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Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington

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Abstract

Understanding the mechanisms of bioaccumulation in food webs is critical to predicting which food webs are at risk for higher rates of bioaccumulation that endanger the health of upper-trophic predators, including humans. Mercury and organochlorines were measured concurrently with stable isotopes of nitrogen and carbon in key fishes and invertebrates of Lake Washington to explore important pathways of bioaccumulation in this food web. Across the food web, age and trophic position together were highly significant predictors of bioaccumulation. Trophic position was more important than age for predicting accumulation of mercury, ∑DDT, and ∑-chlordane, whereas age was more important than trophic position for predicting ∑PCB. Excluding age from the analysis inflated the apparent importance of trophic position to bioaccumulation for all contaminants. Benthic and pelagic habitats had similar potential to bioaccumulate contaminants, although higher ∑-chlordane concentrations in organisms were weakly associated with more benthic carbon signals. In individual fish species, contaminant concentrations increased with age, size, and trophic position ($\delta^{15}N$), whereas relationships with carbon source ($\delta^{13}C$) were not consistent. Lipid concentrations were correlated with contaminant concentrations in some but not all fishes, suggesting that lipids were not involved mechanistically in bioaccumulation. Contaminant concentrations in biota did not vary among littoral sites. Collectively, these results suggest that age may be an important determinant of bioaccumulation in many food webs and could help explain a significant amount of the variability in apparent biomagnification rates among food webs. As such, effort should be made when possible to collect information on organism age in addition to stable isotopes when assessing food webs for rates of biomagnification.

Keywords: Biomagnification; Bioaccumulation; Mercury; Organochlorines; Food web; Lake Washington

1. Introduction

Understanding the mechanisms of bioaccumulation in food webs is critical to predicting which food webs are at risk for higher rates of bioaccumulation that endanger the health of upper-trophic predators, including humans. Bioaccumulation of persistent contaminants is a complex phenomenon, potentially controlled by a myriad of physiological and environmental factors. Often, these factors are confounding, making it difficult

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and sometimes impossible to tease out those factors acting in a particular system and their relevant contributions to bioaccumulation.

Bioaccumulation in aquatic organisms results from the net uptake of contaminants from water (bioconcentration) and from food (trophic transfer or dietary accumulation). Age-related accumulation occurs when the rate of contaminant uptake from all sources is greater than the rate of elimination, a phenomenon well established for mercury and organochlorines. Although some debate continues over whether bioaccumulation of persistent contaminants in aquatic organisms results primarily from bioconcentration or dietary accumulation (Leblanc, 1995; Randall et al., 1998; Gray, 2002), most researchers agree that food is the dominant uptake pathway in the majority of situations, especially when considering less hydrophilic contaminants (Bruggeman et al., 1984; Thomann, 1989; Hall et al., 1997; Fisk et al., 2003; Borga et al., 2004). Feeding at higher trophic levels will therefore result in greater bioaccumulation. Dietary accumulation that results in an individual attaining a greater contaminant concentration than its food is termed biomagnification (Gobas and Morrison, 2000) and will therefore be a net process of time-related and diet-related accumulation.

Factors that may influence time-related and diet-related accumulation include lipids, sex, seasonality, and spatial variability in contaminant concentrations. Lipids are often correlated with concentrations of lipophilic contaminants such as organochlorines, but recently the assumption of a causal relationship between the two has been challenged, with numerous studies showing either no relationship between lipid content and organochlorine concentrations (Jackson et al., 2001; Manchester-Neesvig et al., 2001; Crimmins et al., 2002; Davis et al., 2002), or suggesting that such relationships are spurious (Stow, 1995; Stow et al., 1997). Large seasonal changes in contaminant concentration have been observed in temperate freshwater systems for mercury (Ward and Neumann, 1999; Farkas et al., 2003) and for organochlorines (Vanderford and Hamelink, 1977; Raldua et al., 1997). Seasonal differences may be due in part to the effects of reproduction, if contaminants are redistributed to the gonads (Niimi, 1983). Differences in contaminant concentration between male and female fish have been observed in some populations (Nicoletto and Hendricks, 1988; Lange et al., 1994; Johnston et al., 2002). Spatial variability in contamination concentrations of two kinds – between basins (Madenjian et al., 1998; Dufour et al., 2001) and between benthic and pelagic habitats (Campbell et al., 2000; Stapleton et al., 2001; Power et al., 2002) – has been implicated in increased contaminant variability in some systems.

Mercury and organochlorines readily bioaccumulate in aquatic biota and are responsible for the majority of fish consumption advisories issued in the United States (US EPA, 2005). Understanding the mechanisms of bioaccumulation in food webs is critical to predicting which food webs are at risk for higher rates of bioaccumulation. Elevated levels of mercury and organochlorines were recently measured in the food web of Lake Washington, U.S.A. (McIntyre, 2004), prompting the Washington State Department of Health to issue an interim fish consumption advisory for upper-trophic level fishes in this system. Lake Washington has been well characterized ecologically by diet and stable isotope studies (Beauchamp et al., 1992; Chigbu and Sibley, 1998b,a; Beauchamp et al., 2004; Nowak et al., 2004; McIntyre et al., 2006). The lake contains a complex food web with four distinct trophic levels and apex predators that show well-defined ontogenetic transitions from lower to higher trophic levels, accompanied by shifts between bentho-littoral and pelagic habitats (McIntyre et al., 2006). Extensive paired contaminant and stable isotope measurements combined with age and other biological information in the Lake Washington data set provide an excellent opportunity to explore bioaccumulation of persistent contaminants in a complex freshwater food web.

The goal of this research was to determine the dominant factors governing bioaccumulation of mercury and organochlorines in the food web of Lake Washington and to quantify their relative importance. We focused on the role of age, trophic position, feeding habitat (benthic versus pelagic), and lipids, factors that are typically confounding in food web studies. We also tested for spatial differences in contaminant concentration in demersal biota at three littoral sites spanning the range of typical shoreline conditions. Stable nitrogen isotopes were used as an indicator of trophic position because the lighter isotope is preferentially excreted, leaving consumers with $\delta^{15}N$ values approximately 3.4‰ enriched in the heavier isotope compared to their prey (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001). Stable carbon isotopes were used to differentiate carbon derived from pelagic versus benthic-littoral sources because pelagic carbon is depleted in the heavier isotope compared to carbon that is derived from benthic-littoral sources (France, 1995).

1.1. Site description

Lake Washington is a large, monomictic, mesotrophic lake located in the Puget Sound basin of the Pacific Northwest, adjacent to the city of Seattle, Washington,

Table 1
Stable nitrogen and carbon values and concentrations of methylmercury (MHg) and organochlorines (OC) in fishes and invertebrates from Lake Washington

Species	Size	TL (mm)	Weight (g)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	N (MHg)	MHg (μg kg^{-1})	N (OC)	∑DDT (μg kg^{-1})	∑PCB (μg kg^{-1})	∑CHL (μg kg^{-1})
N. pikeminnow	L	459±53	907±347	16.7±0.2	−27.1±0.3	10	413±45	10	258±95	1071±541	40±17
	S	234±44	118±72	14.5±0.6	−26.0±0.5	10	57±12	9	45±26	140±88	7±4
Cutthroat trout	L	429±44	869±368	17.6±0.2	−28.0±0.5	10	194±32	10	168±68	377± 156	44±25
	S	248±38	107±54	14.0±0.4	−26.9±0.5	10	39±5	10	47±61	79±67	15±20
Yellow perch	L	304±20	404±86	15.8±0.1	−25.5±0.3	9	204±19	9	59±20	191±55	16±8
	M	244±13	181±42	15.6±0.2	−28.0±0.4	10	86±19	10	49±21	66±45	10±4
	S	136±13	26±10	13.9±0.2	−30.6±0.3	10	30±2	10	14±3	47±16	6±2
Smallmouth bass	L	419±42	1247±262	15.8±0.2	−22.9±0.6	3	261±13	3	63±19	371±62	10± 5
Juv. sockeye	–	121±2	13±1	14.9±0.1	−31.0±0.1	20	46±2	6*	24±2	37±14	5±2
Stickleback	–	69±2	4±0.5	16.3±0.2	−31.3±0.2	20	39±1.3	4*	44±10	165±41	17±5
Longfin smelt	–	89±3	5±2	17.0±0.1	−29.7±0.3	31	50±5	11*	53±15	195±54	21±9
Mysids	–	–	–	11.5±0.3	−28.9±0.5	10*	15±2	5*	7±0.7	ND [a] (12–16)	ND (2)
Bulk zooplankton [b]	–	–	–	12.7±0.7	−29.1±0.8	10*	4±0.4 [c]	9*	4±0.8	ND (6–16)	1.1±0.3
Daphnia	–	–	–	10.3±0.1	−30.2±0.6	3*	9±0.9	3*	ND (3)	ND (8)	ND (2)
Leptodora	–	–	–	11.9±0.5	−27.0±1.0	2*	5±1.8	2*	NA [d]	NA	NA
Prickly sculpin	L	122±5	22±3	13.0±0.1	−24.3±0.4	34	55±8	16	9±1.3	158±57	5±1.1
	S	32±0.2	0.3±0.01	12.7±0.2	−19.8±0.4	13*	14±1.0	4*	ND (6)	17±0.2	ND (4)
Signal crayfish	L	74±4	14±3	NA	NA	21*	23±4	4*	ND (3–9)	ND (7–8)	ND (2–5)
	S	28±0.3	0.46±0.02	9.8±0.2	−17.8±0.3	6	12±0.7	–	NA	NA	NA
Trichopteran larvae	–	22±0.3	0.32±0.01	8.3±0.1	−16.8±0.7	9*	6±0.6	–	NA	NA	NA

