fluorescence spectrometry. The detection limit for this method was 0.6 pg of MeHg and the accuracy of the method was confirmed by analyzing different National Research Council of Canada standards (DORM-1 and TORT-1).

2.3. Data analysis

Parametric tests were used for normally distributed samples. Paired *t*-test analysis was used to test the level of difference for plankton size, taxonomic composition of zooplankton groups, and Chl-*a* concentrations between SHL and SOL, as well as EFA and MeHg concentrations of plankton between the two study lakes (lake effect). We conducted linear regression analysis, using analysis of covariance (ANCOVA) to correct for the effect of sampling station (used as fixed factor), to examine the relationships between plankton size (explanatory variable) and their EFA as well as MeHg concentrations (response variables). Subsequently, we tested for equality of slopes of several regression lines (Sokal and Rohlf, 1995). Because sample biomass changes naturally rather than randomly, non-parametric Kruskal–Wallis tests were used to examine the effect of season (non-random variation) on (a) EFA and MeHg concentrations of the planktonic food web, and (b) the biomass distribution of phytoplankton taxa within the seston size class. The significance level for all statistical analyses was set at $p < 0.05$.

To examine how concentrations of MeHg and EFA changed from April to September with increasing plankton size, we calculated concentration changes, [Δ], of MeHg (ng g^{-1}) and EFA (mg g^{-1}) per µm plankton size increment for the planktonic size spectrum between seston and macrozooplankton, using the following formula:

$$\left[\Delta\right] = \frac{[x]_i - [x]_{ii}}{L_i - L_{ii}},$$

where [*x*] are MeHg or EFA concentrations, and *L* is plankton size (µm) of organisms from macrozooplankton ($_i$) and seston ($_{ii}$) size groups.

3. Results

The mean (± SD) epilimnetic water temperatures were 9.2 °C (± 0.3), 20.7 °C (± 1.0), 19.9 °C (± 0.5), and 20.3 °C (± 0.5) for April, June, August, and September sampling, respectively. The water columns of SHL and SOL were isothermal in April, but stratified at 6 m in June, at 7 m (SHL) and 8 m (SOL) in August, and at 9 m in September. Throughout the sampling season, the lake columns were circumneutral (pH ~7) and well oxygenated (>7.5 mg DO L^{-1}). Both lakes were oligotrophic and mean DOC concentrations were similar throughout the sampling season (<4 mg L^{-1}; Table 1). Chl-*a* concentrations, used as a proxy for algal biomass, were significantly ($p < 0.001$) lower in SOL than SHL (Table 1).

The mean (±SD of samples from the two sampling stations for each lake) length for macrozooplankton was 1119 (± 79) µm (SHL) and 1159 (± 102) µm (SOL). Organisms from the meso- and micro-zooplankton size fraction were larger than the mesh size (i.e., 701 ± 54 and 224 ± 25 µm as well as 713 ± 108 and 239 ± 31 µm at SHL and SOL, respectively) in which they were collected likely because larger, fusiform-shaped, organisms could pass, head-first, through the 500 and 200 µm mesh, respectively. There was

Table 1
Water chemistry measured at sampling stations A and B in Shawnigan (SHL) and Sooke Lake (SOL) between April and September 2002

	T[a] (°C)	pH	Chl-*a*	Secchi depth (m)	DO[a]	DOC[a]
April						
SHL	9.5 ± 0.1	6.5	0.9 ± 0.3	5.5	11.2	2.5
SOL	8.9 ± 0.1	7.2	0.5 ± 0.1	5.5	11.0	2.9 ± 0.4
June						
SHL	21.0 ± 0.2	7.1	1.5 ± 1.3	5.0 ± 1.0	7.6 ± 0.1	3.7
SOL	20.4 ± 0.9	7.1	0.6 ± 0.3	6.5 ± 0.5	7.5	3.0 ± 0.1
August						
SHL	20.2 ± 0.1	6.5	1.3 ± 1.0	7.0	10.8 ± 0.5	3.1 ± 0.1
SOL	19.5 ± 0.3	6.8	0.4 ± 0.3	9.0 ± 1.0	12.0 ± 2.2	2.3 ± 0.1
September						
SHL	20.4 ± 0.1	6.8 ± 0.1	1.2 ± 0.7	7.5	8.2 ± 0.1	2.7
SOL	20.2 ± 0.4	6.8	0.4 ± 0.2	8.0	7.4 ± 0.5	2.3 ± 0.1

T, temperature; Chl-*a*, chlorophyll *a* data are mean values (µg L^{-1}) of epi-, meta-, and hypolimnetic Chl-*a* measurements, ± standard deviation (SD); DO, dissolved oxygen (mg L^{-1}); DOC, dissolved organic carbon (mg L^{-1}).

