North Coast Cascades Network
Science Learning Network
Grant Report
Cooperative Agreement No. H8W07110001

A Stable Isotope Approach to Understanding Contaminant Distribution in Food Webs of Montane Lakes

Ariana Chiapella, Angela Strecker (PI)
Portland State University, Center for Lakes and Reservoirs

Executive Summary

This study investigates the variables that influence contaminant levels in fish from mountain lakes in North Cascades National Park, as well as Olympic and Mount Rainier National Parks. While the original goal was to assess differences in PCB distribution between fishless and fish-containing lakes, the low biomass of invertebrate species in the lakes rendered this goal unobtainable. In addition, the method used for the processing and analysis of PCBs was not sufficient for these systems. As a result, the focus of the project shifted to mercury (Hg) as the contaminant of interest (which requires much less biomass for analysis), and the variables that affect its accumulation in fish. Mercury can supply similar information as PCBs because it is persistent, and thus follows trophic pathways, resulting in higher concentrations at higher trophic positions (Morel et al. 1998, McIntyre & Beauchamp 2007). In 2014, we sampled food webs from 8 lakes in North Cascades National Park, and in 2015 we sampled 16 lakes – 6 in Mount Rainier, 6 in Olympic, and 4 in North Cascades National Parks. We hypothesized that there would be a relationship between fish feeding habitat and THg burden, with benthic diets leading to higher concentrations of THg than pelagic diets. However, the opposite was true for most lakes – pelagic diets were typically indicative of higher Hg. In addition, factors such as water temperature and lake morphometry were also important. This information provides insight into which lakes are safest for angling and fish consumption, and may also be useful for informing decisions around mountain lake fisheries management.

Introduction

Aquatic food web structure and the flow of energy through and across trophic levels is a concept ecologists have been striving to comprehend for decades (Schindler and Lubetkin 2004). An understanding of ecosystem structure and trophic dynamics is vital for determining how humans are affecting the environment. For instance, by evaluating how anthropogenic stressors affect aquatic systems, the fate of ecosystem health and human health may be determined (Figueiredo et al. 2013). Persistent pollutants are of particular interest because they accumulate in aquatic food webs, and often have negative health effects for both wildlife and humans. However, they can also be used as a tracer to better understand food web structure and energy flow (Rasmussen & Vander Zanden 2004). For example, polychlorinated biphenyls (PCBs) and mercury (Hg) bind to lipid and protein structures, and are therefore transferred along energy pathways. Using contaminants like PCBs and Hg as tools to understand food web dynamics is a technique that could be of particular use in alpine and sub-alpine lakes.

Montane lakes are sensitive ecosystems, and their response to stressors can be early indicators of how more complex systems may be affected. The simple food web structure in high-elevation lakes provides for an optimum study system, with fewer confounding variables. Additionally, these lakes are important to understand because they are the lifelines of ecosystems downstream. Similar to arctic regions, mountains receive inputs of contaminants due to cold-condensing atmospheric deposition (Kallenborn 2006). These seemingly pristine ecosystems unfortunately suffer exposure to the same chemicals as urban watersheds, and PCBs and Hg are just two of many chemical compounds. These chemicals can also accumulate in fish, because many mountain lakes have been stocked with non-native trout, despite these systems being historically fishless.

Trout invasions in historically fishless systems have been shown to have repercussions on a community-wide level. Amphibian and macroinvertebrate populations decline (Knapp et al. 2001), large-bodied, grazing zooplankton populations are diminished, and phytoplankton populations surge (Eby et al. 2006). Meanwhile, trout transport nutrients from the benthos (where they feed on macroinvertebrates) to the pelagic zone, further propagating phytoplankton growth and increasing dissolved organic carbon (DOC) concentrations (Leavitt et al. 1994, Eby et al. 2006). These altered conditions also favor higher rates of contaminant bioaccumulation in food webs (St. Louis et al. 1994, Ullrich et al. 2001). Since trout are top predators, they can accumulate relatively high levels of contaminants in seemingly pristine ecosystems such as mountain lakes. However, the level of contaminants like PCBs and Hg in trout is unpredictable because while some factors like fish age and trophic position are known to affect contaminant levels (e.g. McIntyre & Beauchamp 2007), the effect of environmental variables are poorly understood in mountain lakes. These factors can include lake water quality conditions (like temperature and nutrients) and morphometry (Clayden et al. 2013, Lavoie et al. 2013), as well as trophic dynamics within the food web. The effect of these environmental factors may vary, depending on the ecological context of the system (Clements et al. 2012). There is also evidence that fish diet can influence mercury concentrations (Eagles-Smith et al. 2008, Stewart et al. 2008)

Stable isotopes can be a useful tool in understanding how food web dynamics may influence contaminant levels in fish. Stable carbon isotopes can be used to identify feeding habitat (e.g. benthic, littoral, profundal), while nitrogen isotopes can be used to determine trophic position. By using stable carbon and nitrogen isotopes in mixing models, relationships between contaminant burden and feeding habitat can be explored. This study evaluates how lake water quality, morphometry, and trophic dynamics interact to influence contaminant levels in fish from mountain lakes. Specifically, we sought to determine (1) which trophic and environmental variables are important predictors of mercury in mountain lakes, and (2) if these factors change from a landscape (state-wide, multiple mountain ranges) to local (discrete area- i.e. National Park - within a single mountain region) scale. We predicted that in addition to well-known environmental predictors (e.g. temperature, nutrients), trophic factors (such as diet) would also be important predictors of Hg concentration in fish, and that these factors would be more important at the local scale, due to the importance of ecological context (Clements et al. 2012).

Methods

Field collection and laboratory analysis

Food web components were sampled from twenty lakes in 2014 and 2015 in North Cascades (NOCA), Mount Rainier (MORA), and Olympic (OLYM) National Parks (MORA & OLYM in 2015 only) (Table 1). Lakes were sampled in August and September, using stratified random sampling along an elevation gradient; sampling started at the lowest elevations, and progressed in altitude, since lakes at higher elevations thaw later in the season. A YSI was used to record the temperature profile of the lake and determine the epilimnion (mixed surface layer, <1°C per meter, Wetzel 2001). Percent dissolved oxygen (%DO), temperature, pH, and specific conductance (SPC) were measured with a YSI probe at each lake (pH and SPC in 2015 only). Integrated lake water samples for total phosphorus (TP), total nitrogen (TN), and chlorophyll-*a* (chl. *a*) were collected from the epilimnion using tygon tubing. A known volume of lake water was concentrated onto glass fiber filters using a vacuum pump to measure total and edible (<35 µm) fractions of chlorophyll-*a*. Periphyton was scrubbed off of rocks or logs with a toothbrush in a plastic bin, then concentrated onto glass fiber filters using a vacuum pump. Filters were stored in aluminum foil in Whirl-pack bags on ice or snow.

Macroinvertebrates were collected from each type of substrate (rocks, sediment, macrophytes) in the littoral zone of each lake using a D-frame kick net. Net contents were placed in plastic bins and invertebrates were picked out and sorted using ice cube trays. Invertebrates were then placed into 100-ml glass jars with Teflon-lined lids. Zooplankton were collected with an 80-µm mesh zooplankton net at the deep part of each lake. Zooplankton community composition samples were collected via vertical tows, and preserved in 120-ml plastic jars to a final concentration of 70% ethanol. Zooplankton samples for stable isotope and mercury analysis were collected via horizontal tows, and stored in 100-ml glass jars with Teflon-lined lids. Fish were collected via multi-filament mesh gill nets, which were placed perpendicular to shore and then monitored until at least five fish were caught. Fish were removed, measured, and weighed, then wrapped in foil and kept on snow or ice in the field, and placed in a freezer upon return. For each site, elevation and surface area (hectares) was determined using ArcGIS.

