

# Bioaccumulation patterns of methyl mercury and essential fatty acids in lacustrine planktonic food webs and fish<sup>☆</sup>

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## Abstract

Organisms of the planktonic food web convey essential nutrients as well as contaminants to animals at higher trophic levels. We measured concentrations of methyl mercury (MeHg) and essential fatty acids (EFAs, key nutrients for aquatic food webs) in four size categories of planktonic organisms – seston (10–64  $\mu\text{m}$ ), micro-(100–200  $\mu\text{m}$ ), meso-(200–500  $\mu\text{m}$ ), and macrozooplankton (>500  $\mu\text{m}$ ) – as well as total mercury (THg) and EFAs in rainbow trout (*Oncorhynchus mykiss*) in coastal lakes. We demonstrate that, in all lakes during this summer sampling, MeHg concentrations of planktonic organisms increase significantly with plankton size, independent of their taxonomic composition, and that their MeHg accumulation patterns predict significantly THg concentrations in rainbow trout ( $R^2=0.71$ ,  $p<0.05$ ). However, concentrations of total EFAs do not follow this pattern. Total EFAs increased from seston to mesozooplankton but decreased in the largest zooplankton size fraction. Moreover, concentrations of individual EFA compounds in rainbow trout are consistently lower, with the exception of docosahexaenoic acid, than those in macrozooplankton. The continuous increase of MeHg concentrations in aquatic organisms, therefore, differs from patterns of EFA accumulation in zooplankton and fish. We interpret these contrasting accumulation patterns of MeHg and EFA compounds as the inability of aquatic organisms to regulate the assimilation of dietary MeHg, whereas the rate of EFA retention may be controlled to optimize their physiological performance. Therefore, we conclude that bioaccumulation patterns of Hg in these aquatic food webs are not controlled by lipid solubility and/or the retention of EFA compounds.

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**Keywords:** Methyl mercury; Essential fatty acids; Planktonic food web; Rainbow trout

## 1. Introduction

The planktonic food web plays an important ecological and ecotoxicological role as it conveys nutrients as well as contaminants to organisms at higher trophic levels. Such plankton-derived nutrients include fatty acids (FAs), in particular some polyunsaturated FA

(PUFAs), which play an important role for providing metabolic energy for somatic development and reproduction of daphnids (Müller-Navarra et al., 2000) and fish (Copeman et al., 2002). Besides essential nutrients, organisms of the planktonic food web bioaccumulate the powerful neurotoxin methyl mercury (MeHg; Kainz et al., 2002), which is consequently taken up by phagotrophic organisms at higher trophic levels (e.g., Cabana and Rasmussen, 1994). However, accumulation patterns of MeHg and essential nutrients within the planktonic food web and in fish are not well understood and warrant further investigation and comparison.

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Besides proteins, lipids and their constituent FAs are one of the major organic components of fish. Although there is laboratory evidence that some fish can synthesize saturated FAs de novo (and perhaps some mono-unsaturated FAs) from lipid and non-lipid precursors (e.g., Lin et al., 1977), it has been suggested that most PUFAs in fish have to be obtained preformed from the diet (Henderson, 1996). Similarly, it is assumed that most FA in daphnids (Goulden and Place, 1990), and perhaps also in other zooplankton, are dietary in origin. Within the dietary lipid pool, some PUFAs have been identified as essential nutrients because they have structural roles in phospholipids of all cells, promoting membrane viscosity and permeability (e.g., Spector, 1999), and because they cannot be fully synthesized by phagotrophic organisms to meet their physiological requirements. Results from laboratory tests suggest that physiological benefits of such dietary essential FA (EFAs) include enhanced somatic development and reproduction of zooplankton (e.g., Becker and Boersma, 2003; Wacker and von Elert, 2001; Müller-Navarra et al., 2000) and fish (Ballantyne et al., 2003; Tocher, 2003). Moreover, some EFAs are involved in the regulation of hormonal processes in fish (Bell et al., 1991). Most of the EFAs in the aquatic food web are synthesized by algae (Wainman et al., 1999) and subsequently transferred to phagotrophic organisms at higher trophic levels (Kainz et al., 2004). In this paper, we use the following PUFAs as EFAs: linoleic (LIN; C18:2 $\omega$ 6),  $\alpha$ -linolenic (ALA; C18:3 $\omega$ 3), arachidonic (ARA; C20:4 $\omega$ 6), eicosapentaenoic (EPA; C20:5 $\omega$ 3), and docosahexaenoic (DHA; C22:6 $\omega$ 3) acid.

The dietary uptake of EFAs and MeHg by zooplankton and fish is likely a concurrent process because several lines of evidence suggest that diet conveys MeHg to zooplankton (Montgomery et al., 2000; Paterson et al., 1998; Watras and Bloom, 1992) and fish (Hall et al., 1997). MeHg in lake zooplankton bioaccumulates as MeHg concentrations increase with body size of zooplankton (Masson and Tremblay, 2003; Kainz et al., 2002). As a result, macrozooplankton, the preferred prey size for planktivorous fish (Brooks and Dodson, 1965), transfers the highest MeHg concentrations from the planktonic food web to planktivorous fish. At the same time, macrozooplankton should also provide the required amount of essential nutrients for optimal somatic development of planktivorous fish. Consequently, the amount of MeHg and EFAs taken up by planktivorous fish depends on how efficiently MeHg and EFAs, in particular growth-enhancing EFAs, are accumulated in organisms of the planktonic food web.

Our first objective of this study is to examine accumulation patterns of MeHg and EFAs in organisms of the planktonic food web. We test the hypothesis that accumulations of dietary MeHg and EFA compounds co-vary in organisms of the planktonic food web. Secondly, we examine the effect of planktonic MeHg and EFA concentrations on higher trophic levels. As it has been suggested that diet is the major source of total Hg (THg; presumably mostly in its methylated form; Spry and Wiener, 1991) and FAs to fish, we hypothesize that MeHg and EFA concentrations in macrozooplankton can predict THg and EFA concentrations in planktivorous fish. To test these hypotheses we conducted a field study and analyzed MeHg and EFA concentrations in different size classes of planktonic organisms and in rainbow trout (*Oncorhynchus mykiss*, a widespread species in Canadian lakes and rivers) from coastal lakes of British Columbia.

## 2. Materials and methods

The study was conducted in June 2002 in six monomictic coastal lake systems on southern Vancouver Island, British Columbia, Canada. Shawnigan Lake (SHL; N48° 37', W123° 38') and Elk Lake (ELL; N48° 31', W123° 23') are natural lakes used for recreational activities including sport fishing. Council Lake (COL; N48° 31', W123° 40'), Sooke Reservoir (SOL; N48° 33', W123° 41'), Goldstream Reservoir (GOL; N48° 30', W123° 38'), and Butchard Reservoir (BUL; N48° 32', W123° 39') are located in the protected Capital Regional District watershed area. SOL, GOL and BUL are drinking-water reservoirs. The lake morphometries of SHL and SOL are very similar and both lakes have a shallow (-A) and a deep (-B) basin. Some physico-chemical characteristics of these lakes are listed in Table 1.