[a] Epilimnetic values.

no significant difference ($p > 0.3$) in plankton size (>100 μm) between the two sampling stations of each lake and between the two lakes.

The largest size-fraction was comprised of cladocerans including mostly *Daphnia* spp. as well as *Holopedium gibberum* and, to a lesser extent, of calanoid and cyclopoid copepods. However, mesozooplankton consisted mainly of calanoid and cyclopoid copepods, followed by *Daphnia* spp. and *Bosmina longirostris*. In the macrozooplankton size-fraction, cladoceran biomass shares were consistently higher than that of copepods, whereas the reverse was the case in the mesozooplankton size-fraction (Fig. 1). The taxonomic composition of zooplankton (>200 μm; number of animals per zooplankton group) did not vary significantly (cladocerans, $p = 0.6$; copepods, $p = 0.5$) between the two lakes. The microzooplankton size-fraction was mainly composed of small daphnids, copepod nauplii, rotifers (mostly *Keratella* spp.), and some larger phytoplankton. The seston size-fraction contained most of the major classes of freshwater algae including Cryptophyceae, Dinophyceae, Bacillariophyceae, Chrysophyceae, Myxophyceae, and Chlorophyceae; algal class-specific biomass varied significantly ($H = 8.351$, df = 3, $p = 0.04$) during this sampling period.

3.1. EFA and MeHg concentrations

EFA concentrations (mean ± SD) increased from seston (10.7 ± 2.9 and 10.7 ± 2.6 mg g^{-1}) to mesozooplankton (28.3 ± 5.1 and 26.6 ± 6.7 mg g^{-1}), and decreased slightly in macrozooplankton (26.2 ± 4.8 and 25.1 ± 4.6 mg g^{-1}) at SHL and SOL, respectively. There was no significant effect of sampling periods (non-random factor) on the variability of total EFA concentrations ($H = 0.938$, df = 3, $p = 0.8$) or concentrations of LIN ($H = 3.994$, $p = 0.2$), ALA ($H = 4.814$, $p = 0.2$), ARA ($H = 3.935$, $p = 0.3$), EPA ($H = 1.262$, $p = 0.7$), and DHA ($H = 0.895$, $p = 0.8$). In macrozooplankton, there was no significant difference of either total EFA or individual EFA concentrations between SHL and SOL. Because the variance of EFA compound concentrations increased with increasing plankton size (μm), we log-transformed the concentrations of LIN, ALA, ARA, EPA, and DHA (dependent variables) and plankton size (explanatory variable). Log-concentrations of the following EFA were significantly ($p < 0.05$) and linearly correlated with plankton size with no significant effect of lake type ($p > 0.1$; Fig. 2): LIN ($R^2 = 0.36$, $F = 18.8$), ALA ($R^2 = 0.45$, $F = 24.1$), ARA ($R^2 = 0.78$, $F = 110.1$), EPA ($R^2 = 0.68$, $F = 67.3$). However, DHA concentrations in the planktonic food web were not significantly related with increasing plankton size ($R^2 = 0.03$, $F = 0.08$, $p = 0.9$), but were highest in mesozooplankton.

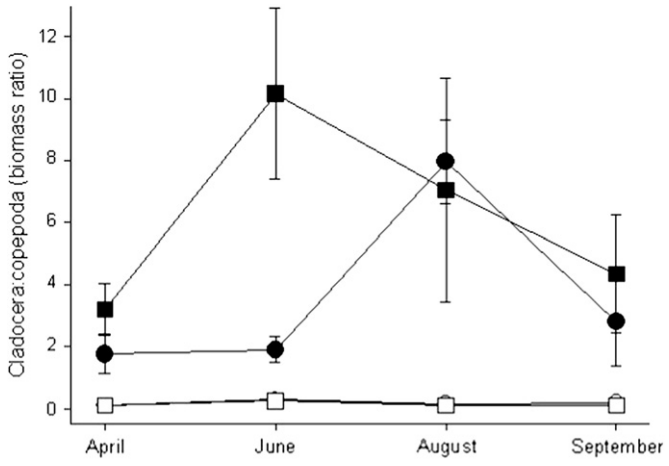

Fig. 1. Seasonal changes of macro- (open symbols) and mesozooplankton (dark symbols) biomass ratios (mean ± SD) between cladocerans (*Bosmina longirostris*; *Holopedium gibberum*; *Daphnia* spp.) and copepods (calanoids, cyclopoids, copepodites, nauplii) of Shawnigan Lake (SHL; boxes) and Sooke Lake (SOL; circles).