Chlorophyll *a* was analyzed at the Center for Lakes and Reservoirs at Portland State University (EPA method 445.0), and nutrient analysis for total N and total P were conducted by the University of Washington Marine Chemistry Laboratory (EPA method SM 4500-P J). Samples for isotope and contaminant analyses were freeze-dried, then homogenized with a mortar and pestle. Subsamples of the homogenate of each sample were weighed into tin capsules and analyzed for stable carbon and nitrogen isotopes at the Yale Isotope lab (2014) and UC Davis Isotope Lab (2015) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Mercury analysis was conducted at USGS in Corvallis, OR. Fish were analyzed for total Hg (THg), since the majority of Hg in salmonid species is the persistent and bioavailable form, methylmercury (MeHg) (Bloom 1992). Invertebrates were analyzed for MeHg. Total Hg was analyzed using a direct mercury analyzer (DMA), and MeHg was determined after digestion with KOH–ethanol, followed by aqueous phase ethylation, then gas chromatography separation, isothermal decomposition, and analysis by atomic fluorescence (as in Stewart et al. 2008). Percent moisture content of fish muscle varies between fish species, therefore, to standardize concentrations, Hg is reported based on dry-weight (ng/g), unless specified otherwise. Samples for PCBs were extracted using the Agilent QuEChERS method. Extractions were then transferred to GC vials then analyzed on a GC-ECD at Pacific University in Forest Grove, OR. Chromatograph peaks were quantified using calibration curve, by comparing the peak areas of PCBs to those of the standard solutions.

Statistical methods

Statistical analyses were completed using R, version 3.2.1 (© 2015 The R Foundation for Statistical Computing). Lakes were baselined for stable nitrogen isotopes by calculating trophic position of each fish (Equation 1); this allows comparison of fish from all lakes, and corrects for variation in the nitrogen values of primary producers from different lakes (Post 2002).

Equation 1. Trophic position (TP) calculation (Post 2002). λ represents the trophic position of the organism used to estimate $\delta^{15}N_{B1}$ (in this case, a primary consumer was used, so $\lambda = 2$). $\delta^{15}N_{C}$ is the nitrogen signature of the consumer of interest (in this case, fish), $\delta^{15}N_{B1}$ is the nitrogen signature of the base of food web 1 (in this case, a benthic primary consumer), and $\delta^{15}N_{B2}$ is the nitrogen signature of base 2 (in this case, zooplankton). α is the estimated proportion of nitrogen derived from the base of food web 1 (see Equation 2), and Δ_n is the enrichment of nitrogen per trophic level (typically 3.5 ‰, Peterson & Fry 1987).

$$TP = \lambda + (\delta^{15}N_C - (\delta^{15}N_{B1} \times \alpha + \delta^{15}N_{B2} \times (1-\alpha))) / \Delta_n$$

After transforming variables for normality, a series of one-way ANOVAs were used to determine differences in THg concentration and trophic position between fish species, and between parks. Tukey's Honest Significant Difference (HSD) post-hoc tests were used to determined which species and parks were significantly different from each other. Stable carbon and nitrogen isotopes were plotted on isotope biplots to visually depict trophic position and functional feeding in isotope space.

The proportion of each fish's diet (from benthic and pelagic resources) was estimated using an isotope mixing model, which uses the carbon signatures of consumers and their prey to partition dietary resources. A two-end member mixing model (Equation 2) – with zooplankton as pelagic end-member and benthic primary consumer (Ephemeroptera or certain species of Trichoptera) as benthic end-member – was used to assess feeding habits of fish (Post 2002), with diets represented as percent benthic diet).

Equation 2. Two-end member mixing model (Post 2002). α is the proportion of the consumer's diet from food web 1 (in this case, the benthic food web). $\delta^{13}C_C$ represents the carbon signature of the consumer of interest (fish), $\delta^{13}C_{B1}$ is the carbon signature of the base of food web 1 (in this case, a benthic primary consumer), and $\delta^{13}C_{B2}$ is the carbon signature of base 2 (in this case, zooplankton).

$$\alpha = (\delta^{13}C_C - \delta^{13}C_{B2}) / (\delta^{13}C_{B1} - \delta^{13}C_{B2})$$

These models did not include terrestrial diet sources, and thus make the assumption that diet is 100% lake-derived, which likely introduces some error. In order to remove for the influence of trophic position on THg concentrations in analyses, a simple linear model was used to correct THg concentrations in fish for trophic position. Mercury concentrations in fish were corrected for trophic position before analyses by taking the residual values from a THg ~ trophic position model. The residuals were used as the trophic position-corrected THg concentrations of fish. The corrected concentrations were used along with the isotope mixing model results in mixed effects models (lme4 package, R version 3.2.1), with lake as a random factor, to determine the influence of food web dynamics and lake variables on THg concentrations in fish (Table 1). A combination of Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) were used for model selection (lmerTest package, R version 3.2.1.). Four final mixed effects models were used to determine which trophic and environmental factors were important predictors of THg in fish at the landscape scale (all lakes, model 1), as well as at the more local, park scale (lakes within each park, models 2-4).

Table 1. Model parameters used in the linear mixed-effects models. Minimum, maximum, and mean values are given for each parameter at landscape and local (for each park) scales. Factor types include trophic (relating to fish characteristics and diet), and environmental (Env). Some parameters were not available for every lake, due to sampling and analysis difficulties. For instance, maximum depth values were only available for one lake in OLYM, and TN & TP values were only available for one lake in NOCA (denoted by *). Since the species parameter was categorical, no numerical values are listed. Since rainbow trout were present in all parks, this species was used as the predictor variable in the model (e.g. does THg concentration differ if the species is, or is not, a rainbow trout).

Parameter	**Type**	**Landscape**			**NOCA**			**MORA**			**OLYM**		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
% benthic diet	Trophic	0	100	67	6.5	100	67	0	100	64	15	100	70
fish weight (g)	Trophic	2	360	143	40	360	215	12	210	85	2	118	61
fish length (mm)	Trophic	20	339	178	52	339	184	60	296	186	20	214	158
species	Trophic	--	--	--	--	--	--	--	--	--	--	--	--
elevation (m)	Env	1227	1747	1418	1227	1747	1378	1373	1680	1498	1372	1540	1394
surface area (ha)	Env	0.5	55	6.1	1.6	55.1	11.6	1.6	7.6	3.4	0.5	9.2	4.6
temperature (°C)	Env	6.5	19	14.5	6.5	19	14.2	13.7	17.9	15.7	8.3	13.4	11.9
max depth (m)	Env	2.8	79	13.5	4.9	79	19.7	2.8	12.3	5.8	4.2*	--	--
% DO	Env	101	118	107	105	112	108	105	118	111	101	110	104
chl. *a* (total)	Env	0.15	2.21	0.52	0.18	0.95	0.60	0.15	0.50	0.32	0.18	2.21	0.65
SPC (µS)	Env	4.7	139	53.3	19	97.8	35.8	8.8	56.7	28	41.9	139	81.9
TN (µg/L)	Env	111	249	179	161*	--	--	111	248	153	152	249	199
TP (µg/L)	Env	3.4	11.8	7	4.9*	--	--	3.4	7.8	6.1	5.7	11.8	7.8
pH	Env	5.3	7.8	6.8	6.3	7.0	6.7	6.2	7.4	6.6	7.0	7.8	7.4