Zooplankton samples were collected vertically at the deepest lake stations or deepest spots of lake basins using a 64  $\mu$ m net. The organisms were rinsed with filtered (0.45  $\mu$ m) lake water to remove adhered matter and size fractionated using 100-, 200-, and 500  $\mu$ m meshes. For seston, lake water was sampled using an integrated sampling tube (10 m length), filtered through a 64- $\mu$ m mesh and collected in a 10  $\mu$ m mesh-size filter cup. Size-separated zooplankton organisms and seston were transferred in polypropylene vials and immediately put on dry ice. All samples were kept frozen at  $-80^{\circ}\text{C}$  until lyophilization, then again stored at  $-80^{\circ}\text{C}$  until analysis.

For fish sampling, we have chosen rainbow trout (*O. mykiss*) as a model fish species for THg and EFA

Table 1

Water chemistry measured in natural lakes and drinking-water reservoirs of southern Vancouver Island, British Columbia. DO = dissolved oxygen; DOC = dissolved organic carbon; Chl-*a* = chlorophyll *a* data are mean values of epi-, meta-, and hypolimnetic chl-*a* values,  $\pm$  standard deviation (SD)

Station	Station depth (m)	Secchi depth (m)	DO <sup>a</sup> (mg · L <sup>-1</sup> )	Chl- <i>a</i> mean $\pm$ SD ( $\mu$ g · L <sup>-1</sup> )	DOC (mg · L <sup>-1</sup> )
<i>Natural lakes</i>					
SHL-A	24.0	4.5	7.9	1.7 $\pm$ 0.5	3.7
SHL-B	49.0	6.0	7.6	1.3 $\pm$ 1.2	3.7
COL	20.0	7.0	7.3	1.0 $\pm$ 0.3	2.5
ELL <sup>b</sup>	13.0	4.5	9.1	3.2 $\pm$ 0.6	6.0
<i>Reservoirs</i>					
SOL-A	15.5	6.5	7.5	0.5 $\pm$ 0.2	2.9
SOL-B	67.0	7.0	7.6	0.7 $\pm$ 0.3	3.1
GOL	28.0	7.5	8.5	0.4 $\pm$ 0.2	2.4
BUL	41.0	8.0	6.7	0.5 $\pm$ 0.4	2.4

<sup>a</sup> Epilimnetic values.

<sup>b</sup> ELL turned anoxic below 9 m water depth.

analysis because it is widespread in Canadian lakes and rivers and also a target species for sport fishing. Fish were collected using gillnets, but only dorsal muscle samples of fish of similar length (25.6  $\pm$  2.0 cm) and weight (182  $\pm$  37 g wet weight) from SHL-A ( $n=1$ ), SHL-B ( $n=3$ ), SOL-A ( $n=2$ ), SOL-B ( $n=3$ ), and ELL ( $n=3$ ) were used for THg and lipid analysis, so that our results would not be biased by size or weight. Results of gut analysis indicated that these rainbow trout were planktivores (R. McMackin, personal communication).

Sampling and analytical methods for chl-*a*, dissolved organic carbon (DOC), and pH have been described previously (Kainz et al., 2004).

### 3. Analyses

#### 3.1. Zooplankton classification

Zooplankton were transferred to a counting wheel for identification, enumeration and measurement as well as subsequent biomass estimation under a microscope using Z-Counts<sup>®</sup> software (Version 2.3, Voila Data Inc., Gloucester, Ontario). Dissolved oxygen (DO) and temperature profiles were measured using an YSI Model 3800 multisampler (YSI Yellow Springs, Ohio).

#### 3.2. Lipid and fatty acid analysis

Lipids from homogenized, freeze-dried zooplankton samples (5–10 mg) and dorsal muscle samples of rain-

bow trout (25–35 mg) were extracted as described by Parrish (1999). Briefly, the samples were sonicated and vortexed 4 $\times$  in a 4:2:1 chloroform–methanol–water mixture and the organic layers were removed and pooled. Total lipid concentrations were determined gravimetrically after a subsample of the lipid extract had evaporated.

FAs were analyzed as methyl esters (FAME) using a gas-chromatograph (GC; Varian CP-3800, Varian, Inc., Palo Alto, Calif.). The methyl esters were prepared by transesterifying the lipid extract in BF<sub>3</sub>/CH<sub>3</sub>OH (for details on FAME formation, see Kainz et al., 2002). FAME were analyzed on a 2560 Capillary Column (100 m, 0.25 mm i.d., 0.2  $\mu$ m film thickness; Supelco<sup>™</sup> (Sigma-Aldrich), Inc., Bellefonte, Pa.). Helium was used as the carrier gas (1 mL min<sup>-1</sup> flow rate). The following temperature ramp was employed: 65 °C for 0.5 min, hold at 195 °C for 15 min after ramping at 40 °C min<sup>-1</sup>, and hold at 240 °C for 10 min after ramping at 2 °C min<sup>-1</sup>. Detection was by flame ionization (FID). Helium (make-up gas) and air (combustion) had flow rates of 30 and 300 mL min<sup>-1</sup>, respectively. The FID was isothermal at 260 °C, whereas the injector was programmed to rise to 250 °C at a rate of 200 °C min<sup>-1</sup> after holding at 150 °C for 0.5 min. FAME were identified by comparison of retention times with known standards (37-component FAME mix, Supelco<sup>™</sup>). Quantification of individual FAME components was calculated on the basis of known amounts of injected standard dilutions (2000, 1000, 500, 250, 100, 50, and 2.5 ng  $\mu$ L<sup>-1</sup>).

#### 3.3. Methyl and total mercury analysis

For MeHg analysis, each freeze-dried zooplankton fraction was ground to a powder using a glass rod. 0.5–1 mg (DW) of a sample was digested in 0.5 mL of a KOH/MeOH (1 g 4 mL<sup>-1</sup>) solution for 8 h at 68 °C as described by Pichet et al. (1999). MeHg was separated by GC and then quantified using atomic fluorescence spectrometry. Each sample was analyzed 3 $\times$  and mean concentrations ( $\pm$  SD) were reported. The detection limit for this method was about 0.6 pg of MeHg, which corresponds to 0.3 ppb for a typical 2 mg sample; the accuracy of this method was positively tested by analyzing different National Research Council of Canada standards (DORM-1 and TORT-1).

For THg analysis, freeze-dried dorsal fish samples (50 mg) were homogenized, digested in HCl (3 parts) and HNO<sub>3</sub><sup>-</sup> (1 part); to ensure that all Hg is converted into and kept in the non-volatile oxidized state (Hg<sup>2+</sup>), an additional oxidant of 0.01% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was added to

the solution. Subsequently, the digestion tubes (polypropylene Falcon centrifuge tubes) were loosely capped until the reaction died down (usually 5 h), then tightly capped and vortexed (1 min). Thereafter, the caps were loosened to release pressure and the tubes were placed in a water bath (80 °C for 5 h). The digest was then diluted (50 mL final volume and weighed) using 0.01%  $K_2Cr_2O_7$  in de-ionized water. Finally, digests were vortexed (1 min) and centrifuged (10 min) to separate the solution from any residue. Each sample was analyzed 3× by Cold Vapour Atomic Fluorescence Spectrometry (CV-AFS) using a PS Analytical Millennium Merlin/Galahad Mercury Analyzer. Calibration standards were made from  $HgCl_2$  and diluted in 5% Aqua-Regia with 0.01%  $K_2Cr_2O_7$ . Precision and accuracy were tested by repeat analysis of standards (NRC Dog Fish Liver Tissue standard, DOLT; Institute for Reference Materials and measurements BCR-CRM 422 Cod Muscle; and IAEA-405 Estuarine Sediment). Blank runs contained no detectable Hg concentrations. The limit of detection (LOD) for the method was 0.05 ng g dry weight<sup>-1</sup> and the instrumental LOD was ~5 pg Hg.