Methyl Hg concentrations increased significantly with increasing plankton size, i.e., from seston (4–28 ng g^{-1}) to macrozooplankton (71–218 ng g^{-1}). Due to the increasing variance of MeHg concentrations with increasing plankton size, MeHg concentrations and plankton size were log-transformed resulting in a significant ability to predict log-MeHg concentrations from plankton size ($R^2 = 0.80$; $F = 123.2$, $p < 0.0001$; Fig 3). There was no significant difference ($p = 0.3$) in MeHg concentrations of the planktonic food web between the two study lakes and different sampling periods had no significant effect on the variability of MeHg concentrations ($H = 3.474$, df = 3, $p = 0.3$). When only testing differences of MeHg concentrations in macrozooplankton, the preferred diet size for planktivorous fish (Brooks and Dodson, 1965), MeHg concentrations were significantly higher at SOL (155 ± 52 ng g^{-1}) than at SHL (105 ± 14 ng g^{-1}; $p = 0.032$).

The increase of MeHg concentrations per μm size increment from seston to macrozooplankton was significantly ($p = 0.034$) higher at SOL (0.133 ± 0.05 ng g^{-1}) than at SHL (0.088 ± 0.01 ng g^{-1}; Fig. 3). Time of sampling (spring, summer, and early fall) did not significantly affect the MeHg increase per μm plankton size increment at SHL ($H = 4.667$, $p = 0.2$) and SOL ($H = 4.000$, $p = 0.3$). Increases of EFA concentrations per μm size increment were highest during spring sampling (23.9 ± 7.1 μg g^{-1}, April), followed by 14.1 ± 1.7 μg g^{-1} (June), 9.3 ± 1.8 μg g^{-1} (August), and 9.3 ± 4.3 μg g^{-1} (September). There was no significant difference in concentration changes of EFA ($p = 0.6$), LIN ($p = 0.4$), ALA ($p = 0.8$), ARA ($p = 0.6$), EPA ($p = 0.4$), and DHA ($p = 0.3$) from seston to macrozooplankton between SHL and SOL. Increases of EFA compound concentrations per μm size increment were highest for EPA, followed by ARA (except in April), whereas DHA concentrations decreased consistently per μm size increment in both lakes (Fig. 4). No consistent pattern was observed for LIN and ALA concentration changes.

The slopes of regression lines (Table 2) between plankton size and concentrations of MeHg and ARA were not significantly different ($p = 0.28$), whereas slopes of all other EFA were significantly flatter ($p < 0.0001$) than the slope between plankton size and MeHg concentrations.