Statistics reported are means±one standard error of the mean. In the sample size column, * indicates samples analyzed as composites. All concentrations are on a wet weight basis. TL=total length.
[a] ND=not detected, showing analytical detection limits in brackets for the sum of congeners.
[b] Stable isotope values adjusted for algal content.
[c] Total mercury concentration.
[d] NA=not analyzed due to insufficient sample mass.

U.S.A. The lake drains an area of 1274 km^2 and covers an area of 87.6 km^2, with a length of 21 km, an average width of 2.4 km, average depth of 33 m, and maximum depth of 65 m (Anderson, 1954). Thermal stratification occurs in Lake Washington from late March to early November with a thermocline forming around 16 m, separating maximum epilimnetic temperatures (~24 °C) from hypolimnion temperatures that remain 7–9 °C year-round. Dissolved oxygen concentrations in the water column remain above 5 mg/L throughout the year.

The food web of Lake Washington is dominated by the piscivores cutthroat trout (*Oncorhynchus clarki*), northern pikeminnow (*Ptychocheilus oregonensis*), and yellow perch (*Perca flavescens*). Pelagic forage fishes include longfin smelt (*Spirinchus thaleichthys*) with a 2-year life span, threespine stickleback (*Gasterosteus aculeatus*) with a 1-year life span, and juvenile sockeye salmon (*Oncorhynchus nerka*), which reside 1.5 years in the lake before migrating to the ocean. The pelagic invertebrate community is dominated by mysid shrimp (*Neomysis mercedes*), *Daphnia* spp., and calanoid and cyclopoid copepods. The benthic fish community is dominated by relatively sedentary prickly sculpin (*Cottus asper*). Average stable isotope values for individuals used in the current study are presented in Table 1.

2. Materials and methods

2.1. Field collections

Fishes and invertebrates were collected from Lake Washington during October 2001 through July 2003. Predatory fishes including northern pikeminnow, cutthroat trout, yellow perch, and smallmouth bass (*Micropterus dolomieui*) were captured opportunistically throughout the lake by gillnets and angling throughout the year. Pelagic planktivorous forage fishes were collected by mid-water trawl during October and March. Zooplankton and mysid shrimp (February, May, August, December) were collected from the top 20 m in the pelagic zone with a 35-cm diameter 153-μm mesh net (bulk zooplankton) and a 1-m diameter 1-mm mesh net (mysids, *Daphnia*, *Leptodora*). Prickly sculpin and benthic invertebrates including signal crayfish (*Pacifasticus leniusculus*) and trichopteran larvae (family: Limnephilidae) were collected from the littoral zone (1–5 m depth) at up to three fixed locations including one relatively undisturbed site (St. Edwards State Park in

the NE), and two sites with significant shoreline disturbance (Magnuson Park in the NW and Baker Beach in the SW). Prickly sculpin were collected by electroshocking and angling in May and August, signal crayfish by minnow trap and by hand in August, and trichopterans by hand in August.

Most fishes were stratified into size classes, based on size-specific differences in food habits (Mazur, 2004; McIntyre et al., 2006). Northern pikeminnow and cutthroat trout were separated into large (>350 mm total length) or small (<300 mm) size classes, yellow perch were large (>275 mm), medium (200–275 mm), or small (<200 mm), prickly sculpin were large (>90 mm) or small (<50 mm), and crayfish were large (>50 mm) or small (<30 mm). Smallmouth bass were all large (>350 mm). All predatory fishes were analyzed as individuals with a maximum goal of ten individuals per size class to minimize costs. Mercury analysis required less sample mass than organochlorine analysis (see below), therefore the larger forage fishes (juvenile sockeye salmon, stickleback, smelt, large prickly sculpin) and invertebrates (crayfish) could be analyzed as individuals for mercury but required compositing for organochlorine analysis. When composite samples were necessary a minimum of three replicates were used. Limiting predatory fishes to ten individuals per size class was justified by the high mobility observed for northern pikeminnow (Brocksmith, 1999) and cutthroat trout (Nowak and Quinn, 2002) in Lake Washington, a lack of spatial heterogeneity in contaminant concentrations among sedentary littoral benthic species in this study (see below), and relatively low variability in trophic level and benthic orientation among similar-sized individuals collected from different areas of the lake (McIntyre et al., 2006). Replicates of three composites were justified by the low variability in contaminant concentration among composites for this study (see below).

2.2. Sample preparation

Fish and crayfish were measured to the nearest millimeter and weighed to the nearest 0.01 g. Approximately 0.5 g of muscle tissue was removed from the anterior dorsal area of individual fish for stable-isotope analysis. Otoliths and scales were removed for age and growth analysis. Trichopteran larvae were removed from their cases. Fish and crayfish were wrapped in aluminum foil and stored in plastic bags at −20 °C until analyzed for contaminants. Pelagic invertebrates were blotted dry through a filter to remove excess water and stored in plastic bags at −20 °C.

2.3. Contaminant analysis

Whole bodies were analyzed for contaminants. Large fish were cut into pieces while still partially frozen and homogenized with liquid nitrogen in a Hobart food cutter (Model 18416; Troy, OH, U.S.A.). Smaller fish (<150 mm) were homogenized in smaller blenders (Model 911; Hamilton Beach Commercial) and invertebrates were homogenized in their sample bag using a rolling pin. Contaminants were extracted from wet tissue and all contaminant concentrations are on a wet weight basis.

Total mercury and a short list of organochlorines including total DDT (∑ DDD, DDE, DDT), total PCB (∑ Aroclor 1254, 1260), total chlordane (∑CHL = α-chlordane + γ-chlordane), and total hexachlorocyclohexane (∑HCH = α-HCH + β-HCH) were analyzed at the King County Environmental Laboratory (KCEL) in Seattle, Washington, U.S.A. An aliquot of each sample homogenized for total mercury analysis was sent to Frontier Geosciences Inc. in Seattle, Washington, U.S.A. for methylmercury analysis. Detection limits were 0.35–4 ppb for total Hg, 1.1–5 ppb for MeHg, 3–6 ppb for ∑DDT, 6–20 ppb for ∑PCB, 2–4 ppb for ∑CHL and 2–6 ppb for ∑HCH. Aroclors 1016, 1221, 1232, 1242, and 1248 were not detected in any sample. Hexachlorocyclohexanes (∑HCH) were not detected in any sample. We assigned congeners a value equal to one-half the detection limit if a congener was detected in other samples of the same species. A value of zero was assigned if a congener was undetectable in any sample for a species.