## 4. Results

### 4.1. Lake and reservoir characteristics

The mean ( $\pm$  SD) epilimnetic water temperature was 21 °C ( $\pm$  1) and all lakes and reservoirs were thermally stratified (thermocline started on average at 6 m depth). The water columns were generally well oxygenated ( $>2$  mg DO L<sup>-1</sup>, however, ELL turned anoxic below 9 m). pH values at all lake systems were ~7 and mean chl-*a* concentrations in reservoirs ( $<1$   $\mu$ g L<sup>-1</sup>) were lower than those of many natural lakes ( $2.1$   $\mu$ g L<sup>-1</sup>  $\pm$  1.1; Table 1).

### 4.2. Size fraction and taxonomic composition of plankton

The mean ( $\pm$  SD) body length of macrozooplankton was 1144  $\mu$ m ( $\pm$  172) and 1102  $\mu$ m ( $\pm$  228) for natural lakes and reservoirs, respectively. Although meso- and micro-zooplankton were collected using mesh sizes between 200 and 500  $\mu$ m and 100 and 200  $\mu$ m, respectively, their average body lengths were larger (i.e.,  $633 \pm 104$   $\mu$ m and  $205 \pm 10$   $\mu$ m;  $623 \pm 142$   $\mu$ m and  $237 \pm 21$   $\mu$ m for natural lakes and reservoirs, respectively) probably because body flexibility allowed them to pass through smaller mesh sizes. There were no significant differences in body size of zooplankton in

any size fractions between natural lakes and reservoirs (paired *t*-Test;  $p > 0.05$ ). The taxonomic composition of seston (10–64  $\mu$ m) was not verified microscopically.

Macrozooplankton was mainly comprised of Calanoid copepods and *Daphnia* spp., and the cladoceran *Holopedium gibberum* was also collected at COL, SHL-A and -B, GOL, BUL, and SOL-A. The mesozooplankton size fraction consisted of Calanoid and cyclopoid (missing at ELL) copepods, *Daphnia* spp., and copepod nauplii. Organisms in the microplanktonic size fraction were composed of copepod nauplii, *Keratella* spp., and phytoplankton (*Asterionella formosa*, *Tabellaria fenestrata* et *T. flocculosa*, *Cyclotella* spp., *Ceratium hirundinella*, and *Dinobryon divergens*). The seston size-fraction was mainly comprised of algae (e.g., *A. formosa*, *T. fenestrata*, and *Chryso-sphaerella longispina*). The taxonomic composition of macro- and mesozooplankton, as tested by hierarchic cluster analyses (see Kainz et al., 2004), were similar among the lakes. However, the biomass shares of these size fractions differed; the biomass of the macrozooplankton size class was highest for *H. gibberum* and *Daphnia* spp., and for mesozooplankton, biomass shares differed among the lakes with the exception of COL, at which *H. gibberum* clearly dominated (Fig. 1).

### 4.3. MeHg and EFAs in zooplankton

MeHg concentrations (ng g dry weight<sup>-1</sup>) increased from seston ( $13 \pm 15$ ), to micro- ( $24 \pm 14$ ), to meso- ( $63 \pm 28$ ), to macrozooplankton ( $142 \pm 55$ ; Fig. 2). In macrozooplankton, MeHg concentrations were lowest in mesotrophic ELL ( $94 \pm 2$ ) and highest in oligotrophic GOL ( $240 \pm 19$ ). Variations of the measured plankton size fractions could significantly predict MeHg concentrations in all study lakes ( $R^2 = 0.81$ ,  $p < 0.0001$ ; ANCOVA to correct for the effect of sampling station). However, there was no significant correlative evidence ( $p > 0.05$ ; ANOVA) that biomass of different zooplankton genera could predict MeHg concentrations of the meso- and macrozooplankton size classes. MeHg bioaccumulation factors increased with increasing size fractions (from seston to macrozooplankton) of planktonic organisms and were smallest at SOL-A (5.0) and highest at GOL (54.4). Using linear correlation analysis, MeHg of each size-fraction of the planktonic food web was not significantly ( $p > 0.1$ ) related with pH, DOC, and chl-*a* of lake water. However, these parameters were somewhat invariant across the lakes.

Total lipid concentrations (mg g dry weight<sup>-1</sup>) increased significantly from seston ( $120 \pm 36$ ) to micro-

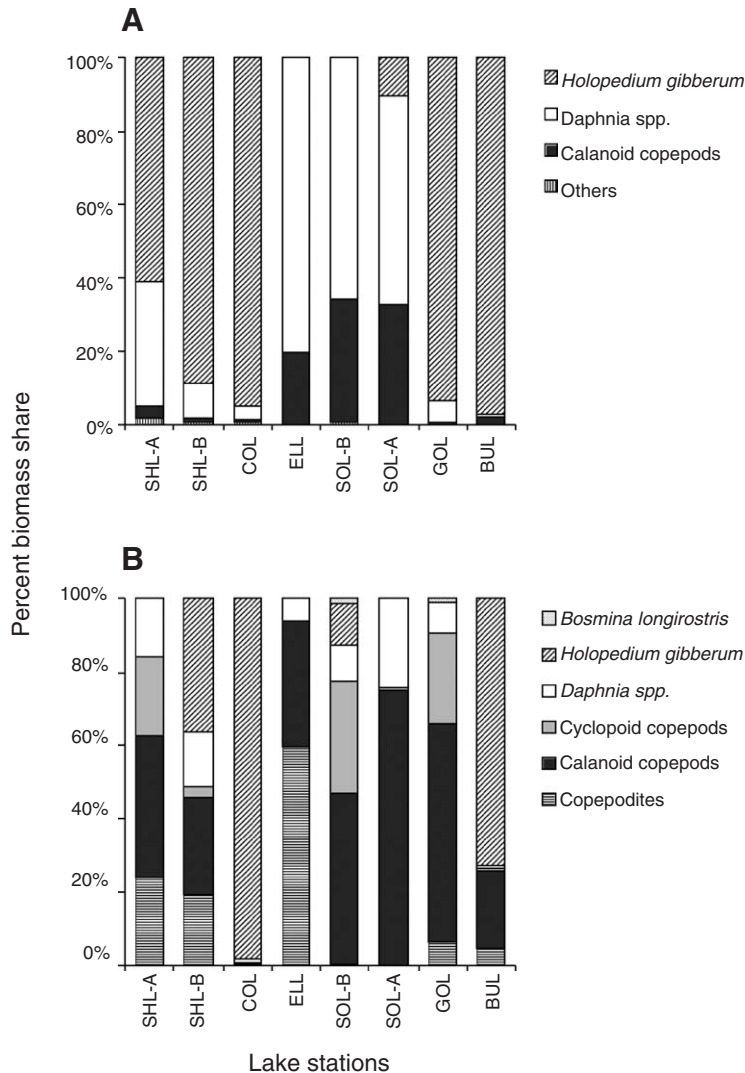


Fig. 1. Percentages of biomass shares (cumulative bars) of (A) macrozooplankton (>500 µm mesh size) and (B) mesozooplankton (200–500 µm mesh size) from Shawnigan Lake, Station-A and -B (SHL-A and-B), Council Lake (COL), Elk Lake (ELL), Sooke Lake, Station-A and -B (SOL-A and-B), Goldstream Lake (GOL), and Butchard Lake (BUL). Figure modified from Kainz et al. (2004).