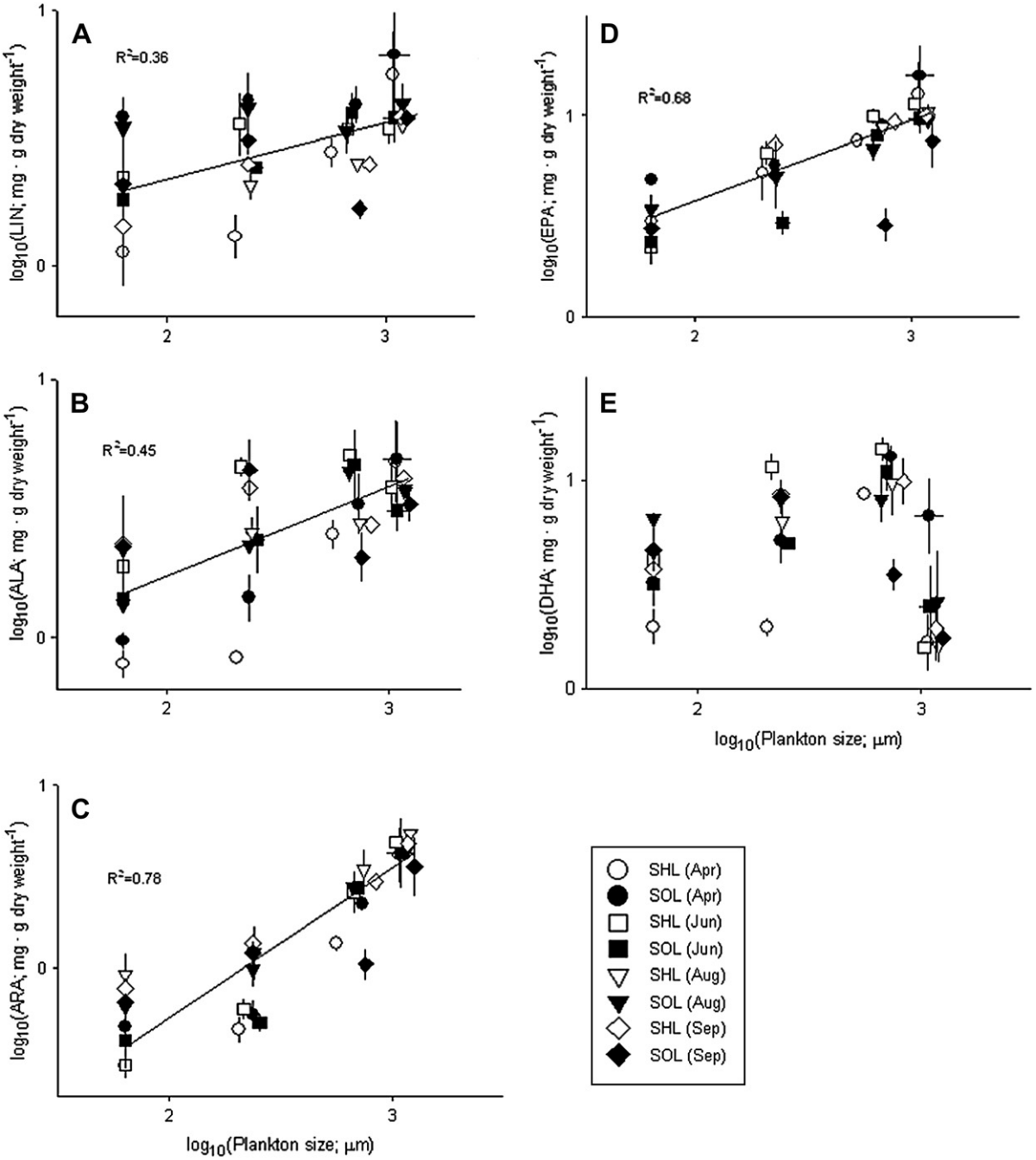

Fig. 2. Variation of log-transformed: (A) linoleic, LIN; (B) α-linolenic, ALA; (C) arachidonic, ARA; (D) eicosapentaenoic, EPA; and (E) docosahexaenoic acid, DHA, in log-transformed seston (10–64 μm), micro- (100–200 μm), meso- (200–500 μm) and macro- (>500 μm) zooplankton size-classes of Shawnigan Lake and Sooke Lake. Variation of LIN, ALA, ARA, and EPA concentrations were significantly ($p < 0.05$) predicted by plankton size.

4. Discussion

This field study demonstrates that EFA concentrations in lacustrine macrozooplankton, the preferred diet size for planktivorous fish, are on average 3.8×10^5, 1.7×10^5, 1.8×10^5 and 1.5×10^5 fold higher than MeHg concentrations in April, June, August and September, respectively. The higher concentration differences in spring than in early fall clearly show that macrozooplankton in spring, from a nutritional point of view, are more desirable for consumers than later in the season as they contain higher EFA relative to MeHg concentrations. Although such mostly algal-derived EFA in zooplankton can trigger enhanced somatic growth as shown in laboratory-raised daphnids (e.g., Müller-Navarra et al., 2000), it is important to note that these field data do not provide unequivocal evidence that EFA concentrations in zooplankton may functionally affect MeHg concentrations in consumers, as previously demonstrated by MeHg biodilution in consumers through increased algal biomass (e.g., Pickhardt et al., 2002). Our results demonstrate that the retention of dietary essential nutrients