Total mercury was measured by cold vapor atomic absorption (CVAA) using a modified EPA Method 245.6. Approximately 1.25 g of tissue was digested in nitric and sulfuric acid in the presence of potassium permanganate and potassium persulfate at 100 °C for 2.0–2.5 h. Sodium chloride hydroxylamine hydrochloride was added after digestion to reduce the sample and stannous chloride was added immediately before analysis. Certified reference materials were DORM-2 (dogfish muscle), DOLT-2 (dogfish liver), and TORT-2 (lobster tomalley) from the Institute for National Measurement Standards (Ottawa, Ontario, Canada), and Peach Leaves from the National Institute of Standards and Technology (Gaithersburg, MD, U.S.A). Methylmercury was measured by cold vapor atomic fluorescence spectroscopy (CVAFS) after digestion of 0.5 g of tissue in a KOH–methanol reagent followed by gas chromatography. The certified reference material was DORM-2 (Institute for National Measurement Standards). For organochlorines, homogenized wet tissue

was ground with diatomaceous earth to absorb excess water. High molecular weight Decachlorobiphenyl (DBC) and low molecular weight Tetrachloroxylene (TCX) surrogates were added prior to extraction with a 50:50 methylene chloride-acetone solvent. The samples were cleaned first by gel permeation chromatography (GPC) then further cleaned by Alumina. A small aliquot was set aside for analysis of pesticides (DDTs, chlordanes, benzene hexachlorides). The remainder, for analysis of PCBs, was digested with sulfuric acid and reduced in volume. Chlorinated compounds were analyzed by gas chromatography with electron capture detection (GC-ECD). Quality assurance and control measures included method blanks (diatomaceous earth + surrogates + solvents), spike blanks (method blank + analytes), two matrix spikes (spike blank + tissue), and a laboratory duplicate (method blank + tissue). P,p′-DDE, p,p′-DDD, p,p′-DDT, Aroclor 1016, Aroclor 1260, α- and γ-chlordane, and α-BHC, β-BHC, γ-BHC, and δ-BHC were calibrated using an internal standard method. Leptophos and Isodrin were used as internal standards and retention time markers and added just prior to injection. PCBs were identified and quantified by five non-interfering peaks. Each peak was calibrated using an internal standard method by injecting known concentrations over a range. The concentrations of the five peaks were averaged to determine the Aroclor concentration. The PCBs and pesticides were analyzed by primary and secondary column confirmation. Surrogates and standards were obtained from Restek Chromatography Products (Bellfonte, PA, U.S.A.). Matrix spike recoveries were 94 ± 10% for the pesticides, and 78 ± 4% for PCBs. Relative percent difference between replicate samples was 20 ± 12% for the pesticides and 9 ± 3% for the PCBs.

2.4. Stable-isotope analysis

Muscle tissue for individual fish and whole bodies of age-0 sculpin and invertebrates were dried in a convection oven at 50–60 °C. For composite samples, tissue for isotope analysis was also composited. Dried samples were ground to a fine powder in a porcelain mortar, and weighed to 1.00 ± 0.02 mg in a tin capsule on a Cahn electrobalance. Stable isotopes were measured via continuous flow using a Carlo Erba 2100 elemental analyzer interfaced with a Thermo-Finnagan Delta^{plus} isotope ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. Stable isotope values were expressed as a ratio (R) of the heavy to the light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) standardized with respect to internationally recognized reference materials as follows:

$$\delta(‰) = [(R_{sample}/R_{reference}) - 1] \times 1000.$$

Every 10th sample was analyzed in duplicate yielding an average standard deviation of 0.11‰ $\delta^{13}C$ and 0.07‰ $\delta^{15}N$ between replicates. Reference materials were Vienna Pee Dee belemnite limestone for carbon and atmospheric N_2 for nitrogen.

Relative ^{13}C-depletion of lipids compared with other tissues (Deniro and Epstein, 1977) can affect interpretation of stable isotopes in ecological contexts because they suggest a diet that is more depleted in ^{13}C than similar tissue with less lipid. However, because lipid-extraction can also alter stable nitrogen ratios (Pinnegar and Polunin, 1999), lipid-extraction was not performed on Lake Washington samples. Carbon isotope values were arithmetically normalized for lipids using the method developed by McConnaughey and McRoy (1979) and validated by Kline et al. (1998) in which the atomic ratio of C/N is used as a proxy for lipid content. This adjustment resulted in an average shift in $\delta^{13}C$ of +0.31‰ (SD = 0.90‰) across all samples.

2.5. Mercury normalization

Methylmercury and total mercury concentrations were normalized to their respective average matrix spike recoveries, resulting in an average increase in total mercury concentrations of 6% and decrease in methylmercury concentrations of 11%. In 77% of samples, the ratio of methyl to total mercury (M/T) was >1, indicating that all of the mercury was present as methylmercury. Because of the higher analytical variability accepted for methylmercury (±25%) than total mercury (±20%), analyses were performed using total mercury concentrations for samples with M/T > 1. For the 23% of samples with M/T < 1, methylmercury values were used in statistical analyses. All results are presented as methylmercury unless otherwise noted.

Methylmercury concentrations in all zooplankton samples were at or below detection limits, making methyl mercury to total mercury ratios for bulk zooplankton approximate at best. Mercury concentrations in bulk zooplankton are therefore presented as total mercury, despite the fact that methylmercury likely represented less than 100% of total mercury in these samples.

2.6. Age analysis

After storage in ethanol, sagittal otoliths of northern pikeminnow, cutthroat trout, and prickly sculpin were placed on a dark background in distilled water and prominent annual rings were counted under reflected light using a dissecting microscope. Otoliths of yellow perch were cracked through the nucleus along the ventral-dorsal plane. Open halves were passed over a pure alcohol flame to enhance the contrast between dense winter growth and less dense summer growth. Open halves were covered with light emersion oil and dark annular rings counted under reflected light. When available, both otoliths and scales were examined. Preferred scales (DeVries and Frie, 1996) of northern pikeminnow, cutthroat trout, and yellow perch were cleaned of debris, pressed onto acetate sheets, and annular rings counted under magnification. Lake age for cutthroat trout, the number of years spent in the lake after migrating from tributaries, was the number of annuli after growth rate appreciably increased, analogous to the pattern shown by salmon migrating from tributaries to the ocean. If scales did not show a period of obvious increase in growth rate for cutthroat trout, no lake age was assigned. Ages were not analyzed for smallmouth bass ($n=3$).

2.7. Statistical analyses

Contributions of age, trophic position, and carbon source to bioaccumulation in the food web of Lake Washington were assessed by backwards multiple linear regression analysis. Lipids were not included as a possible independent variable because our analysis (see below) suggested that relationships between lipids and contaminant concentrations were spurious. Only samples for which all three metrics were available were used in the analysis. Small signal crayfish and trichopteran

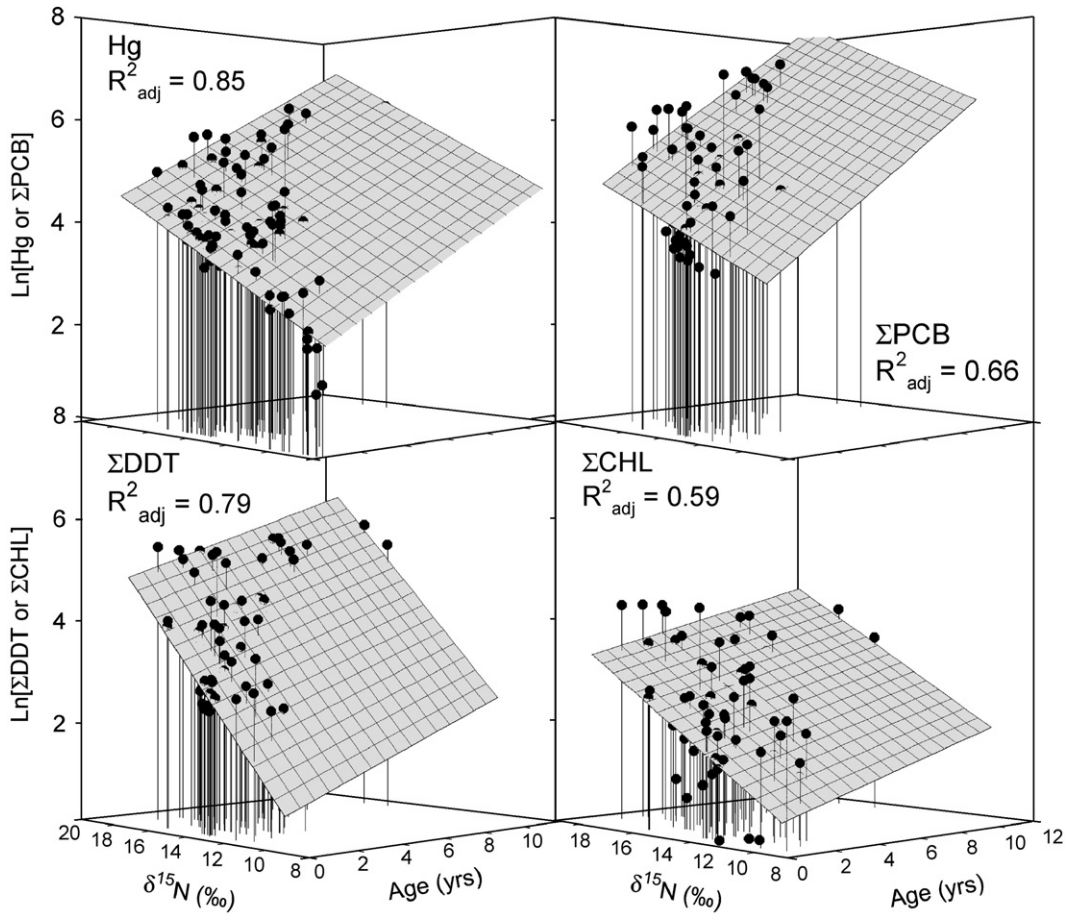

Fig. 1. Concentrations of Hg, ∑DDT, ∑PCB, ∑CHL (natural log of μg/kg wet weight) in the food web of Lake Washington as a function of individual trophic position (δ^{15}N) and age. The plane in each plot represents the three dimensional surface of the multiple regression of contaminant concentration on age and δ^{15}N. Axes are the same for all plots.