zooplankton ( $144 \pm 35$ ) to mesozooplankton ( $228 \pm 95$ ; analysis of variance, ANOVA, using size-fractions as covariates;  $p < 0.05$ ) and decreased significantly in macrozooplankton organisms ( $183 \pm 41$ ; ANOVA with linear contrasts;  $p < 0.05$ ). The relative amount of EFA in the total lipid mass of the planktonic food web decreased from seston ( $22.3\% \pm 9.9$ ), to microzooplankton ( $11.6\% \pm 5.0$ ), mesozooplankton ( $10.5\% \pm 4.5$ ), and macrozooplankton ( $10.4\% \pm 1.6$ ).

EFA concentrations ( $\text{mg g dry weight}^{-1}$ ) increased from seston ( $9.5 \pm 1.7$ ) to microzooplankton ( $19.1 \pm 6.2$ ) and mesozooplankton ( $30.0 \pm 5.7$ ), but decreased thereafter by 20% in macrozooplankton ( $24.3 \pm 3.6$ ). Results from quadratic regression analysis ( $y = 6830.4 +$

$59.6x - 0.0383x^2$ ) showed that EFA concentrations decreased significantly with zooplankton sizes  $> 778 \mu\text{m}$  ( $R^2 = 0.72$ ;  $p < 0.001$ ).

Mean concentrations ( $\pm$  SD;  $\text{mg g dry weight}^{-1}$ ) of ARA, EPA, and LIN increased from the seston to macrozooplankton size fraction (ARA:  $0.4 \pm 0.2$ – $4.3 \pm 1.3$ ; EPA:  $2.6 \pm 1.0$ – $10.8 \pm 1.8$ ; LIN:  $1.5 \pm 0.8$ – $3.4 \pm 0.7$ ). However, mean concentrations of DHA only increased from seston ( $2.8 \pm 1.2$ ) to mesozooplankton ( $10.2 \pm 4.5$ ), and decreased sharply in the macrozooplankton size fraction ( $2.2 \pm 1.5$ ). Similarly, ALA concentrations increased from seston ( $2.1 \pm 1.9$ ) to mesozooplankton ( $5.0 \pm 2.4$ ), but decreased in macrozooplankton ( $3.6 \pm 0.9$ ; Fig. 2).

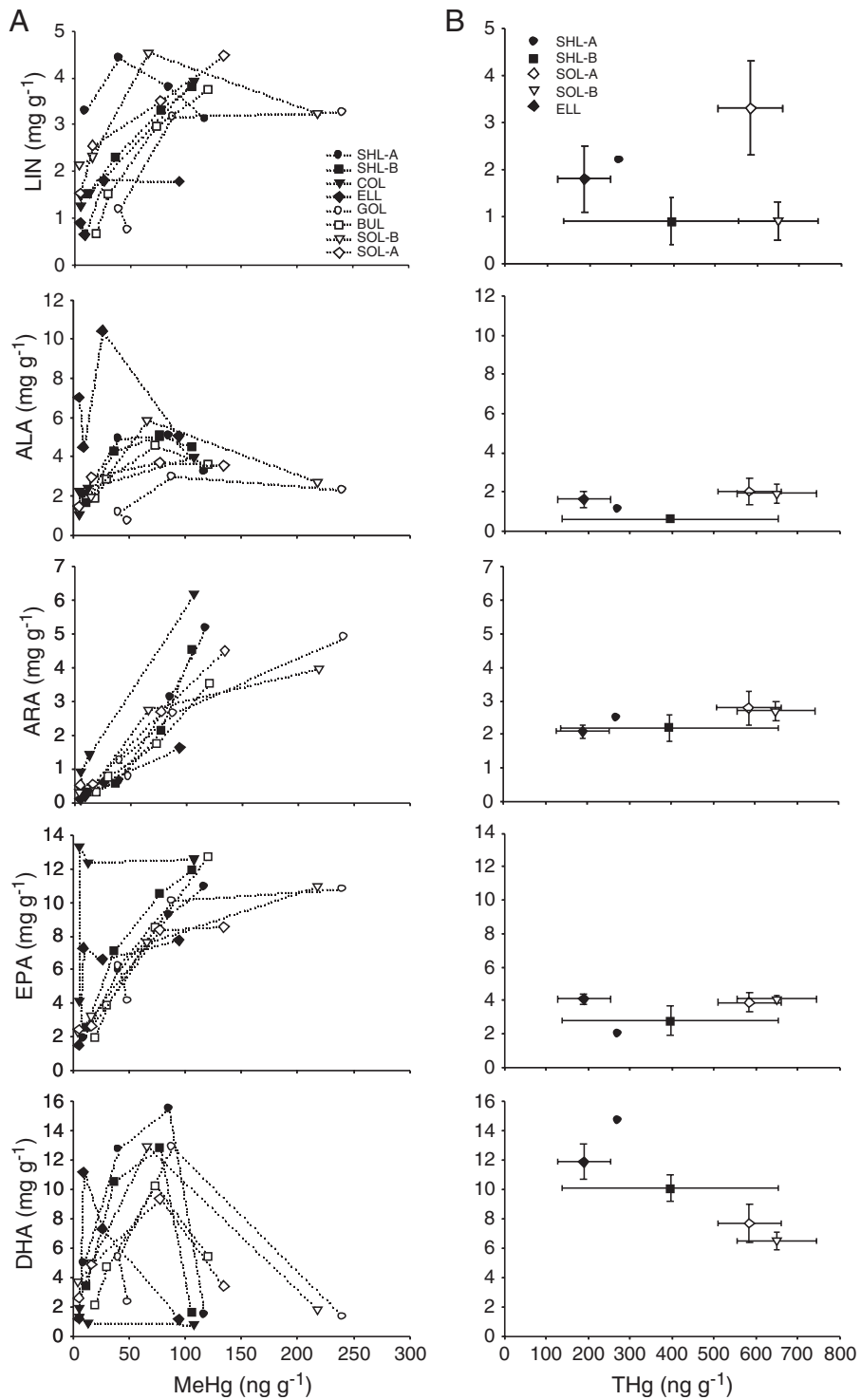


Fig. 2. Concentrations of methyl mercury (MeHg) and essential fatty acids (A) in seston, micro-, meso-, and macrozooplankton body sizes and (B) in rainbow trout (*O. mykiss*) of SHL-A ( $n=1$ ), -B ( $n=3$ ), ELL ( $n=3$ ), SOL-A ( $n=2$ ) and -B ( $n=3$ ).