Results

Between 1 and 7 fish were collected from each lake. Fish species included rainbow trout (*Oncorhynchus mykiss*), Eastern brook trout (*Salvelinus fontinalis*), and cutthroat trout (*Oncorhynchus clarkii*), however most lakes only contained one species of fish. Only cutthroat and rainbow trout were caught in North Cascades lakes, whereas only rainbow and Eastern brook trout were caught in Mount Rainier and Olympic lakes. Invertebrate species typically consisted of some combination of mayflies (Ephemeroptera Baetidae), dipteran flies (Diptera Chironomidae and sometimes Tabanidae), dragonflies (Odonata Aeshnidae & Corduliidae), caddisflies (Trichoptera Limnephilidae & Polycentropodidae), and aquatic beetles (coleopteran dytiscidae). Some lakes contained pea clams (Bivalvia Sphaeridae), a long-lived filter feeder, and many lakes from Olympic, as well as Deadwood lake from Mount Rainier, contained amphipods (Amphipoda Gammarus). In many lakes, it was not possible to collect enough biomass for stable isotope and contaminant analyses of some invertebrates. Therefore, we did not have values for zooplankton from Lower Anderson, Bench, Louise, Lower Palisades, Snow, and Lunch Lake, and were not able to produce isotope biplots for Doubtful, Hidden, and Upper Watson lakes (Table 2). In addition, we were unable to collect enough biomass for isotope analyses of periphyton in some lakes. At Lunch Lake, sample collection was incomplete due to hazardous weather.

Table 2. Summary of each lake, including fish species collected, invertebrates analyzed (excludes invertebrates that were present, but with not enough biomass for contaminant analyses), and average dry weight THg concentration of fish (+/- SD). Values with a * indicate a sample size of one, thus no standard deviation could be calculated.

Park	Lake	Fish species	Invertebrates analyzed	Average fish [Hg] (ng/g)
NOCA	Lower Anderson	*O. mykiss*	Odonata, Ephemeroptera, Diptera, Coleoptera	114 (+/- 34.7)
NOCA	Doubtful	*O. clarkii*	none	460*
NOCA	Hidden	*O. mykiss*	none	170*
NOCA	Monogram	*O. clarkii*	zooplankton, Coleoptera, Trichoptera, Diptera	233 (+/- 175)
NOCA	Sauk	*O. mykiss*	zooplankton, Ephemeroptera, Diptera, Coleoptera, Bivalvia	240 (+/- 86)
NOCA	Thornton	*O. clarkii*	none	342 (+/- 155)
NOCA	Lower Watson	*O. mykiss*	zooplankton, Odonata, Ephemeroptera, Diptera, Trichoptera	199 (+/- 87)
NOCA	Upper Watson	*O. mykiss*	none	202 (+/- 31)
MORA	Bench	none	Odonata, Ephemeroptera, Diptera, Bivalvia	n/a
MORA	Deadwood	*O. mykiss*	zooplankton, Odonata, Coleoptera, Amphipoda, Diptera	442 (+/- 137)
MORA	Louise	*S. fontinalis*	Odonata, Diptera, Coleoptera, Bivalvia	255 (+/- 67)
MORA	LP-19	*S. fontinalis*	zooplankton	612 (+/- 420)
MORA	Lower Palisades	*S. fontinalis*	Diptera, Coleoptera, Bivalvia	352 (+/- 154)
MORA	Snow	*S. fontinalis*	Odonata, Diptera, Coleoptera, Bivalvia	163 (+/- 82)
OLYM	Gladys	*S. fontinalis*	zooplankton, Diptera, Coleoptera, Bivalvia	216 (+/- 56)
OLYM	Grand	*S. fontinalis*	zooplankton, Odonata, Coleoptera, Trichoptera, Amphipoda	369 (+/- 75)
OLYM	Upper Lena	*O. mykiss*	zooplankton, Amphipoda, Trichoptera, Bivalvia	231 (+/- 71)
OLYM	Lunch	*S. fontinalis*	Diptera, Coleoptera	259 (+/- 290)
OLYM	Moose	*S. fontinalis*	zooplankton, Amphipoda, Trichoptera, Bivalvia, Diptera	352*
OLYM	PJ	*O. mykiss*, *S. fontinalis*	zooplankton, Ephemeroptera, Diptera, Amphipoda	472 (+/- 377

Stable Isotopes: trophic position and dietary composition of fish

There were no significant differences in trophic position (mean = 3.5) between fish species from all lakes ($p= 0.75$, $F= 0.29$, $df= 2$, one-way ANOVA, Figure 1B). There were, however, significant differences in trophic position between parks ($p= 0.01$, one-way ANOVA, Figure 1A)); fish from Mount Rainier held higher trophic positions than fish from Olympic ($p= 0.01$, Tukey's HSD post-hoc test) and North Cascades, although the difference was only weakly significant ($p=0.07$, Tukey's HSD post-hoc test). There were no differences in fish trophic position between OLYM and NORA ($p=0.92$, Tukey's HSD post-hoc test). On average, fish diet was predominantly composed of benthic organisms, as determined by the two-end member mixing models (mean = 67% benthic), but this differed by park (Figure 2). At North Cascades and Mount Rainier, rainbow trout fed on a significantly more benthic diet than cutthroat (NOCA, $p= 0.03$, $t= -2.44$, $df= 12$) or Eastern brook trout (MORA, $p= 0.3*10^{3}$, $t= -4.29$, $df= 23$), respectively, and at Olympic, Eastern brook trout fed on a more benthic diet compared to rainbow trout ($p= 0.02$, $t= 2.49$, $df= 17$) (Figure 2C).

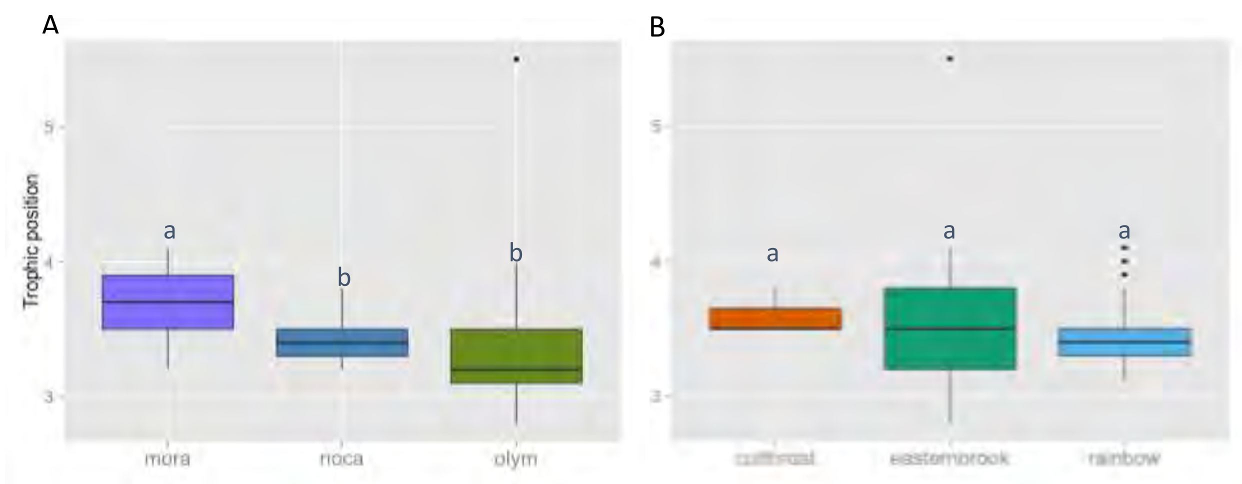


Figure 1. Comparison of trophic position across the three parks (A), and between the three trout species (B). Mean trophic position was 3.5. Fish from Mount Rainier lakes had significantly higher trophic positions than fish from the other parks, but there were no significant differences in trophic position between species.