larvae were assigned an age of 0.5, because they were less than 1-year old and were captured midway through the growing season. Multiple regressions excluding age (i.e., $\delta^{15}N$ and $\delta^{13}C$ only) were also conducted to compare the effect of including age with the more common approach of excluding age. Spatial differences in contaminant concentrations among benthic biota at the three littoral sites were tested by ANCOVA with length as a covariate (prickly sculpin and crayfish) and by ANOVA (trichopteran larvae). All statistical analyses were performed in SPSS 11.5 (www.spss.com).

3. Results

3.1. General contaminant patterns in the food web

Contaminant concentrations varied considerably among species in Lake Washington (Table 1), with higher average concentrations generally associated with larger, more trophically-elevated species. Across all the species examined in the food web of Lake Washington, age and trophic position explained a significant amount of the variability in concentrations of mercury and organochlorines among individual samples (Fig. 1). Age and trophic position together explained 85% of the variability in concentrations of Hg and 66% for ∑PCB, whereas age, trophic position, and carbon source were all included in the best model for concentrations of ∑DDT and ∑CHL, explaining 79% and 59% of variability respectively. The contributions of age and trophic position were highly significant in all models (Table 2; regression coefficients all $p<0.001$). In contrast, the contribution of carbon source was minor when it appeared in the overall model (Table 2; regression coefficients $p(\sum DDT)=0.070$, $p(\sum CHL)=0.029$). Standardized regression coefficients suggested that across the range of ages and trophic positions present in the Lake Washington food web, the relative importance of trophic position compared to age ($\beta\delta_{15N}/\beta_{age}$) was 1.2 for Hg, 1.8 for ∑DDT, 0.5 for ∑PCB, and 1.7 for ∑CHL (Table 2). Results for specific congeners (i.e., DDE, α-chlordane) or groups (i.e., Aroclor 1254) did not explain more variability in contaminant concentration than for the sum of the congeners.

Excluding age from the regression analysis yielded models that explained less of the variability in contaminant concentration (15% less for Hg, 10% less for ∑DDT, 27% for ∑PCB, and 8% for ∑CHL), and resulted in considerably higher regression coefficients for the contribution of trophic position (60% higher for Hg, 29% for ∑DDT, 103% for ∑PCB, and 36% for ∑CHL).

For ∑CHL, higher concentrations in the food web were very weakly associated with benthic feeding (higher $\delta^{13}C$), but the standardized regression coefficients suggested that age and trophic position together were five times more important than contributions from carbon source ($\beta\delta_{13C}:\beta_{age}:\beta\delta_{15N}=1:2:3$; Table 2).

Adjusted R^2 was higher for mercury than for the organochlorines and tended to be highest for ∑CHL. This was partly due to the greater precision in measuring mercury than in measuring organochlorines. Therefore, measurement precision affected how much of the variability in contaminant concentration was accounted

Table 2
Multiple linear regression results for the natural log of contaminant concentrations (μg kg^{-1} wet weight) on age, $\delta^{15}N$, and $\delta^{13}C$ across the food web of Lake Washington

Contaminant	R^2_{adj}	Variable	Coefficient	SE	p	Std. coefficient (β)
MHg	0.853	Constant	−0.226	0.231	0.330	
		$\delta^{15}N$	0.256	0.018	<0.001	0.581
		Age	0.253	0.022	<0.001	0.472
∑DDT	0.790	Constant	−4.757	0.851	<0.001	
		$\delta^{13}C$	−0.051	0.028	0.070	−0.100
		$\delta^{15}N$	0.425	0.039	<0.001	0.662
		Age	0.206	0.034	<0.001	0.366
∑PCB	0.661	Constant	0.591	0.708	0.406	
		$\delta^{15}N$	0.213	0.049	<0.001	0.320
		Age	0.337	0.040	<0.001	0.624
∑CHL	0.586	Constant	−6.428	1.144	<0.001	
		$\delta^{13}C$	−0.086	0.039	0.029	−0.178
		$\delta^{15}N$	0.385	0.062	<0.001	0.546
		Age	0.169	0.047	<0.001	0.313

Statistics presented are the adjusted coefficient of determination (R^2_{adj}), the regression coefficient for each independent variable and its respective standard error, the p-value testing the difference of the regression coefficient from a value of zero, and the standardized regression coefficient (β).

for by variability in age and trophic position. Higher variance for ∑CHL was also expected because concentrations overall were lower than for the other organochlorines and were often near detection limits for one or both congeners.

3.2. Contaminant patterns in predatory fishes

Concentrations of all contaminants were positively correlated with trophic position for individual northern pikeminnow (all contaminants $p \leq 0.002$), cutthroat trout (all $p \leq 0.001$), and yellow perch (all $p \leq 0.003$). Contaminant concentrations were positively correlated with carbon source in yellow perch (all $p < 0.001$), but were negatively correlated with carbon source in cutthroat trout ($p \leq 0.016$) and northern pikeminnow ($p_{Hg} = 0.041$, $p_{organochlorines} = 0.077–0.111$). (Fig. 2). The difference in the direction of correlation between carbon source and contaminant concentration among species reflected the ontogenetic dietary shift from benthic invertebrates to pelagic prey fishes by cutthroat trout and northern pikeminnow versus the shift from pelagic invertebrates to benthic prey fishes by yellow perch, as indicated by the positive significant correlation between carbon isotope values and nitrogen isotope values for yellow perch ($r = 0.671$) compared to the negative correlations for cutthroat trout ($r = -0.627$) and northern pikeminnow ($r = -0.724$; all $p \leq 0.003$).

Mercury and organochlorine concentrations increased with fish length, weight (not shown), and age for northern pikeminnow, cutthroat trout, and yellow perch (Table 3). Total age was a better predictor of contaminant concentration than was total length or weight for northern pikeminnow and yellow perch, whereas for cutthroat trout, lake age, the number of years spent in the lake, was the strongest predictor for mercury concentration, and length was the best predictor for organochlorines (Table 3). The positive correlations between contaminant concentrations and fish weight were similar to, but usually weaker than, those with fish length. Poorer correlations with total age for cutthroat trout likely resulted from variable rates of growth and contaminant bioaccumulation among tributaries and between tributaries and the lake, because cutthroat trout in the Lake Washington basin spend from 1 to 3 years in tributaries before migrating to the lake (Scott et al., 1986; Nowak et al., 2004). The slope of the relationship of fish length or fish age with contaminant concentration was statistically similar