Bioaccumulation factors for MeHg and EFA compounds were calculated as the quotients of MeHg and EFA concentrations between different (larger and smaller) plankton size classes, and between rainbow trout and macrozooplankton samples of lakes from which fish were collected. Factors  $>1$  indicate that concentrations of MeHg or EFAs from a smaller to the next larger size class or from macrozooplankton to fish have increased, and factors  $<1$  show that MeHg or EFA concentrations have decreased (Fig. 3). Between seston to macrozooplankton, the mean ( $\pm$ SD) accumulation factor was higher for MeHg 20 ( $\pm 16$ ) than for total EFA compounds 4.7 ( $\pm 0.8$ ). For MeHg, bioaccumulation factors increased steadily from seston to macrozooplankton. For individual EFA compounds, the mean ( $\pm$ SD) accumulation factor was highest for ARA (13.2  $\pm$  4.3), followed by EPA (4.5  $\pm$  1.4), LIN (2.9  $\pm$  1.5), and ALA (2.3  $\pm$  1.0). No significant increase of DHA concentrations from seston to macrozooplankton was detected. Concentrations of LIN, ARA, EPA, and DHA in planktonic organisms were not significantly ( $p > 0.05$ ) related to mean chl-*a* concentrations of the study lakes.

Using analysis of covariance (ANCOVA), we corrected for the effect of sampling stations to investigate linear relationships between MeHg and EFA concentrations along the size gradient of planktonic food web.

Plankton size fractions correlated significantly with MeHg concentrations and have not been included as a covariate to avoid problems with collinearity. Because EFA concentrations decreased in macrozooplankton, the relationship between EFA and MeHg concentrations was tested using a quadratic regression model ( $y = 11.404 + 0.266x - 0.001x^2$ ), which revealed that EFAs were significantly ( $R^2 = 0.51$ ;  $p < 0.0001$ ) related to MeHg concentrations. In particular, MeHg concentrations were significantly ( $p < 0.01$ ) related to the following EFA concentrations: EPA ( $R^2 = 0.65$ ), ARA ( $R^2 = 0.80$ ), and LIN ( $R^2 = 0.64$ ). Because concentrations of DHA and ALA decreased in macrozooplankton, we used quadratic regression analysis to calculate the maximum MeHg concentration at which ALA and DHA concentrations started to decrease. The highest concentrations of DHA and ALA in zooplankton occurred at 94 and 109 ng MeHg  $g^{-1}$ , respectively; however, results of this regression model were not significant (DHA,  $p = 0.06$ ; ALA,  $p = 0.22$ ). Lastly, MeHg and EFA bioaccumulation factors were significantly different (using planned contrast ANOVA;  $p > 0.05$ ) among these size classes of the planktonic food web. MeHg concentrations bioaccumulated at higher rates than EFA concentrations along the planktonic food web as demonstrated by significantly increasing MeHg<sub>(ng/g)</sub>:EFA<sub>(mg/g)</sub> concentration ratios (Fig. 4).

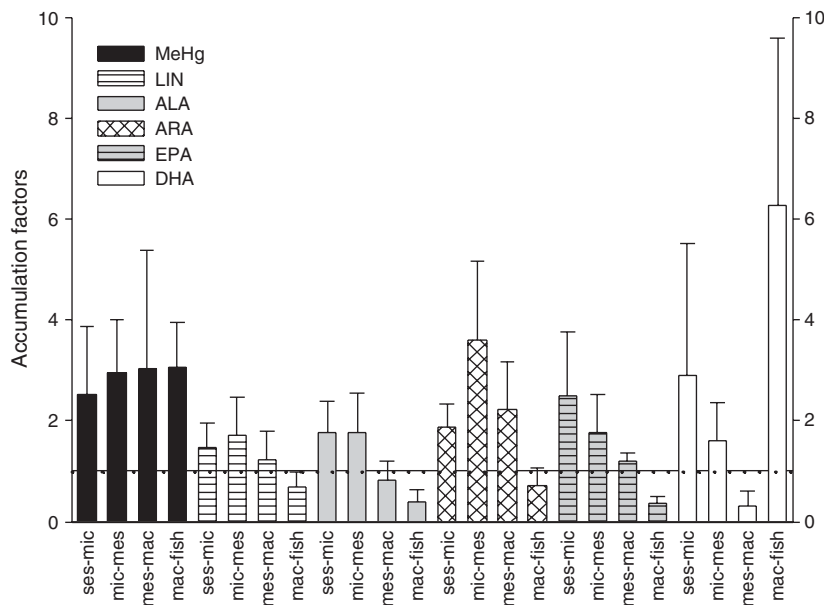


Fig. 3. Accumulation factors (mean values;  $\pm$ SD) of MeHg, linoleic (LIN),  $\alpha$ -linolenic (ALA), arachidonic (ARA), eicosapentaenoic (EPA), and docosahexaenoic (DHA) acids between different size classes of the planktonic food web (seston, ses; microzooplankton, mic; mesozooplankton, mes; and macrozooplankton, mac) and fish (rainbow trout).

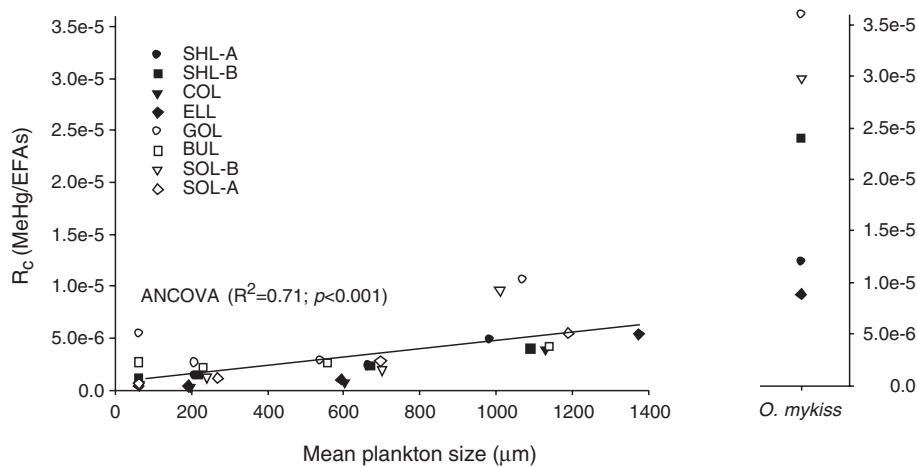


Fig. 4. Concentration ratios ( $R_c$ ) between MeHg ( $\text{ng g dry weight}^{-1}$ ) and EFAs ( $\text{mg g dry weight}^{-1}$ ) in organisms of the planktonic food web and in rainbow trout (*O. mykiss*).

#### 4.4. THg and EFAs in rainbow trout (*O. mykiss*)

Mean concentrations of THg ( $\text{ng g dry weight}^{-1}$ ;  $\pm$  SD) in fish were highest at SOL (SOL-A:  $585 \pm 77$ ; SOL-B:  $650 \pm 95$ ), lower at SHL (SHL-A:  $396 \pm 258$ ; SHL-B: 271), and lowest at mesotrophic ELL ( $189 \pm 63$ ). The THg concentration of one fish sample (SHL-A: 101) was lower than any of the ELL samples and THg concentrations were not significantly different from each other ( $t$ -test;  $p > 0.05$ ). Results from linear regression analysis (using ANCOVA to correct for the effect of sampling station) showed that MeHg concentrations in macrozooplankton could significantly predict THg concentrations in rainbow trout ( $R^2 = 0.71$ ,  $p = 0.048$ ).