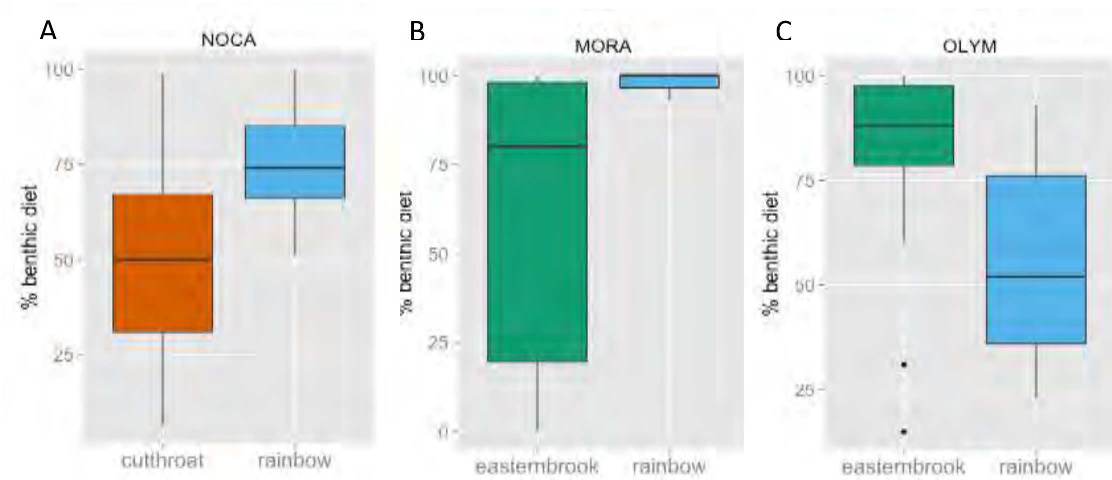


Figure 2. Proportion of fish diet from benthic origin, as determined by two-end member isotope mixing models, by species (in order to fit axis labels, "trout" has been removed from fish names). Fish from North Cascades (A), Mount Rainier (B), and Olympic (C) National Parks typically derived the majority of their diet from benthic sources (67%). However, diet proportions varied both by species, as well as within species (middle line=median, whiskers = upper and lower quartiles, dots = outliers).

PCBs

We were not able to collect sufficient biomass (5-8g wet weight) of invertebrates to analyze for PCBs (Serrano et al. 2003, Norli et al. 2011). In fish, calculated PCB concentrations were unrealistically high: 23.3 – 349.3 ng/g (ppb) compared to <20 ng/g in other studies on high elevation lakes (Moran et al. 2007, Landers et al. 2008). Chromatographs had elevated background noise, and showed evidence of an unidentified compound that mimicked PCB congeners, which led to inaccurate quantification of concentrations. As a result, we were unable to obtain accurate concentrations of PCBs in fish. Mercury was used instead, as a proxy for PCBs, because it is also persistent in fish tissue, and is passed up the food chain via trophic transfer.

Mercury in fish

Mercury was detected in fish (THg) and invertebrates (MeHg) from all mountain lakes. Mount Rainier and Olympic had the highest THg concentrations in fish, while fish from lakes in North Cascades had significantly lower THg concentrations than fish from MORA and OLYM (p= 0.01).

Wet weight mercury concentrations in all fish remained below the EPA's water quality criterion of 300 ppb (i.e., 300 ng/g), but often exceeded the 49 ppb threshold for subsistence fishing (Figure 3A). Trophic position was a significant predictor of THg concentration in fish, but the relationship was weak ($p=0.03*10^3$, $R^2= 0.18$, $t=3.8$, $df = 60$, Figure 4B). Rainbow trout had the lowest THg concentrations of all species ($p= 0.01*10^2$), while Eastern brook and cutthroat trout had similar THg concentrations (Figure 3).

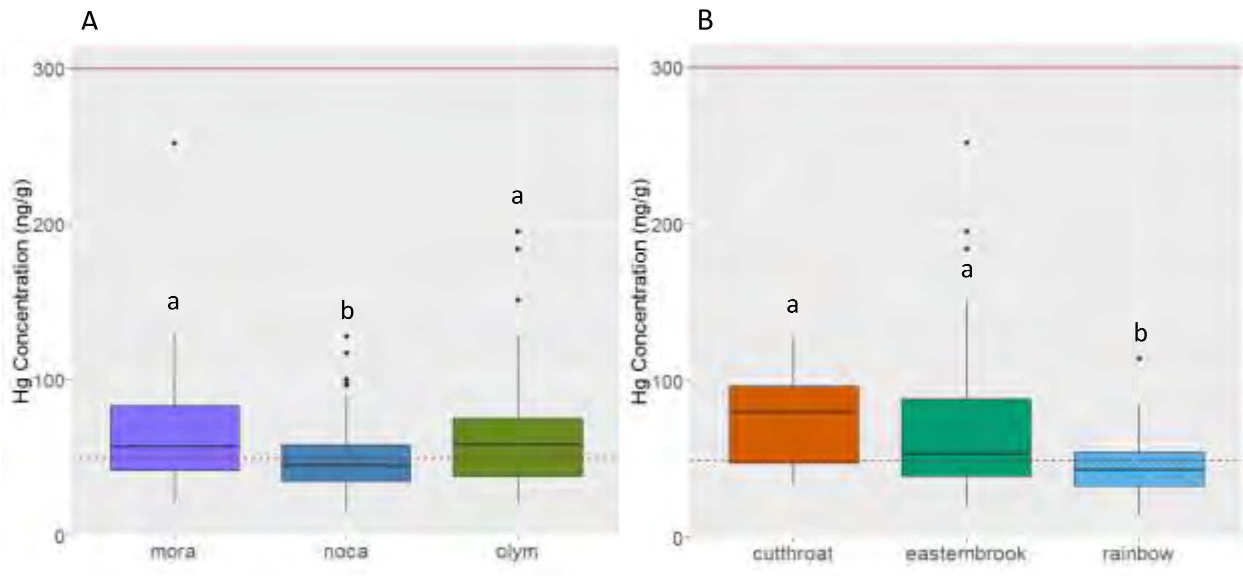


Figure 3. Distribution of THg concentrations in fish (wet weight), as compared between parks (A) and between species (B). The solid red line indicates the EPA mercury water quality criterion for protecting human health (300 ng/g), while the dotted line indicates the threshold for subsistence fishing (49 ng/g) (EPA 2001). (A) Fish from NOCA had significantly lower THg than fish from MORA and OLYM. (B) Rainbow trout had significantly lower THg than cutthroat trout ($p=0.2*10^2$, Tukey's post-hoc HSD) and Eastern brook trout ($p=0.09*10^3$ Tukey's post-hoc HSD).