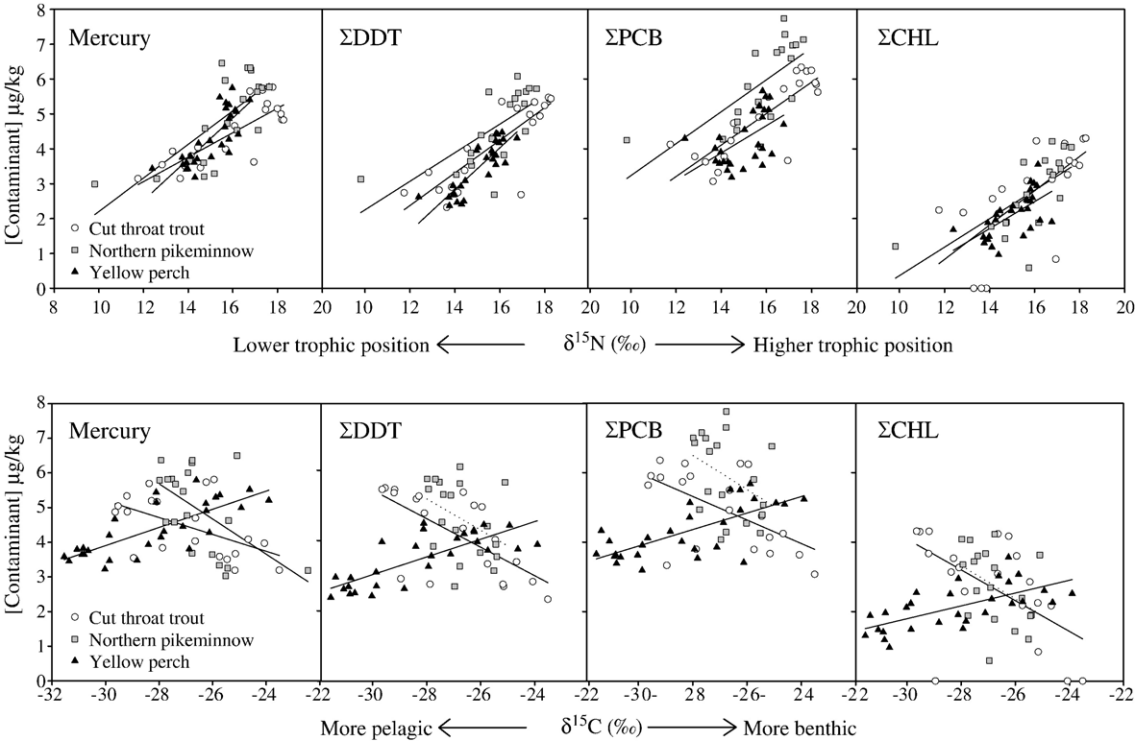

Fig. 2. Contaminant concentrations (natural log of µg/kg wet weight) in individual cutthroat trout, northern pikeminnow, and yellow perch from Lake Washington as a function of trophic position ($\delta^{15}N$) and carbon source ($\delta^{13}C$). Solid lines through the data represent significant correlations and dotted lines represent non-significant correlations.

Table 3
Regression equations for mercury and organochlorine concentrations (μg/kg wet weight) on total length (TL) and on age in Lake Washington northern pikeminnow (NP), cutthroat trout (CT), yellow perch (YP), prickly sculpin (PS), and crayfish (CF)

Species	MeHg (μg/kg ww)	r^2	∑DDT (μg/kg ww)	r^2	∑PCB (μg/kg ww)	r^2	∑CHL (μg/kg ww)	r^2	n
Total length (mm)									
NP	$4\cdot10^{-6}\cdot TL^{3.00}$	0.897	$9\cdot10^{-5}\cdot TL^{2.391}$	0.747	$6\cdot10^{-6}\cdot TL^{3.082}$	0.785	$1\cdot10^{-6}\cdot TL^{2.812}$	0.804	19
CT	$5\cdot10^{-5}\cdot TL^{2.475}$	0.702	$9\cdot10^{-7}\cdot TL^{3.125}$	0.702	$8\cdot10^{-7}\cdot TL^{3.277}$	0.749	$3\cdot10^{-7}\cdot TL^{3.038}$	0.564	20
YP	$9\cdot10^{-4}\cdot TL^{2.111}$	0.732	$3\cdot10^{-3}\cdot TL^{1.711}$	0.714	$4\cdot10^{-2}\cdot TL^{1.383}$	0.419	$3\cdot10^{-2}\cdot TL^{1.033}$	0.447	29
PS	$5\cdot10^{-1}\cdot TL^{0.995}$	0.728	$p=0.123$	–	$1\cdot10^{-1}\cdot TL^{1.342}$	0.448	$p=0.292$	–	46[a]
CF	$9\cdot10^{-1}\cdot TL^{0.738}$	0.443	–	–	–	–	–	–	27
Age (years)									
NP	$11.75\cdot Age^{1.756}$	0.930	$14.88\cdot Age^{1.341}$	0.836	$29.80\cdot Age^{1.699}$	0.829	$1.65\cdot Age^{1.519}$	0.815	19
CT	$16.55\cdot Age^{1.573}$	0.625	$10.86\cdot Age^{1.797}$	0.513	$23.20\cdot Age^{1.806}$	0.493	$2.29\cdot Age^{1.906}$	0.362	20
CT	$33.10\cdot(L.Age+1)^{1.345}$	0.817	$25.25\cdot(L.Age+1)^{1.465}$	0.594	$52.51\cdot(L.Age+1)^{1.542}$	0.658	$5.67\cdot(L.Age+1)^{1.587}$	0.422	19
YP	$28.29\cdot Age^{1.33}$	0.939	$15.50\cdot Age^{0.955}$	0.774	$34.40\cdot Age^{0.972}$	0.663	$4.77\cdot Age^{0.723}$	0.549	25
PS	$13.80\cdot(Age+1)^{0.869}$	0.704	$p=0.295$	–	$15.18\cdot(Age+1)^{1.332}$	0.443	$p=0.628$	–	46[a]

Correlation coefficients (r^2) follow regression equations. All regressions were significant ($p<0.002$) unless otherwise noted. Cutthroat ages are total age and lake age (L.Age) as described in the text. Organochlorines were not detected in crayfish samples.
[a] Sample sizes were 46 for mercury, 20 for ∑PCB, and 16 for ∑DDT and ∑CHL.

for all three species for all contaminants but tended to be lower for yellow perch. Mercury and organochlorine concentrations in smallmouth bass were intermediate to those of similar-sized northern pikeminnow and cutthroat trout (Table 1).

Percent lipid was significantly correlated with contaminant concentration for all contaminants in cutthroat trout and for Hg, ∑DDT, and ∑CHL in yellow perch, with significant Pearson correlation coefficients ranging from 0.59 to 0.72 (Table 4). Percent lipid did not correlate significantly with any contaminant in northern pikeminnow. For all three species, percent lipid was more highly correlated with fish weight than with any contaminant. Correlations between percent lipid and fish length differed very little from those with fish weight, and were therefore not shown.

Table 4
Pearson correlation coefficient for relationships between the natural log of percent lipids and the natural log of contaminant concentration or weight for three predatory fishes (cutthroat trout, northern pikeminnow, yellow perch) and a benthic forage fish (prickly sculpin) in Lake Washington

Fish species	n	Ln [MeHg]	Ln [∑DDT]	Ln [∑PCB]	Ln [∑CHL]	Ln (W)
Cutthroat trout	18	0.695 *	0.659 *	0.723 *	0.634 *	0.796 *
Northern pikeminnow	12	0.428	0.361	0.330	0.528	0.594 **
Yellow perch	25	0.587 *	0.787 *	0.370	0.748 *	0.815 *
Prickly sculpin	13	0.049	0.635.	0.242	0.484	0.207

Asterisks indicate significance levels of correlations. Correlations that were not significant have no asterisk ($p>0.05$). Contaminant concentrations are on a wet weight basis.
* $p\leq0.001$.
** $p\leq0.01$.

3.3. Pelagic fishes and invertebrates

Mercury and organochlorine concentrations in pelagic forage fishes were higher in March than for the same cohort in the previous October (Fig. 3). Organochlorine concentrations were correlated with seasonal body-lipid content in longfin smelt but not in stickleback or juvenile sockeye salmon (Fig. 3). For individual juvenile sockeye salmon, neither trophic position ($p=0.172$) nor carbon source ($p=0.224$) correlated significantly with mercury concentration.