Total lipid concentrations ( $\text{mg g dry weight}^{-1}$ ) from dorsal muscle tissue of *O. mykiss* were highest at SHL-A ( $207$ ;  $n = 1$ ) and lower at SOL (-A:  $201 \pm 51$ ,  $n = 2$ ; -B:  $197 \pm 76$ ,  $n = 3$ ), ELL ( $98 \pm 24$ ;  $n = 3$ ), and SHL-B ( $74 \pm 22$ ;  $n = 3$ ). THg concentrations were not significantly related with total lipid concentrations in rainbow trout (ANOVA;  $p = 0.25$ ). Mean concentrations of EFAs ( $\text{mg g dry weight}^{-1}$ ;  $\pm$  SD), in contrast to THg concentrations, were highest in samples from ELL ( $21.4 \pm 1.8$ ), followed by SOL ( $18.7 \pm 1.5$ ), and SHL ( $18.0 \pm 3.0$ ). The highest concentrations of individual EFAs were measured in DHA ( $9.6 \pm 2.8$ ), followed by EPA ( $3.5 \pm 0.9$ ), ARA ( $2.4 \pm 0.4$ ), LIN ( $2.2 \pm 1.1$ ), and ALA ( $1.4 \pm 0.7$ ). Fish samples from ELL had significantly higher EFA concentrations in their dorsal muscles than fish from the other lakes ( $p < 0.05$ ), whereas EFA concentrations in fish samples of SOL and SHL did not differ significantly from each other ( $p > 0.05$ ). THg continued to increase between macrozooplankton

and fish (bioaccumulation factor:  $3.1 \pm 0.9$ ), and MeHg:EFA concentration ratios in fish were higher than those of the respective macrozooplankton size fraction (Fig. 4). For EFAs, DHA concentrations increased on average  $6.3\times$  between macrozooplankton and fish, but all other EFA concentrations decreased with respect to LIN, ALA, ARA, and EPA concentrations in macrozooplankton on average by 31%, 60%, 29%, and 65%, respectively (Fig. 3).

## 5. Discussion

According to the principle of MeHg bioaccumulation in organisms of aquatic food webs (e.g., Watras et al., 1998; Cabana and Rasmussen, 1994), we should expect that Hg concentrations increase with increasing body size as a result of food uptake. Consequently, we should expect that the dietary uptake of MeHg is consistent, or at least similar, with the dietary uptake of essential nutrients that promote somatic growth of zooplankton and fish. Here we discuss bioaccumulation patterns in different size classes of plankton organisms and in fish and demonstrate that MeHg concentrations increase steadily with increasing body size (from seston to fish), whereas EFAs show irregular retention patterns along the planktonic food web and in fish.

### 5.1. MeHg and EFAs in the planktonic food web

Increasing MeHg concentrations with increasing plankton size classes follow the concept of MeHg bioaccumulation in aquatic organisms and are consistent with previous studies of temperate lakes (Masson and Tremblay, 2003; Kainz et al., 2002). The steady

accumulation of MeHg identified during this summer sampling was not significantly affected by the taxonomic composition or by the standing stock biomass of zooplankton genera, which indicates that MeHg bioaccumulation patterns are independent of the taxonomic composition of these planktonic food webs. Thus, it is evident that bioaccumulation of MeHg is related to somatic growth of planktonic organisms.

However, concentrations of EFA compounds, some of which promote somatic growth of daphnids (von Elert, 2002), increased only from seston to mesozooplankton and decreased thereafter in macrozooplankton, demonstrating that MeHg in these plankton size classes is retained differently than algal-derived EFAs. As evidenced by continuously increasing MeHg:EFA concentration ratios along the planktonic food web, we argue that MeHg in macrozooplankton, the preferred diet for planktivorous fish, is more efficiently retained than EFA compounds.

### 5.2. Eicosapentaenoic acid (EPA) and MeHg

There is laboratory evidence that dietary EPA increases somatic growth of daphnids (e.g., Müller-Navarra et al., 2000). Kainz et al. (2004) have recently demonstrated that EPA concentrations accumulate in zooplankton and suggested that EPA is required throughout the lifespan of zooplankton and possibly enhances their reproductive success (see also Becker and Boersma, 2003). The significant relationship between EPA and MeHg concentrations indicates that this particular growth-enhancing nutrient and MeHg are both efficiently retained in all of these planktonic food webs, regardless of their taxonomic composition. Furthermore, the continuous accumulation of EPA and MeHg concentrations suggests that MeHg accumulation and possibly selective EPA retention by zooplankton is independent of the taxonomic composition of edible algae in these study lakes.

Although EPA and MeHg concentrations increase along the planktonic food web, MeHg bioaccumulates at higher rates than EPA. From an ecotoxicological viewpoint, it is tempting to ask whether increasing dietary MeHg concentrations affect the retention and/or assimilation of EPA in zooplankton. Based on this field study, however, it is unlikely to identify any effect of dietary MeHg on the retention efficiency of EPA or any other EFA in planktonic organisms. Because EPA and MeHg accumulate with increasing plankton size, macrozooplankton convey the highest concentrations of this somatic growth-enhancing nutrient and of MeHg to organisms at higher trophic levels.

### 5.3. Docosahexaenoic acid (DHA) and MeHg

The highly unsaturated FA DHA is primarily found in phospholipids of cell membranes and is essential to some physiological key processes. For example, dietary DHA-additions stimulate somatic growth of fish more than any other EFA (e.g., Copeman et al., 2002) and, although at lower rates than EPA, DHA also improves somatic growth of daphnids (Wacker and von Elert, 2001; Müller-Navarra, 1995).

The relationship between DHA and MeHg concentrations clearly differed from that between EPA and MeHg. The high MeHg and low DHA concentrations in macrozooplankton indicate that DHA is retained differently than EPA in the planktonic food web. As shown in Fig. 1, the macrozooplankton size class was dominated by cladocerans, in particular by daphnids and *H. gibberum*. The different taxonomic composition between meso- and macrozooplankton suggests that cladocerans retain little DHA (see Ballantyne et al., 2003), however, continue to bioaccumulate MeHg with increasing body size. Following the size-selective feeding concept (Brooks and Dodson, 1965), these different accumulation patterns of DHA and MeHg concentrations along the planktonic food web clearly demonstrate that macrozooplankton convey the highest concentrations of MeHg to planktivorous fish, whereas DHA is provided by organisms of the mesozooplankton size class. The increasing MeHg and decreasing DHA concentrations in macrozooplankton show furthermore that DHA is probably not a required somatic growth-enhancing nutrient for this cladoceran-dominated size class because MeHg gets linearly bioaccumulated with zooplankton size. Accordingly, these contrasting patterns suggest that MeHg bioaccumulation is independent of physiological DHA requirements in the planktonic food web. Therefore, retention of dietary DHA, in contrast to EPA, appears to be determined by the taxonomic composition of the planktonic food web, whereas increasing MeHg concentrations are not specific to zooplankton species of these study lakes.