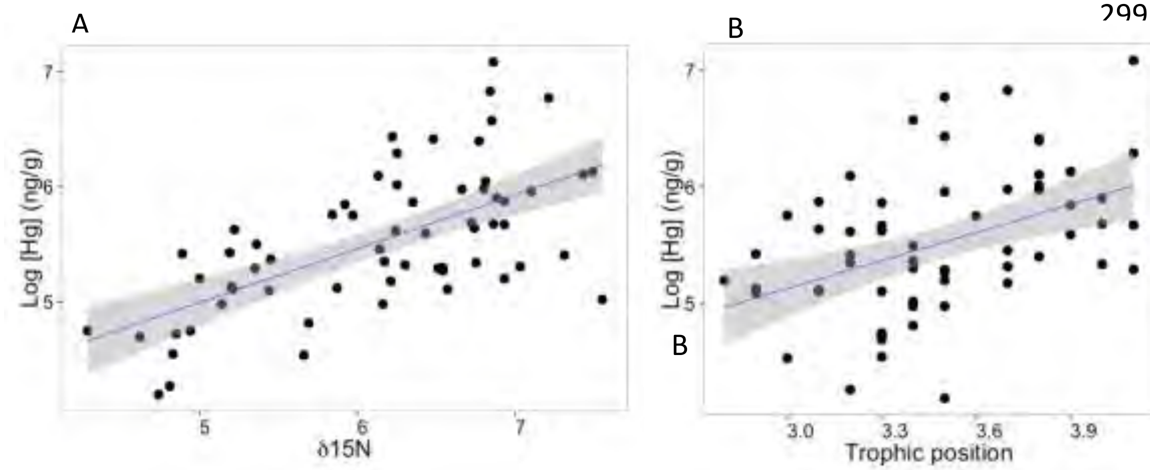


Figure 4. Relationship between trophic status and THg concentrations in all fish. (A) Linear regression of THg ~ $\delta^{15}N$, which represents trophic level (log[Hg] = 0.47*d15N + 2.65). (B) Linear regression after $\delta^{15}N$ has been converted to trophic position, which creates a baseline between study lakes and provides ease of comparison between lakes (log[THg] = 0.8*trophic position + 2.73).

While diet (as described by $\delta^{13}C$) was not important for explaining fish THg concentrations at the landscape scale ($p= 0.26$, $t= -1.13$, $df= 106.5$), diet explained some variability in THg concentrations in fish at the park scale. At Mount Rainier and North Cascades National Parks, there was a negative relationship between feeding habitat ($\delta^{13}C$) and THg concentration in fish, indicating that fish with a more pelagic diet typically had higher concentrations of THg than fish with predominantly benthic diets. This trend was significant in North Cascades lakes ($p= 0.02$, $R^2 = 0.14$, $t= -2.33$, $df= 61$) (Figure 5A) and nearly significant at Mount Rainier National Park lakes ($p= 0.06$, $R^2= 0.14$, $t= -2.03$, $df=41$) (Figure 5B). This trend was not significant in Olympic National Park lakes ($p = 0.23$) (Figure 5C).

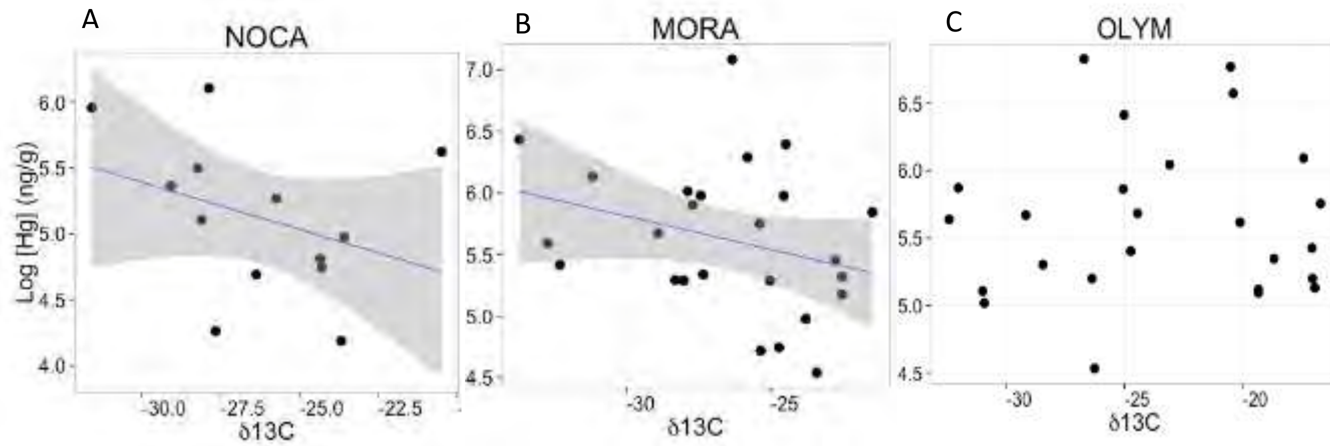


Figure 5. Relationships between feeding habitat (^{13}C) and THg concentrations in fish from (A) North Cascades (log[Hg] = -0.05 X $\delta^{13}C$ + 1.92), (B) Mount Rainier (log[Hg] = -0.08 X $\delta^{13}C$ + 0.76)), and (C)Olympic National Park lakes. NOCA and MORA lakes had a significant negative trend between $\delta^{13}C$ and THg; fish with diets more enriched in $\delta^{13}C$ (benthic invertebrates) had lower THg concentrations than fish with diets depleted in $\delta^{13}C$ (zooplankton). Olympic lakes had no trend between $\delta^{13}C$ and THg in fish.

These THg and $\delta^{13}C$ relationships are also reflected on the isotope biplots for each lake (Figure 6). In North Cascades and Mount Rainier lakes, fish tend to have higher THg concentrations when they align above the zooplankton resource in isotopic space (Figure 6, A-B). In Olympic, however, the THg concentrations of fish were much more variable; in some lakes, high-THg fish aligned with zooplankton resources, while in other lakes, they aligned with benthic resources (Figure 6, C-D).