Among pelagic invertebrates, methylmercury concentrations were highest in mysids, followed by *Daphnia*, *Leptodora*, and bulk zooplankton (Table 1). Seasonal variation in mercury concentration was evident in mysids (ANOVA; $p<0.001$) and bulk zooplankton (ANOVA; $p=0.015$), with the highest values in winter (24 μg/kg in mysids and 7 μg/kg in zooplankton) and the lowest in spring (8 μg/kg in mysids and 3 μg/kg in zooplankton). Zooplankton were dominated by omnivorous cyclopoid copepods in the winter sample and were predominantly herbivorous calanoid copepods (*Diaptomus)* and *Daphnia* in the spring. Organochlorines in pelagic invertebrates were often below or near detection limits (Table 1). ∑DDT was most frequently detected; total DDT ranged 5–10 μg/kg in mysids and 1–9 μg/kg in bulk zooplankton but seasonal differences were not statistically significant for mysids (ANOVA; $p=0.235$) or bulk zooplankton (ANOVA; $p=0.078$). PCBs were uniformly not detected in pelagic invertebrates, likely

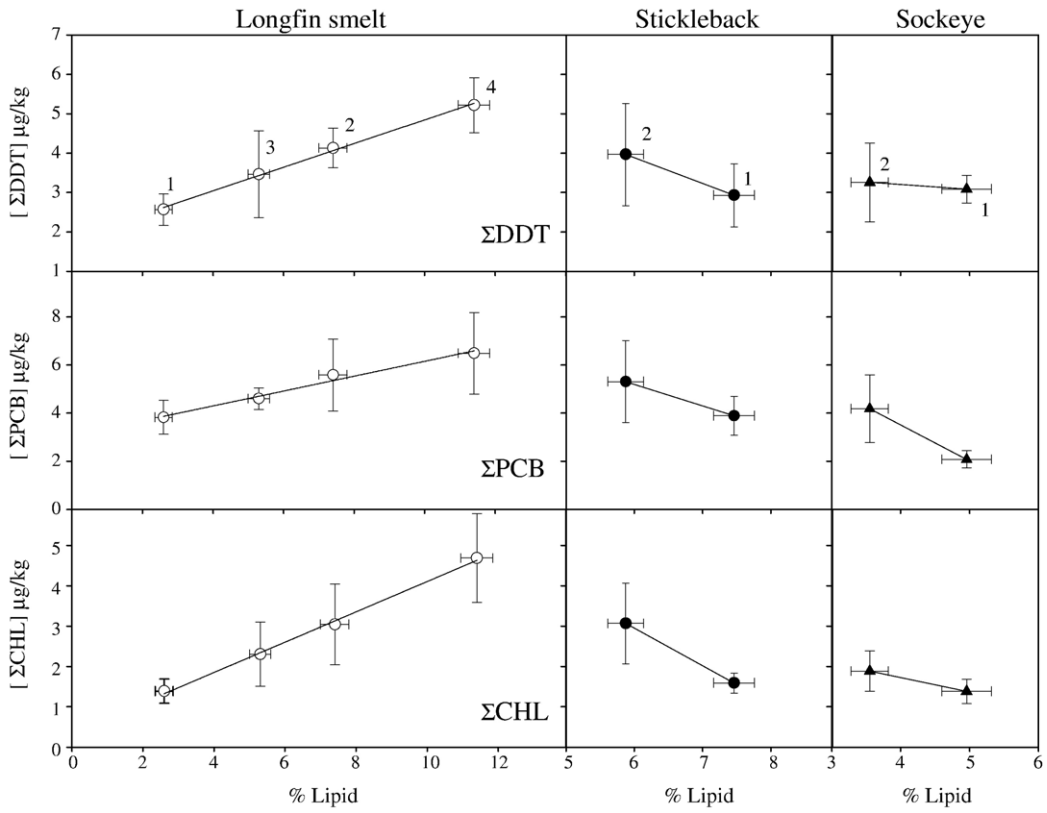

Fig. 3. Organochlorine concentration (natural log of µg/kg wet weight) as a function of lipid content in pelagic forage fishes captured during consecutive sampling periods in Lake Washington. Numbers associated with symbols represent their time of collection and apply to all panels: 1=age-0 fish in October, 2=age-1 fish in March, 3=age-1 fish in October, and 4=age-2 fish in March. Open circles are for longfin smelt, closed circles for stickleback, closed triangles for juvenile sockeye salmon. Error bars represent one standard deviation.

because of the relatively high analytical detection limits for PCB congeners in these samples (3 to 8 µg/kg). Total chlordane was only detected in one autumn bulk zooplankton sample (4 µg/kg). All *Daphnia* samples were below detection limits for ∑DDT (<3 µg/kg).

3.4. Benthic fish and invertebrates

Contaminant concentrations for prickly sculpin were not correlated with trophic position ($p=0.106$–0.935), and carbon source was only weakly correlated with mercury concentration ($r=-0.400$, $p=0.043$) but was not correlated with organochlorine concentrations (all $p=0.059$–0.586). Paired samples of contaminants and stable isotopes were not available for other benthic invertebrates.

Mercury concentrations were positively correlated with body length and weight for both prickly sculpin and signal crayfish, and with age for prickly sculpin (Table 3). Correlations with body weight were similar to but weaker than correlations with body length. Organochlorine concentrations in prickly sculpin generally increased with body length (and weight) and age (Table 1); however, the relationship was only significant for ∑PCB because small sculpin contained detectable levels of ∑PCB, but not ∑DDT or ∑CHL (Table 3). The positive correlation between ∑PCB and sculpin weight was slightly lower than the correlation with length. Percent lipid was not significantly correlated with any metric in prickly sculpin (Table 4).

Mercury concentrations were similar among the three littoral sites (ANCOVA, length as a covariate, $p=0.132$ for prickly sculpin; $p=0.369$ for crayfish; ANOVA, $p=0.297$ for trichopteran larvae). Organochlorine concentrations in large prickly sculpin (>90 mm) were also not significantly different among sites (ANOVA; $p=0.150$ for ∑DDT; $p=0.163$ for ∑PCB; $p=0.315$ for ∑CHL). None of the crayfish samples contained detectable levels of organochlorines, despite analytical

detection limits of 1–4 μg/kg. Trichopteran larvae were not analyzed for organochlorines due to insufficient sample mass.

4. Discussion

Indicators of trophic position have often been used to quantify biomagnification rates within species (Cabana et al., 1994; Kidd et al., 1995; Vander Zanden and Rasmussen, 1996; Kidd et al., 1998) and within food webs (Broman et al., 1992; Kidd et al., 1995, 2001; Kiriluk et al., 1995; Jarman et al., 1996; Atwell et al., 1998; Campbell et al., 2000; Power et al., 2002; Borga et al., 2004; Braune et al., 2005). A goal of biomagnification studies is to better understand the mechanisms of biomagnification in order to predict which food webs will experience higher rates of contaminant accumulation. Biomagnification rates can be highly variable among food webs, even for the same organochlorine congener (e.g., review by Hoekstra et al., 2003).

In Lake Washington, both age and trophic position contributed significantly to biomagnification of mercury and organochlorines in the food web. Based on the standardized regression coefficients, the relative importance of age and trophic position to biomagnification varied among contaminants; trophic position was similar to or more important than age for mercury, and was more important than age for ∑DDT and ∑CHL, whereas age was twice as important as trophic position for biomagnification of ∑PCB. To our knowledge, ours is the first study to quantify the relative contribution of age to biomagnification in a food web.

Given the importance of age to biomagnification in the Lake Washington food web, age may be important in explaining variability in rates of biomagnification observed among other food webs. Considering trophic position while ignoring age in the Lake Washington food web tended to explain less of the variation in contaminant concentrations and artificially attributed more dependence on trophic position. If trophic position does not overwhelm the influence of age on contaminant bioaccumulation within a food web, the age of individuals at the top of the trophic chain will affect the apparent rate of biomagnification in the food web. In other words, older individuals among apex predators will yield higher apparent rates of biomagnification in the food web and younger individuals among apex predators will yield lower apparent rates of biomagnification. Effects such as these could explain at least some of the variability in biomagnification rates observed among food webs. Therefore, while age is not typically considered in food web studies, for practical, economic, or logistical reasons, age should be acknowledged for the important role it may play in bioaccumulation in many food webs.

Predatory fishes in Lake Washington shift both trophic position and feeding habitat as they grow (Beauchamp et al., 1992; Nowak et al., 2004; McIntyre et al., 2006). All three predatory fishes included in this study shifted from feeding on invertebrates to fish prey, whereas trends in feeding habitat change differentially among the species; cutthroat trout and northern pikeminnow shifted from feeding in benthic to pelagic habitats, whereas yellow perch shifted from pelagic to benthic habitats. Lower rates of accumulation of contaminants with fish size and age of yellow perch compared with cutthroat trout and northern pikeminnow were probably related to the slower increase in trophic position as a function of size for this species (McIntyre et al., 2006). Studies that capture a shift to piscivory in a population find a concurrent increase in contaminant concentration, whether as a function of size or age (Maccrimmon et al., 1983; Mathers and Johansen, 1985; Driscoll et al., 1994; McIntyre et al., 2006) or trophic position (Vander Zanden and Rasmussen, 1996; Bowles et al., 2001). When the shift to piscivory is not captured or when there is no change in dietary habits, there tends to be no relationship between trophic position and contaminant concentration; for example, there was no relationship between δ^{15}N and concentrations of mercury or organochlorines among same-aged arctic charr that were already piscivorous (Dufour et al., 2001). Mercury concentration increased with δ^{15}N for omnivorous and piscivorous fishes in Lake Murray, Papua New Guinea, but not for planktivorous species, despite similar variability in δ^{15}N (Bowles et al., 2001). Similarly in Lake Washington, mercury and organochlorine concentrations were positively correlated with trophic position across the food web as a whole as well as for northern pikeminnow, cutthroat trout, and yellow perch, species that underwent ontogenetic shifts to piscivory. However, the relationship between trophic position and contaminant concentration was very weak or absent for prickly sculpin and juvenile sockeye salmon, species that did not shift towards piscivory for the size classes studied (McIntyre et al., 2006). Large shifts in trophic position make it easier to detect biomagnification in a species or a food web.