### 5.4. Arachidonic acid (ARA) and MeHg

ARA, an  $\omega$ -6 PUFA, is an essential precursor for eicosanoid synthesis and a constituent of membrane phospholipids involved in signal transduction (Smith and Fitzpatrick, 1996). Physiological functions of ARA include the formation of cortisol in fish, which allows fish to alleviate environmental stress (Koven et al., 2001). In contrast to EPA and DHA, ARA may only conditionally promote somatic growth of daphnids, or

perhaps zooplankton in general. For example, while von Elert (2002) demonstrated that dietary ARA does not improve somatic growth or reproduction of *Daphnia galeata*, ARA additions in a recent laboratory study resulted in higher somatic growth rates of *D. magna* (Becker and Boersma, 2005).

ARA and MeHg concentrations increase significantly with increasing plankton size. Between micro- and macrozooplankton, ARA accumulation factors are higher than those of other EFA, which indicate that ARA retention is essential to zooplankton at all life stages. However, our knowledge about physiological requirements for retaining ARA in zooplankton is very limited. It may be possible that ARA retention is required as an essential substance for mitigating stress. Recently, it has been demonstrated that stressors can cause negative physiological effects for zooplankton. For example, zooplankton exposed to toxic stressors such as Cd and Cu showed impaired growth rates and weight loss (Knops et al., 2001), while very high UV-radiation may cause membrane damage (Hessen and Rukke, 2000). More specifically, a recent laboratory study investigating effects of MeHg on astrocytic ARA release has demonstrated that MeHg caused concentration-dependent increases in ARA release from nerve cells (Shanker et al., 2002). It may be possible that the steady increase of ARA concentrations in zooplankton is a physiological stress response to MeHg bioaccumulation. Accordingly, these bioaccumulation patterns of ARA in zooplankton may be based on physiological requirements that are different than those for clearly somatic growth-enhancing nutrients (EPA and DHA) and that ARA concentrations in zooplankton may be sought for optimal cell functioning in the presence of stressors. Although further investigation on such effect–response relationships in organisms of the planktonic food web is clearly required, these results demonstrate that macrozooplankton convey ARA and MeHg most efficiently to organisms at higher trophic levels.

#### 5.5. 18-carbon PUFA (LIN and ALA) and MeHg

LIN and ALA are 18-carbon PUFA compounds, mostly of algal origin (Napolitano, 1999). As it was observed for other EFAs, MeHg bioaccumulates more efficiently along the planktonic food web than LIN and ALA. The bioaccumulation patterns of LIN and ALA are not steady, and thus in contrast to EPA and ARA, along the planktonic food web, suggesting that these 18-C PUFAs have different physiological roles in lake zooplankton than EFAs that increase with increasing

body size. LIN and ALA may be essential substrates for the planktonic food web because, as reported by von Elert (2002) for laboratory-raised *D. galeata*, these 18-C PUFAs can be converted into the somatic growth-enhancing EPA.

It is generally difficult, if at all possible, to link EFA compounds to specific algal-derived diet because no single PUFA can per se identify a single group of algae (Ahlgren et al., 1992, 1990). However, the lower accumulation factors for LIN and ALA than for MeHg suggest that (1) these EFA are less abundant than MeHg concentrations in dietary algae, and/or (2) ingested LIN and ALA concentrations are rapidly converted into other EFAs. Both scenarios would account for different accumulation patterns of 18-C PUFA and MeHg concentrations. Finally, it is not possible to attribute any single EFA compound to one specific group of algae that transfers MeHg to zooplankton, which clearly demonstrates a limit of EFAs for being used as dietary biomarkers in zooplankton.

#### 5.6. Essential fatty acids and THg in higher trophic levels (*O. mykiss*)

The bioaccumulation of EFA and MeHg in planktonic organisms has important nutritional and toxicological implications for higher trophic levels. Because MeHg concentrations increase with body size of planktonic organisms, macrozooplankton, as the preferred prey size for planktivorous fish, convey the highest MeHg concentrations from the planktonic food web to higher trophic levels. Such body-size dependent biomagnification of MeHg throughout the planktonic food web resembles that of lipophilic organic trace pollutants. However, as total lipid concentrations decreased in macrozooplankton, MeHg accumulation in planktonic organisms is not a simple function of total lipid concentrations in prey organisms. In addition, THg concentrations in fish are not significantly related with total lipid concentrations, indicating that accumulation patterns of Hg in aquatic food webs are not controlled by lipid solubility.

Following Brooks and Dodson's concept of size-selective feeding, EFA uptake by planktivorous fish, in contrast to MeHg, is not optimized by dietary uptake of macrozooplankton. While concentrations of ARA and EPA are highest in macrozooplankton, DHA levels peak in mesozooplankton. Because DHA is the most conserved EFA in rainbow trout (see also Ahlgren et al., 1994) and also in juvenile brook trout (*Salvelinus fontinalis*; unpublished data), predation on mesozooplankton would result in the most efficient DHA uptake.

As demonstrated by accumulation factors  $<1$ , most EFA concentrations (i.e., LIN, ALA, ARA, and EPA) in dorsal fish tissues are lower than in macrozooplankton. Because EFA concentrations in rainbow trout cannot be predicted by EFA concentrations of macrozooplankton, some EFAs (i.e., LIN, ALA, ARA, and EPA) from this prey size are not conserved efficiently in fish. Alternatively, such high dietary EFA concentrations may be required for in vivo conversion to DHA—for which ALA and EPA serve as precursors of DHA synthesis in fish (Henderson, 1996)—and are, therefore, less efficiently retained compared to EFA concentrations in their diet. It is also possible that benthic organisms, as an additional diet source, provide further DHA to fish, which would reduce required enzymatic activity for de novo synthesis of DHA in fish. However, for fish preying on planktonic organisms, we conclude that the transfer efficiency of EFAs to higher trophic levels does not increase with increasing body size of the prey, whereas MeHg bioaccumulates steadily along the planktonic food web with highest concentrations in macrozooplankton.

## 6. Conclusions

Results of this summer field study indicate that accumulation patterns of MeHg are different from those EFAs in zooplankton and fish. We interpret these contrasting accumulation patterns of MeHg and EFAs as the inability of aquatic organisms to regulate the assimilation of dietary MeHg, whereas for rainbow trout, and perhaps for fish in general, the rate of EFA retention may be controlled to optimize its physiological performance. As a result, we propose that the observed concentrations of Hg in fish do not seem to limit the ability to regulate EFAs in fish as similar EFA concentrations and patterns in muscle tissue of rainbow trout have been reported in other studies (Ghioni et al., 1997; Ahlgren et al., 1994). We reject our starting hypotheses that dietary MeHg and EFA compounds are accumulated at similar rates. This is because aquatic animals retain EFAs at lower rates than MeHg. Although these presented MeHg and EFA data are restricted to June sampling, patterns of MeHg and EFA concentrations within the planktonic food web remained similar throughout the year, which we attribute to little annual variation of taxonomic zooplankton composition (Kainz, unpublished data). Finally, we conclude that MeHg accumulates continuously with increasing plankton size and at higher trophic levels, as evidenced by planktivorous rainbow trout, whereas the accumulation and/or retention of EFA compounds

appears to be regulated by the taxonomic composition of the planktonic food web and by physiological requirements in fish.