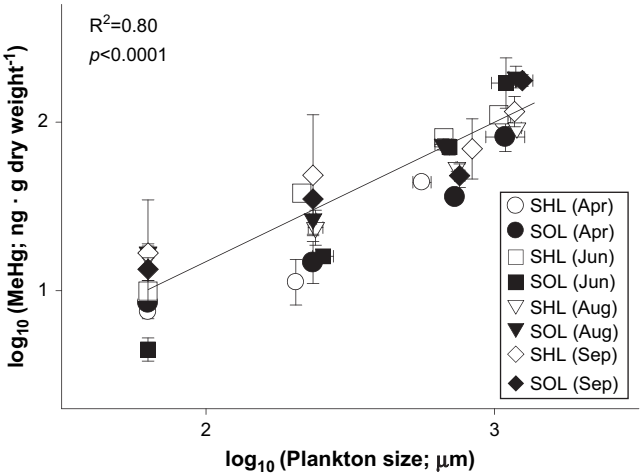

Fig. 3. Variation of log-transformed MeHg concentrations in log-transformed seston (10–64 µm), micro- (100–200 µm), meso- (200–500 µm) and macro- (>500 µm) zooplankton of Shawnigan Lake and Sooke Lake.

(EFA) differs with the taxonomic zooplankton composition, whereas bioaccumulation of MeHg concentrations was clearly related to increasing plankton size in both lakes over this 6-month sampling period.

The increase of total EFA concentrations from seston to macrozooplankton in both lakes indicates that these dietary nutrients are retained and presumably required throughout the sampling period. However, the increase of total EFA concentrations per µm size increment was higher during spring (22.6 and 25.3 µg g^{-1} at SHL and SOL, respectively) than later in the season. Such high EFA retention was not related to higher algal biomass, as suggested by lower Chl-*a* concentrations in April than at later sampling dates at SHL, implying that bulk algal biomass cannot be used as a proxy for the resultant EFA concentrations in zooplankton.

The consistent increase of MeHg concentrations with increasing body size of zooplankton confirms that MeHg bioaccumulates throughout this sampling period. Because the concentration difference of MeHg in the aquatic food web is greatest between water and algae (Mason et al., 1995), we argue that MeHg in zooplankton is mostly obtained from their algal diet, but is not directly related to total algal biomass. Significantly higher MeHg concentrations of macrozooplankton in SOL than in SHL are not related to their taxonomic biomass share (cladocerans vs. copepods) as there was no significant taxonomic difference in the macrozooplankton assemblage between the two lakes. Moreover, we suggest that the higher MeHg concentrations of zooplankton in SOL were too low to exert any toxic effect on the ability of zooplankton to retain EFA concentrations as there was no significant difference of EFA concentrations in zooplankton between SOL and SHL.

Concentrations of the 18-carbon EFA (LIN and ALA) and 20-carbon EFA (ARA and EPA) increase significantly with plankton size demonstrating that these dietary nutrients are increasingly retained in larger organisms and that this effect is evident throughout the sampling period in both lakes. LIN and ALA serve as precursors for longer chain ω-6 FA and ω-3 FA such as ARA (Stanley-Samuelson, 1994) and EPA (Cook and McMaster, 2002), respectively. However, because LIN and ALA concentrations increase from seston to macrozooplankton, these EFA may also be required to optimize zooplankton performance, including increased somatic growth, as has been recently demonstrated for laboratory-raised *D. galeata* (von Elert, 2002). When compared to the consistent increase of MeHg concentrations with increasing plankton size, LIN and ALA concentrations only increase consistently from seston to microzooplankton. Significantly flatter slopes of regressions lines between plankton size vs. LIN and ALA concentrations than between plankton size vs. MeHg concentrations reveal significantly different bioaccumulation patterns between MeHg and these 18-carbon EFA along the planktonic food web ($p < 0.0001$; Table 2). Because the mesozooplankton size class is mainly comprised of copepods, whereas there are mostly cladocerans in macrozooplankton, we argue that the inconsistent patterns of LIN and ALA concentrations are not attributable to particular zooplankton taxa.

EPA concentrations increased more efficiently than any other EFA per µm size increment of planktonic organisms, indicating that this mostly dietary algal-derived nutrient is highly required regardless of zooplankton size and taxonomic composition. Because the mesozooplankton size class was predominantly comprised of copepods, whereas cladocerans were the major taxa (numerically and in terms of biomass) contributing to the macrozooplankton group, we propose that EPA is efficiently retained and MeHg bioaccumulated in zooplankton regardless of taxonomic composition.