In Lake Washington, carbon stable isotope values were strongly correlated with concentrations of most contaminants within the predatory fishes. However, trends were not always in the same direction, a consequence of different ontogenetic shifts in feeding habitats among species rather than an indication of different rates

of bioaccumulation in pelagic or benthic habitats. The inconsistent trends among species due to their different ontogenies contributed to the lack of an influence of carbon source on contaminant concentrations at the food web level and emphasize the importance of exercising caution when interpreting results for single species.

Organochlorine concentrations have often been lipid-normalized in contaminant studies because it was believed that lipids helped determine the degree of bioaccumulation realized by individuals, and that normalized concentrations made inter- and intra-specific comparisons possible. Hebert and Keenleyside (1995) proposed lipid-normalizing only when the correlation between lipids and organochlorine concentration was significant, but this seems imprudent if the relationship is caused by an extraneous factor, a view also expressed by Stow et al. (1997). In Lake Washington, contaminant concentrations were positively correlated with percent lipid in cutthroat trout and yellow perch, but were not correlated with concentrations of any contaminant in northern pikeminnow despite a greater range in lipid content among pikeminnow (8.8%) than for cutthroat trout (8.1%) or yellow perch (5.9%). Mercury is much less lipophilic than are organochlorines, yet percent lipid was just as highly correlated with mercury concentrations as with organochlorine concentrations in predatory fishes. Furthermore, percent lipid was more highly correlated with fish size than with any contaminant concentration for all three species, suggesting that correlations between body lipid and organochlorine levels stem from the strong relationship of size with both lipid and organochlorine levels, not necessarily from a causal relationship between the two latter metrics. Finally, organochlorine concentrations in pelagic forage fishes did not uniformly follow changes in percent lipid, further suggesting that while lipid and organochlorines may co-vary in some cases, they are not necessarily causally related.

Potential sources of variability contributing to bioaccumulation in this food web that were not addressed explicitly include season, sex, and variable ^{15}N fractionation. Forage fishes were collected from Lake Washington both in spring and fall but predatory fish were collected mostly in the late summer and fall. Because not all samples were collected at the same time, seasonal differences may have increased contaminant variability in this food web. The effect of sex on contaminant variability would be greatest among the largest individual predatory fish. The effect of sex could not be adequately considered in our study for three reasons — we only sampled 10 large individuals of each predatory fish species, gonads were relatively undeveloped at the time of year when most samples were collected (late summer/fall), and the predatory fishes in Lake Washington exhibit sexual dimorphism such that the largest fish caught for all three predatory fish species tended to be all female. Finally, it is usually assumed that the $\delta^{15}N$ value of a predator is approximately 3.4‰ greater than its diet, but the trophic increase in $\delta^{15}N$ may actually be variable between individuals or between species within a food web. For example, in freshwater omnivorous fishes, trophic level fractionations of 2.3–3.8‰ have been observed (Vander Zanden and Rasmussen, 2001). Although we do not know the degree of differential fractionation of ^{15}N in the food web of Lake Washington, a stable isotope mixing model estimated that the trophic level fractionation for northern pikeminnow in Lake Washington might be lower than average, possibly as low as 2.1‰ (McIntyre et al., 2006). Because the same degree of fractionation of ^{15}N between an organism and its diet is assumed for all organisms in a food web when using stable nitrogen isotopes to quantify biomagnification, differential trophic fractionation within the food web would obviously increase variability in contaminant concentrations as a function of trophic position, whether or not age is taken into consideration. More species-specific measurements of trophic level fractionation of stable nitrogen isotopes are needed before we can begin to assess the practical effect of variable fractionation rates on assessing biomagnification rates of persistent contaminants in food webs.

In summary, age and trophic position were the most important determinants of contaminant concentration in the food web of Lake Washington. Lipids were correlated with contaminant concentrations, but inconsistent trends allowed us to conclude that they were not a determinant of bioaccumulation. Feeding habitat had little or no influence on contaminant bioaccumulation in this food web and spatial variability is likely not important in this system. Although bioaccumulation in some food webs may be complicated by significant contributions from factors other than age and trophic position, the results of this study demonstrate that age should be considered explicitly in food web bioaccumulation studies.

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References

Anderson GC. A limnological study of the seasonal variation of phytoplankton populations. Ph.D. Dissertation. University of Washington, Seattle, Washington, U.S.A. 1954.

Atwell L, Hobson KA, Welch HE. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. Can J Fish Aquat Sci 1998;55:1114–21.

Beauchamp DA, Vecht SA, Thomas GL. Temporal, spatial, and size-related foraging of wild cutthroat trout in Lake Washington. Northwest Sci 1992;66:149–59.

Beauchamp DA, Sergeant CJ, Mazur MM, Scheuerell JM, Schindler DE, Scheuerell MD, et al. Spatial-temporal dynamics of early feeding demand and food supply for sockeye salmon fry in Lake Washington. T Am Fish Soc 2004;133:1014–32.

Borga K, Fisk AT, Hoekstra PF, Muir DCG. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. Environ Toxicol Chem 2004;23:2367–85.

Bowles KC, Apte SC, Maher WA, Kawei M, Smith R. Bioaccumulation and biomagnification of mercury in Lake Murray, Papua New Guinea. Can J Fish Aquat Sci 2001;58:888–97.

Braune BM, Outridge PM, Fisk AT, Muir DCG, Helm PA, Hobbs K, et al. Persistent organic pollutants and mercury in marine biota of the Canadian Arctic: an overview of spatial and temporal trends. Sci Total Environ 2005;351:4-56.

Brocksmith R. Abundance, feeding ecology, and behavior of a native piscivore northern pikeminnow (*Ptychocheilus oregonensis*) in Lake Washington. M.S. thesis. University of Washington, Seattle, Washington, U.S.A. 1999.

Broman D, Naf C, Rolff C, Zebuhr Y, Fry B, Hobbie J. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in 2 food-chains from the northern Baltic. Environ Toxicol Chem 1992;11:331–45.

Bruggeman WA, Opperhuizen A, Wijbenga A, Hutzinger O. Bioaccumulation of super-lipophilic chemicals in fish. Toxicol Environ Chem 1984;7:173–89.

Cabana G, Tremblay A, Kalff J, Rasmussen JB. Pelagic food-chain structure in Ontario lakes — a determinant of mercury levels in lake trout (*Salvelinus namaycush*). Can J Fish Aquat Sci 1994;51:381–9.

Campbell LM, Schindler DW, Muir DCG, Donald DB, Kidd KA. Organochlorine transfer in the food web of subalpine Bow Lake, Banff National Park. Can J Fish Aquat Sci 2000;57:1258–69.

Chigbu P, Sibley TH. Predation by longfin smelt (*Spirinchus thaleichthys*) on the mysid Neomysis mercedis in Lake Washington. Freshw Biol 1998a;40:295–304.

Chigbu P, Sibley TH. Feeding ecology of longfin smelt (*Spirinchus thaleichthys* Ayres) in Lake Washington. Fish Res 1998b;38:109–19.

Crimmins BS, Brown PD, Kelso DP, Foster GD. Bioaccumulation of PCBs in aquatic biota from a tidal freshwater marsh ecosystem. Arch Environ Contam Toxicol 2002;42:396–404.

Davis JA, May MD, Greenfield BK, Fairey R, Roberts C, Ichikawa G, et al. Contaminant concentrations in sport fish from San Francisco Bay, 1997. Mar Pollut Bull 2002;44:1117–29.

Deniro MJ, Epstein S. Mechanism of carbon isotope fractionation associated with lipid-synthesis. Science 1977;197:261–3.

DeVries DR, Frie RV. Determination of age and growth. In: Murphy BR, Willis DW, editors. Fisheries Techniques. 2nd edition. Bethesda, MD, USA: American Fisheries Society; 1996. p. 483–512.