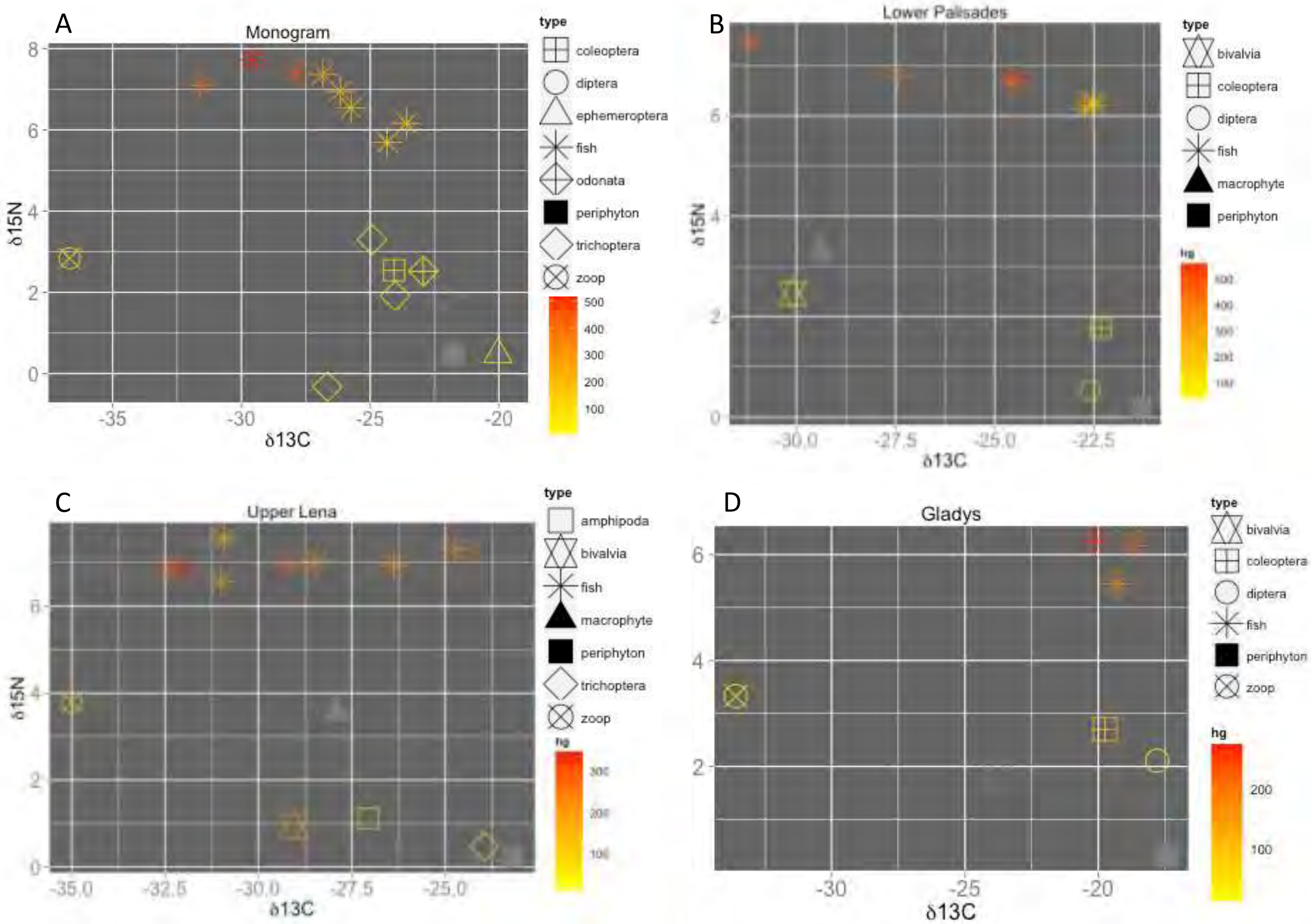


Figure 6. Selection of isotope biplots from study lakes. The ^{15}N axis represents trophic position, and the ^{13}C axis represents feeding habitat. Typically, pelagic resources are depleted in ^{13}C (more negative values), therefore organisms that feed in this habitat are also depleted in ^{13}C. Conversely, benthic resources are typically enriched in 13C relative to pelagic habitats, as are the organisms that feed from the benthos. (A) The Monogram lake (NOCA) biplot illustrates an increase in THg concentration with an increase in trophic position and increasingly pelagic diet (depletion of ^{13}C). (B) The Lower Palisades (MORA) plot shows a similar pattern, with slightly more variability in THg concentration. The bottom two plots – (C) Upper Lena and (D) Gladys lake (OLYM) – show how variability in THg concentrations is not well-aligned with differences in feeding habitat.

Mercury in invertebrates

Methylmercury concentrations in invertebrate prey species were generally below 100 ppb (Figure 7). Zooplankton and amphipods had significantly higher MeHg concentrations than most other invertebrates, which generally had less than 50 ppb MeHg (p=0.01, F=3.26, df= 5, one-way ANOVA with Tukey's HSD post-hoc test). However, fish from lakes that contained amphipods in their food webs (see Table 1) did not have significantly higher mercury concentrations than fish from lakes without amphipods (p= 0.34). Pea clams (*Bivalvia sphaeridae*) had the highest MeHg concentrations, while dytiscid beetles (*Coleoptera dystiscidae*) had concentrations in a similar range to other invertebrate species.

Figure 7. Methylmercury concentrations in invertebrate species from lakes in all parks. Of the prey species, zooplankton and amphipods had the highest MeHg concentrations of all other invertebrates. Pea clams (*bivalvia*), a non-prey species, had the highest MeHg of any invertebrate. Zooplankton had significantly more MeHg than diptera (p= 0.01, Tukey's HSD post-hoc test), odonata (p= 0.01, Tukey's HSD post-hoc test), and trichoptera (p=0.03, Tukey's HSD post-hoc test), and nearly significantly more MeHg than ephemeroptera (p=0.07, Tukey's HSD post-hoc test). Amphipods had significantly higher MeHg than diptera (p=0.02, Tukey's HSD post-hoc test), odonata (p=0.02, Tukey's HSD post-hoc test), and trichoptera (p=0.04, Tukey's HSD post-hoc test). Letters indicate significant codes (same letter = no significant difference, different letter = significant difference), with letters corresponding to the first letter of each taxonomic group.

Mercury model results

Fish characteristics, and lake water chemistry and morphometry variables were important for explaining differences in THg concentrations among fish. When analyzed at the landscape scale (all lakes, all parks), fish species, fish weight, lake size and water temperature were the most important predictors of THg concentration in fish. Heavy cutthroat and Eastern brook trout from small, warm lakes had the highest THg concentrations; fish THg had a significant, negative relationship with lake area (hectares) and a significant, positive relationship with epilimnetic temperature. In North Cascades lakes, the same variables were important, with the addition of maximum lake depth. Heavy cutthroat trout from shallower and warmer lakes had higher THg compared to fish from deeper and colder lakes in NOCA. In Mount Rainier lakes, only temperature and lake depth were important predictors of THg in fish. Fish from warm, shallow lakes had the highest THg concentrations, and fish species was not important

Table 3. Mixed effects model results for the landscape and park (local) models. In the landscape model, the random effect "lake" explained a small amount of the variance (0.003). This is reflected in the small differences in R^2m (fixed effects only) and R^2c (fixed and random effects). The random factor explained no variance in the park models. Selected models were chosen by optimizing the AIC and BIC values, which are listed in row 2 of the model output table.

Landscape model

log[Hg] = 0.20 * SA + 0.07 * weight + 0.06 * temp + 0.02 * jday + -0.52 * taxa(rainbow) + (1|lake)			
n= 82, AIC = 74.2, BIC= 98.3, R^2m = 0.61, R^2c = 0.62, var. explained by random effect = 0.003			
Parameters	**t-stat**	**df**	**p-value**
Surface area (SA) (ha)	-3.70	11	0.008
Fish weight (g)	4.88	43	$0.01*10^{3}$
Epilimnetic temperature	3.93	8	$0.04*10^{1}$
Sample date (Julian day)	4.46	11	$0.09*10^{2}$
Taxa (rainbow)	3.67	6	0.01

Park models

NOCA			**MORA**		**OLYM**	
log[Hg] = 0.02 * jday + -0.61 * taxa(rainbow) + 0.002 * weight + 0.04 * temp + -0.01 * zmax			log[Hg] = 0.004 * weight + 0.13 * temp + -0.14 * SA		log[Hg] = -1.25 * taxa + 0.26 * temp	
n=40, AIC = 36, BIC= 51, R^2 = 0.63, df = 40			AIC = -0.6, BIC = 8, R^2 = 0.91, df=25		AIC = 11, BIC = 15, R^2 = 0.73, df 25	
Parameters	**t-stat**	**p-value**	**t-stat**	**p-value**	**t-stat**	**p-value**
Sample date (Julian day)	2.65	0.01	--	--	--	--
Fish weight (g)	2.70	0.01	4.48	$0.01*10^{2}$	--	--
Epilimnetic temperature	1.98	0.05	4.52	$0.01*10^{1}$	5.50	$0.06*10^{4}$
Max depth	-2.45	0.02	--	--	--	--
Taxa (rainbow)	-3.89	$0.04*10^{2}$	--	--	-6.50	$0.04*10^{2}$
Surface area (ha)	--	--	-6.1	$0.02*10^{8}$	--	--