EPA and ARA are essential precursors for prostaglandin formation and are required for cellular signal transduction (Smith and Murphy, 2002). Both ARA and MeHg bioaccumulate consistently from seston to macrozooplankton, regardless of sampling period and taxonomic composition of the planktonic food web. The slopes of the regression lines between plankton size vs. ARA and MeHg concentrations did not differ significantly from each other, suggesting that ARA retention and MeHg bioaccumulation may be related in planktonic organisms. Although it is as yet not understood whether there is any functional relationship between such consistent ARA and MeHg bioaccumulation, the ability of zooplankton to retain ARA suggests that this ω-6 FA is required (perhaps more than previously assumed) for zooplankton. Our understanding of the physiological role of ARA in zooplankton, however, is only beginning to emerge. Dietary ARA appears to exert different somatic growth effects on laboratory-raised daphnids. For example, von Elert (2002) reported little somatic growth effects of dietary ARA on *D. galeata*, whereas the addition of ARA to the diet enhanced somatic growth of *D. magna* (Becker and Boersma, 2005).

Concentrations of DHA decrease in cladoceran-dominated macrozooplankton compared to copepod dominated mesozooplankton in both lakes during the entire sampling period, which is in clear contrast to patterns observed for MeHg. DHA concentrations are clearly related to the taxonomic composition of the planktonic food web. In both lakes, DHA concentrations increased consistently from seston via

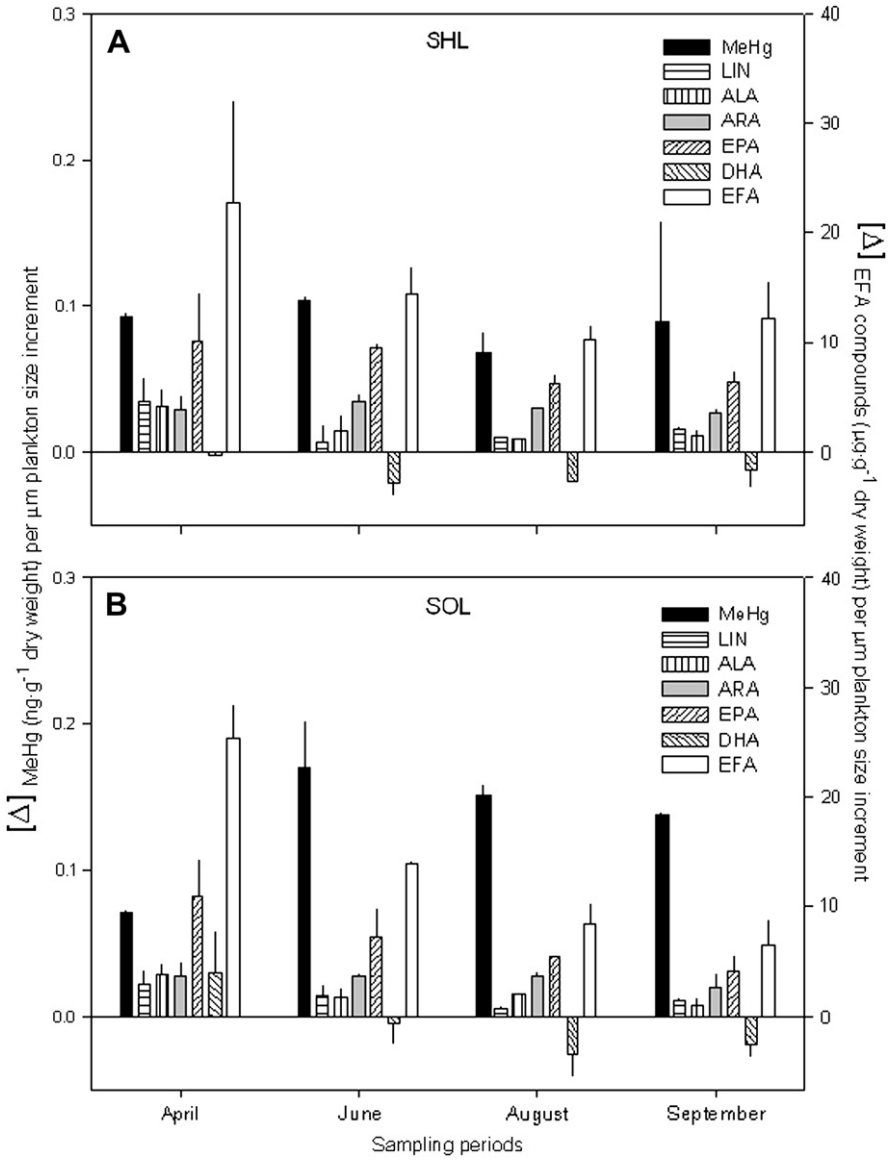

Fig. 4. Concentration changes (mean ± SD) of MeHg, total EFA and individual EFA compounds (i.e., linoleic, LIN; α-linolenic, ALA; arachidonic, ARA; eicosapentaenoic, EPA; and docosahexaenoic, DHA, acids) per µm plankton size increment between seston to macrozooplankton at (A) Shawnigan Lake (SHL) and (B) Sooke Lake (SOL).