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## References

- Ahlgren G, Lundstedt L, Brett M, Forsberg C. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *J Plankton Res* 1990;12:809–18.
- Ahlgren G, Gustafsson I-B, Boberg M. Fatty acid content and chemical composition of freshwater microalgae. *J Phycol* 1992;28:37–50.
- Ahlgren G, Blomqvist P, Boberg M, Gustafsson IB. Fatty-acid content of the dorsal muscle — an indicator of fat quality in fresh-water fish. *J Fish Biol* 1994;45:131–57.
- Ballantyne AP, Brett MT, Schindler DE. The importance of dietary phosphorus and highly unsaturated fatty acids for sockeye (*Oncorhynchus nerka*) growth in Lake Washington—a bioenergetics approach. *Can J Fish Aquat Sci* 2003;60:12–22.
- Becker C, Boersma M. Resource quality effects on life histories of *Daphnia*. *Limnol Oceanogr* 2003;48:700–6.
- Becker C, Boersma M. Differential effects of phosphorus and fatty acids on *Daphnia magna* growth and reproduction. *Limnol Oceanogr* 2005;50:388–97.
- Bell JG, McVicar AH, Park MT, Sargent JR. Effects of high dietary linoleic acid on fatty acid composition of individual phospholipids from tissues of Atlantic salmon (*Salmo salar*): association with stress susceptibility and cardiac lesion. *J Nutr* 1991;121:1163–72.
- Brooks JL, Dodson SI. Predation, body size, and composition of plankton. *Science* 1965;150:28–35.
- Cabana G, Rasmussen JB. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* 1994;372:255–7.
- Copeman LA, Parrish CC, Brown JA, Harel M. Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. *Aquaculture* 2002;210:285–304.
- Ghioni C, Bell JG, Bell MV, Sargent JR. Fatty acid composition, eicosanoid production and permeability in skin tissues of rainbow trout (*Oncorhynchus mykiss*) fed a control or an essential fatty acid deficient diet. *Prostaglandins Leukot Essent Fatty Acids* 1997;56:479–89.

- Goulden CE, Place AR. Fatty acid synthesis and accumulation rates in daphniids. *J Exp Zool* 1990;256:168–78.
- Hall BD, Bodaly RA, Fudge RJP, Rudd JWM, Rosenberg DM. Food as the dominant pathway of methylmercury uptake by fish. *Water Air Soil Pollut* 1997;100:13–24.
- Henderson RJ. Fatty acid metabolism in freshwater fish with particular reference to polyunsaturated fatty acids. *Arch Anim Nutr* 1996;49:5–22.
- Hessen DO, Rukke NA. UV radiation and low calcium as mutual stressors for *Daphnia*. *Limnol Oceanogr* 2000;45:1834–8.
- Kainz M, Lucotte M, Parrish CC. Methyl mercury in zooplankton—the role of size, habitat and food quality. *Can J Fish Aquat Sci* 2002;59:1606–15.
- Kainz M, Arts MT, Mazumder A. Essential fatty acids within the planktonic food web and its ecological role for higher trophic levels. *Limnol Oceanogr* 2004;49:1784–93.
- Knops M, Altenburger R, Segner H. Alterations of physiological energetics, growth and reproduction of *Daphnia magna* under toxicant stress. *Aquat Toxicol* 2001;53:79–90.
- Koven W, Barr Y, Lutzky S, Ben-Atia I, Weiss R, Harel M, et al. The effect of dietary arachidonic acid (20:4n–6) on growth, survival and resistance to handling stress in gilthead seabream (*Sparus aurata*) larvae. *Aquaculture* 2001;193:107–22.
- Lin H, Romsos DR, Tack PI, Leveille GA. Influence of diet on in vitro and in vivo rates of fatty acid synthesis in Coho salmon [*Oncorhynchus kisutch* (Walbaum)]. *J Nutr* 1977;107:1677–82.
- Masson S, Tremblay A. Effects of intensive fishing on the structure of zooplankton communities and mercury levels. *Sci Total Environ* 2003;304:377–90.
- Montgomery S, Lucotte M, Cournoyer L. The use of stable carbon isotopes to evaluate the importance of fine suspended particulate matter in the transfer of methylmercury to biota in boreal flooded environments. *Sci Total Environ* 2000;261:33–41.
- Müller-Navarra DC. Evidence that a highly unsaturated fatty acid limits *Daphnia* growth in nature. *Arch Hydrobiol* 1995;132:297–307.
- Müller-Navarra DC, Brett MT, Liston AM, Goldman CR. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature* 2000;403:74–7.
- Napolitano GE. Fatty acids as trophic and chemical markers in freshwater ecosystems. In: Arts MT, Wainman BC, editors. *Lipids in freshwater ecosystems*. New York–Berlin–Heidelberg: Springer Verlag; 1999. p. 21–44.
- Parrish CC. Determination of total lipid, lipid classes, and fatty acids in aquatic samples. In: Arts MT, Wainman BC, editors. *Lipids in freshwater ecosystems*. New York: Springer Verlag; 1999. p. 4–20.
- Paterson MJ, Rudd JWM, St. Louis V. Increases in total and methylmercury in zooplankton following flooding of a peatland reservoir. *Environ Sci Technol* 1998;32:3868–74.
- Pichet P, Morrison K, Rheault I, Tremblay A. Analysis of total mercury and methylmercury in environmental samples. In: Lucotte M, Schetagne R, Thérien N, Langlois C, Tremblay A, editors. *Mercury in the biogeochemical cycle*. Berlin: Springer Verlag; 1999. p. 41–54.
- Shanker G, Mutkus LA, Walker SJ, Aschner M. Methylmercury enhances arachidonic acid release and cytosolic phospholipase A(2) expression in primary cultures of neonatal astrocytes. *Mol Brain Res* 2002;106:1–11.
- Smith WL, Fitzpatrick FA. The eicosanoids: cyclooxygenase, lipoxigenase, and epoxygenase pathways. In: Vance DE, Vance JE, editors. *Biochemistry of lipids, lipoproteins and membranes*. Amsterdam: Elsevier; 1996. p. 283–308.
- Spector AA. Essentiality of fatty acids. *Lipids* 1999;34:S1–3.
- Spry DJ, Wiener JG. Metal bioavailability and toxicity to fish in low-alkalinity lakes: a critical review. *Environ Poll* 1991;71:243–304.
- Tocher DR. Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews Fish Sci* 2003;11:107–84.
- von Elert E. Determination of limiting polyunsaturated fatty acids in *Daphnia galeata* using a new method to enrich food algae with single fatty acids. *Limnol Oceanogr* 2002;47:1764–73.
- Wacker A, von Elert E. Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. *Ecology* 2001;82:2507–20.
- Wainman BC, Smith REH, Rai H, Furgal JA. Irradiance and lipid production in natural algal populations. In: Arts MT, Wainman BC, editors. *Lipids in freshwater ecosystems*. New York: Springer; 1999. p. 45–70.
- Watras CJ, Bloom NS. Mercury and methylmercury in individual zooplankton: implications for bioaccumulation. *Limnol Oceanogr* 1992;37:1313–8.
- Watras CJ, Back RC, Halvorsen S, Hudson RJM, Morrison KA, Wente SP. Bioaccumulation of mercury in pelagic freshwater food webs. *Sci Total Environ* 1998;219:183–208.