Discussion and recommendations

As predicted, THg concentrations in fish were influenced both by trophic factors, as well as environmental factors, and the importance of these variables differed depending on the scale of analysis. Mercury concentrations in lakes never exceeded the EPA recommended threshold for recreational fishing (300 ppb), but often exceeded the threshold for subsistence fishing (49 ppb) (EPA 2011). North Cascades fish typically had lower mercury than fish from Olympic and Mount Rainier. This result may be explained by the water quality and morphometry factors that influenced THg concentrations in fish. At the landscape scale, heavy cutthroat and Eastern brook trout from small, warm lakes typically had the highest THg concentrations. However, it is unclear if the importance of lake size in temperature is due to differences atmospheric mercury loadings between parks (e.g. as Moran et al. 2007 suggests), or the actual influence of lake size and temperature – which are well-established predictors (e.g. St. Louis et al. 1994, Lavoie et al. 2013). Most of the lakes sampled in North Cascades National Park were quite large and deep, while lakes from Olympic and Mount Rainier were typically smaller and shallower, with a few exceptions (see Table 1). In addition, fish from Mount Rainier National Park had higher trophic positions than fish from other lakes, and there was a highly significant relationship between trophic position and THg in fish in all parks (a trend which is supported by a substantial body of literature, e.g. Borga et al. 2012, Kidd et al. 2012). Maximum lake depth was only important in NOCA lakes, likely because the range in lake depths for MORA and OLYM were too small to have significant variability (see Table 1). In OLYM, fish weight did not matter, likely because most fish we caught were small; an earlier removal effort by park staff in some of the sample lakes resulted in fewer numbers of large fish. At the local scale, surface area was only an important factor in MORA lakes; the explanation for this result is unclear.

At the food web scale, fish with a more pelagic diet (typically Eastern brook and cutthroat trout) had higher THg burdens than fish with a more benthic diet in lakes from North Cascades and Mount Rainier National Park. This was not the case in Olympic National Park, however. This result may be explained by the average MeHg concentrations of invertebrate prey; zooplankton had significantly higher MeHg concentrations than any other invertebrate prey, except for amphipods, which were dominant in Olympic National Park food webs (Table 2, Olympic National Park, unpublished data). Differences in diet composition within each fish species indicates that diet availability, as well as functional feeding may be more important in these lakes. For instance, this could explain why, in Olympic National Park, both species of trout have high THg despite their low trophic position (and for brook trout, a more benthic diet). This could be because the available diet in OLYM lakes includes amphipods, which had significantly higher MeHg than other macroinvertebrates. However, the consistent low THg concentrations in rainbow trout show that functional feeding is still an important factor influencing THg burdens. The data from MORA and NOCA suggest that rainbow trout are predominantly benthic foragers in mountain lakes, despite the availability of zooplankton as prey (which have high [Hg]), suggesting that their functional feeding may buffer their bioaccumulation potential.

While we were unable to determine the PCB levels of fish in mountain lakes, mercury served as a useful indicator for how atmospherically deposited contaminants bioaccumulate in these systems. We were able to contribute significantly to knowledge of mercury levels in the Pacific Northwest, by adding scope and detail to knowledge from existing studies (e.g., Moran et al. 2007, Landers et al. 2008, Eagles-Smith et al. 2014). This research shows that food web dynamics, as well as lake water quality and morphometry are important predictors of THg burdens in mountain lake fish. In addition, we show that THg concentrations in fish do not pose high exposure risk to recreational anglers who consume fish in these lakes. However, for those who may rely on fish for subsistence, the THg levels in fish are concerning, especially at Mount Rainier and Olympic National Parks. It may be important to consider conducting outreach to park visitors about THg in fish, as well as consider these findings when making decisions regarding removal, maintenance, or stocking of fish in lakes.

Acknowledgments

We would like to thank the Center for Lake and Reservoirs at Portland State University, Dr. Collin Eagle-Smith at USGS in Corvallis, OR for assistance with mercury analysis, and Dr. Deke Gunderson at Pacific University in Forest Grove, OR, with assistance with PCB analysis.

References

Bloom, N. S. (1992) On the chemical form of mercury in edible fish and marine invertebrate tissue. Canadian Journal of Fisheries and Aquatic Sciences 49:1010–1017.

Borgå, K., Kidd, K. a., Muir, D. C. G., Berglund, O., Conder, J. M., Gobas, F. a P. C., … Powellkk, D. E. (2012). Trophic magnification factors: Considerations of ecology, ecosystems, and study design. Integrated Environmental Assessment and Management, 8(1), 64–84.

Clayden, M. G., Kidd, K. a., Wyn, B., Kirk, J. L., Muir, D. C. G., & O'Driscoll, N. J. (2013). Mercury biomagnification through food webs is affected by physical and chemical characteristics of lakes. Environmental Science and Technology, 47(21), 12047–12053.

Clements, W., Hickey, C., Kidd, K. (2012). How do aquatic communities respond to contaminants? It depends on the ecological context. Environmental Toxicology and Chemistry, 31(9): 1932-1940.

Eagles-Smith, C., Suchanek, T., Colwell, A. et al. (2008). Mercury trophic transfer in a eutrophic lake: The importance of habitat-specific foraging. Ecological Applications, 18(8):196-212.

Eagles-Smith, C.A., Willacker Jr., J., Flanaga Pritz, C.M. (2014) Mercury in Fisher from 21 National Parks in the Western United States – Inter- and Intra-Park Variation in Concentrations and Ecological Risk. US Geological Survey Open-File Report 2014-1051.

Eby, L. a, Roach, W. J., Crowder, L. B., & Stanford, J. a. (2006). Effects of stocking-up freshwater food webs. Trends in Ecology & Evolution, 21(10), 576–84.

Figueiredo, K., Mäenpää, K., Leppänen, M. T., Kiljunen, M., Lyytikäinen, M., Kukkonen, J. V. K., … Martikainen, P. J. (2013). Trophic transfer of polychlorinated biphenyls (PCB) in a boreal lake ecosystem: Testing of bioaccumulation models. The Science of the Total Environment, 466-467C(1259), 690–698.

Kallenborn, R. (2006). Persistent organic pollutants (POPs) as environmental risk factors in remote high-altitude ecosystems. Ecotoxicology and Environmental Safety, 63(1), 100–7.

Kidd, K. A.; Clayden, M.; Jardine, T. D. (2012). Bioaccumulation and Biomagnification of Mercury in Food Webs. In Environmental Chemistry and Toxicology of Mercury; Liu, G., Cai, Y., O'Driscoll, N., Eds.; John Wiley & Sons, Inc.: Hoboken, NJ; pp 453−499.

Knapp, R. A., Corn, P. S., & Schindler, D. E. (2001). The Introduction of Nonnative Fish into Wilderness Lakes: Good Intentions , Conflicting Mandates , and Unintended Consequences. Ecosystems, 4, 275–278.

Landers, D. H. et al. (2008). The Fate , Transport , and Ecological Impacts of Airborne Contaminants in Western National Parks (USA). EPA/600/R-07/138. U.S. Environmental Protection Agency, Office of Research and Development, NHEERL, Western Ecology Division, Corvallis, Oregon.

Lavoie, R. a, Jardine, T. D., Chumchal, M. M., Kidd, K. a, & Campbell, L. M. (2013). Biomagnification of Mercury in Aquatic Food Webs: A Worldwide Meta-Analysis. Environmental Science & Technology, 47, 13385–13394.