Driscoll CT, Yan C, Schofield CL, Munson R, Holsapple J. The mercury cycle and fish in the Adirondack lakes. Environ Sci Technol 1994;28:A136–43.

Dufour E, Gerdeaux D, Corvi C, Khim-Heang S, Mariotti A. Assessment of the contaminant concentration variability among Lake Geneva arctic char using stable isotopic composition (delta N-15 and delta C-13). Environ Toxicol 2001;16:185–91.

Farkas A, Salanki J, Specziar A. Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. Water Res 2003;37:959–64.

Fisk AT, Hoekstra PF, Borga K, Muir DCG. Biomagnification. Mar Pollut Bull 2003;46:522–4.

France RL. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnol Oceanogr 1995;40:1310–3.

Gobas FAPC, Morrison HA. Bioconcentration and biomagnification in the aquatic environment. In: Boethling RS, Mackay D, editors. Handbook of property estimation methods for chemicals: Environmental health and sciences. Boca Raton, FL, U.S.A: Lewis; 2000. p. 189–231.

Gray JS. Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull 2002;45:46–52.

Hall BD, Bodaly RA, Fudge RJP, Rudd JWM, Rosenberg DM. Food as the dominant pathway of methylmercury uptake by fish. Water Air Soil Poll 1997;100:13–24.

Hebert CE, Keenleyside KA. To normalize or not to normalize — fat is the question. Environ Toxicol Chem 1995;14:801–7.

Hoekstra PF, O'Hara TM, Fisk AT, Borga K, Solomon KR, Muir DCG. Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort–Chukchi Seas. Environ Pollut 2003;124:509–22.

Jackson LJ, Carpenter SR, Manchester-Neesvig J, Stow CA. PCB congeners in Lake Michigan coho (*Oncorhynchus kisutch*) and Chinook (*Oncorhynchus tshawytscha*) salmon. Environ Sci Technol 2001;35:856–62.

Jarman WM, Hobson KA, Sydeman WJ, Bacon CE, McLaren EB. Influence of trophic position and feeding location on contaminant levels in the gulf of the farallones food web revealed by stable isotope analysis. Environ Sci Technol 1996;30:654–60.

Johnston TA, Fisk AT, Whittle DM, Muir DCG. Variation in organochlorine bioaccumulation by a predatory fish: gender, geography, and data analysis methods. Environ Sci Technol 2002;36:4238–44.

Kidd KA, Hesslein RH, Fudge RJP, Hallard KA. The influence of trophic level as measured by delta-N-15 on mercury concentrations in fresh-water organisms. Water Air Soil Poll 1995;80:1011–5.

Kidd KA, Schindler DW, Hesslein RH, Muir DCG. Effects of trophic position and lipid on organochlorine concentrations in fishes from subarctic lakes in Yukon Territory. Can J Fish Aquat Sci 1998;55:869–81.

Kidd KA, Bootsma HA, Hesslein RH, Muir DCG, Hecky RE. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: importance of trophic level and carbon source. Environ Sci Technol 2001;35:14–20.

Kiriluk RM, Servos MR, Whittle DM, Cabana G, Rasmussen JB. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Can J Fish Aquat Sci 1995;52:2660–74.

Kline TC, Wilson WJ, Goering JJ. Natural isotope indicators of fish migration at Prudhoe Bay, Alaska. Can J Fish Aquat Sci 1998;55:1494–502.

Lange TR, Royals HE, Connor LL. Mercury accumulation in largemouth bass (*Micropterus salmoides*) in a Florida lake. Arch Environ Contam Toxicol 1994;27:466–71.

Leblanc GA. Trophic level differences in the bioconcentration of chemicals — implications in assessing environmental biomagnification. Environ Sci Technol 1995;29:154–60.

Maccrimmon HR, Wren CD, Gots BL. Mercury uptake by lake trout, *Salvelinus namaycush*, relative to age, growth, and diet in Tadenac Lake with comparative data from other Precambrian Shield lakes. Can J Fish Aquat Sci 1983;40:114–20.

Madenjian CP, Noguchi GE, Haas RC, Schrouder KS. Sexual difference in polychlorinated biphenyl accumulation rates of walleye (*Stizostedion vitreum*). Can J Fish Aquat Sci 1998;55:1085–92.

Manchester-Neesvig JB, Valters K, Sonzogni WC. Comparison of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in Lake Michigan salmonids. Environ Sci Technol 2001;35:1072–7.

Mathers RA, Johansen PH. The effects of feeding ecology on mercury accumulation in walleye (*Stizostedion vitreum*) and pike (*Esox lucius*) in Lake Simcoe. Can J Zool 1985;63:2006–12.

Mazur MM. Linking visual foraging with temporal prey distributions to model trophic interactions in Lake Washington. Ph.D. Dissertation. University of Washington, Seattle, Washington, U.S.A. 2004.

McConnaughey T, McRoy CP. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Mar Biol 1979;53:257–62.

McIntyre JK. Bioaccumulation of mercury and organochlorines in the food web of Lake Washington. M.Sc. thesis. University of Washington, Seattle, Washington, U.S.A.; 2004.

McIntyre JK, Beauchamp DA, Mazur MM, Overman NC. Ontogenetic trophic interactions and bentho-pelagic coupling in Lake Washington: evidence from stable isotopes and diet analysis. T Am Fish Soc 2006;135:1312–28.

Minagawa M, Wada E. Stepwise enrichment of N-15 along food-chains — further evidence and the relation between delta-N-15 and animal age. Geochim Cosmochim Acta 1984;48:1135–40.

Nicoletto PF, Hendricks AC. Sexual differences in accumulation of mercury in four species of centrarchid fishes. Can J Zool 1988;66:944–9.

Niimi AJ. Biological and toxicological effects of environmental contaminants in fish and their eggs. Can J Fish Aquat Sci 1983;40:306–12.

Nowak GM, Quinn TP. Diel and seasonal patterns of horizontal and vertical movements of telemetered cutthroat trout in Lake Washington, Washington. T Am Fish Soc 2002;131:452–62.

Nowak GM, Tabor RA, Warner EJ, Fresh KL, Quinn TP. Ontogenetic shifts in habitat and diet of cutthroat trout in Lake Washington, Washington. North Am J Fish Manage 2004;24:624–35.

Pinnegar JK, Polunin NVC. Differential fractionation of delta C-13 and delta N-15 among fish tissues: implications for the study of trophic interactions. Funct Ecol 1999;13:225–31.

Power M, Klein GM, Guiguer K, Kwan MKH. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. J Appl Ecol 2002;39:819–30.

Raldua D, Ferrando P, Duran C, Pedrocchi C. The influence of place of capture, sex, and season on the organochlorine pesticide content in barbel (*Barbus graellsi*) from northeastern Spain. Chemosphere 1997;35:2245–54.

Randall DJ, Connell DW, Yang R, Wu SS. Concentrations of persistent lipophilic compounds in fish are determined by exchange across the gills, not through the food chain. Chemosphere 1998;37:1263–70.

Scott JB, Steward CR, Stober QJ. Effects of urban-development on fish population-dynamics in Kelsey Creek, Washington. T Am Fish Soc 1986;115:555–67.

Stapleton HM, Masterson C, Skubinna J, Ostrom P, Ostrom NE, Baker JE. Accumulation of atmospheric and sedimentary PCBs and toxaphene in a Lake Michigan food web. Environ Sci Technol 2001;35:3287–93.

Stow CA. Factors associated with PCB concentrations in Lake Michigan salmonids. Environ Sci Technol 1995;29:522–7.

Stow CA, Jackson LJ, Amrhein JF. An examination of the PCB:lipid relationship among individual fish. Can J Fish Aquat Sci 1997;54:1031–8.

Thomann RV. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ Sci Technol 1989;23:699–707.

US EPA. 2004 National listing of fish advisories. EPA-823-F-05-004. U.S. Environmental Protection Agency, Office of Water; 2005. http://www.epa.gov/waterscience/fish/advisories/fs2004.pdf.

Vander Zanden MJ, Rasmussen JB. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecol Monogr 1996;66:451–77.

Vander Zanden MJ, Rasmussen JB. Variation in delta N-15 and delta C-13 trophic fractionation: implications for aquatic food web studies. Limnol Oceanogr 2001;46:2061–6.

Vanderford MJ, Hamelink JL. Influence of environmental factors on pesticide levels in sport fish. Pestic Monit J 1977;11:138–45.

Ward SM, Neumann RM. Seasonal variation in concentrations of mercury in axial muscle tissue of largemouth bass. North Am J Fish Manage 1999;19:89–96.