microplankton (with exception of April sampling at SHL) to the copepod-dominated mesozooplankton, but decreased sharply toward the clearly cladocerans-dominated macrozooplankton size class throughout the sampling period. Based on DHA concentration changes between seston and macrozooplankton, it is evident that there is a net loss of DHA concentrations in the cladoceran-dominated macrozooplankton when compared to DHA concentrations in seston. Because these size-related taxonomic differences are consistent throughout the sampling period in both lakes, we argue that this sampling period from spring to early fall has little effect on Cladocera's inability or inefficiency to retain dietary DHA. These results are consistent over the entire sampling period and provide therefore additional support for an earlier single-point-in-time field study that reported low DHA concentrations in cladocerans (Kainz et al., 2004). Although it is not clearly understood as to why cladocerans don't retain dietary DHA, it has been shown that DHA increase egg production and egg hatching success of copepods (Arendt et al., 2005). It is thus evident that the taxonomic composition of these zooplankton, in particular macrozooplankton, affects the rate at which DHA, but not MeHg, is conveyed to planktonic consumers at higher trophic levels.

In comparison with seasonal bioaccumulation patterns of MeHg it is clear that dietary ARA and EPA, and in the case of copepod-dominated systems also DHA, are highly retained. Although further studies are needed to understand why zooplankton have different abilities to retain these EFA, yet have little or no taxonomic preference for MeHg bioaccumulation, we demonstrate that cladoceran-dominated macrozooplankton are the most efficient size class in terms of conveying ARA, EPA, and MeHg to organisms at higher

Table 2
Slopes, standard error (SE) of slopes, intercepts, and SE of intercepts of linear log–log regression models of plankton size (independent variable) and concentrations of MeHg and fatty acids

	Slopes	Slopes (SE)	Intercepts	Intercepts (SE)
MeHg	0.8121	0.0515	−0.477	0.131
LIN	0.2089	0.0399	0.064	0.109
ALA	0.3154	0.0456	−0.372	0.116
ARA	0.7364	0.0496	−1.700	0.127
EPA	0.4101	0.0356	−0.258	0.093
DHA	−0.0323	0.0796	0.748	0.203

LIN, linoleic acid; ALA, α-linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

trophic levels. It is thus evident that fish consuming cladocerans have restricted dietary access to essential DHA, their mostly retained EFA, yet taxonomically unregulated dietary access to bioaccumulated MeHg of zooplankton. The ability of algal-derived EFA, EPA in particular, to enhance somatic growth of key herbivores (e.g., Müller-Navarra et al., 2000) opens further research perspectives that may investigate the effect of increasing dietary EFA supply (biochemical algal quality) on reducing MeHg concentrations per unit biomass of zooplankton and other aquatic consumers. Such diet quality effect, as also recently shown for low C:P ratios in green algae lowering MeHg bioaccumulation patterns in *Daphnia pulex* (Karimi et al., 2007), would contrast the algal biomass-induced biodilution of MeHg (effect of algal quantity; Pickhardt et al., 2002).

Although the term ‘‘essential’’ is used for these dietary fatty acids, it must be recognized that these highly beneficial dietary nutrients are subject to various metabolic processes that are regulated in accordance with taxa-specific physiological requirements and intrinsic biosynthetic and catabolic abilities. Results of this field study demonstrate that MeHg bioaccumulation along the planktonic food web is determined by plankton size, whereas the ability of zooplankton to regulate the retention of dietary EFA may result, from a risk-benefit point of view, in a relative smaller EFA than MeHg diet pool for consumers, particularly in the case of DHA-poor cladoceran-dominated lakes. Finally, we conclude that the structure of planktonic food webs clearly affects the composition and thus dietary supply of essential nutrients for planktivorous consumers, whereas this study provides evidence that MeHg bioaccumulation is predicted by increasing plankton size during the seasons examined.

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