Leavitt, P.R., Schindler, D.E., Paul, A.J., Hardie, A.K., Schindler, D.W. (1994). Fossil Pigment Records of Phytoplankton in Trout-stocked Alpine Lakes. Canadian Jounral of Fisheries and Aquatic Sciences, 151(11), 2411, 2423.

McIntyre, J. K., & Beauchamp, D. A. (2007). Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. Science of the Total Environment, 372(2), 571-584.

Moran, P. W., Aluru, N., Black, R. W., & Vijayan, M. M. (2007). Tissue contaminants and associated transcriptional response in trout liver from high elevation lakes of Washington. Environmental Science & Technology, 41(18), 6591–7.

Morel, F. M. M., Kraepiel, A. M. L., & Amyot, M. (1998). The Chemical Cycle and Bioaccumulation of Mercury. Annual Review of Ecology and Systematics, 29(1), 543–566.

Norli, H., Christiansen, A., Deribe, E. (2011) Application of QuEChERS method for extraction of selected persistent organic pollutants in fish tissue and analysis by gas chromatography mass spectrometry. Journal of Chromatography, 1218(41): 7234-7241.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology, 83(3), 703–718.

Rasmussen, J.B., Vander Zanden, M.J. (2004). The Variation of Lake Food Webs across the Landscape and Its Effect on Contaminant Dynamics. In pres: *Food Webs at the Landscape Level*, eds. Polis, G.A., Power, M.E., Huxel, G.R., © 2004 The University of Chicago Press.

Serrano, R., Barreda, M., Pitarch, E. et al. (2003). Determination of low concentrations of organochlorine pesticides and PCBs in fish feed and fish tissues from aquaculture activities by gas chromatography with tandem mass spectrometry. Journal of Separation Science, 26(1-2): 75-86.

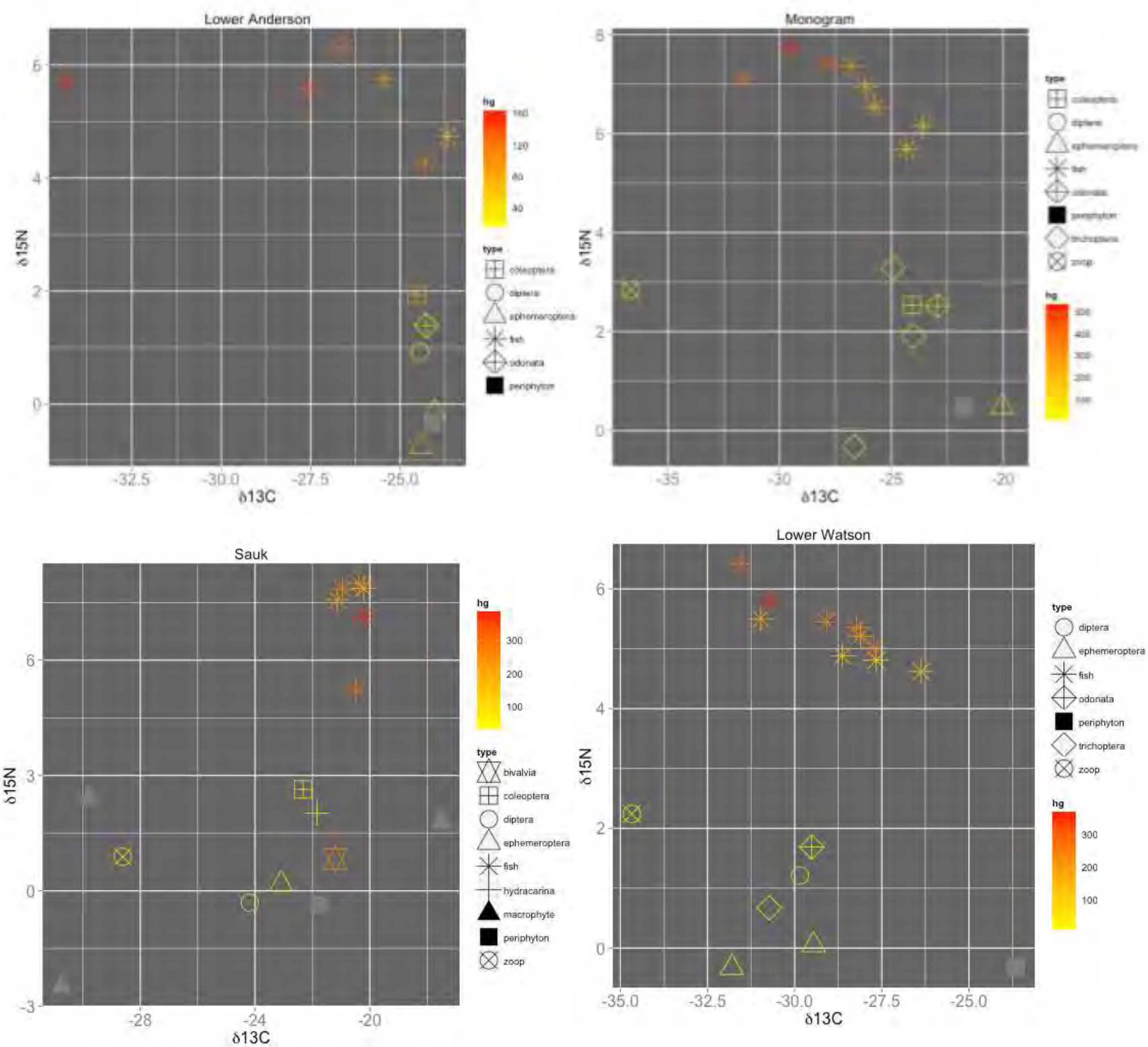
Stewart, A. Saiki, M., Kuwabara, J. et al. (2008). Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. Canadian Journal of Fisheries and Aquatic Sciences, 65: 2351-2366.

St. Louis, V. L., Rudd, J. W. M., Kelly, C. a., Beaty, K. G., Bloom, N. S., & Flett, R. J. (1994). Importance of Wetlands as Sources of Methyl Mercury to Boreal Forest Ecosystems. Canadian Journal of Fisheries and Aquatic Sciences, 51(1), 1065–1076.

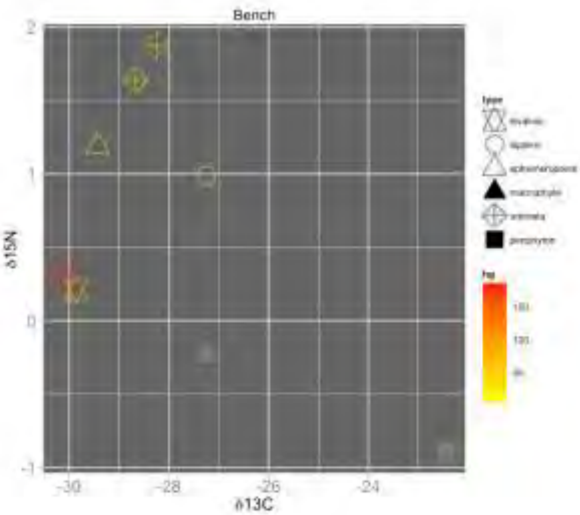
Ullrich, S. M., Tanton, T. W., & Abdrashitova, S. a. (2001). Mercury in the aquatic environment: a review of factors affecting methylation. Critical Reviews in Environmental Science and Technology, 31(3), 241–293.

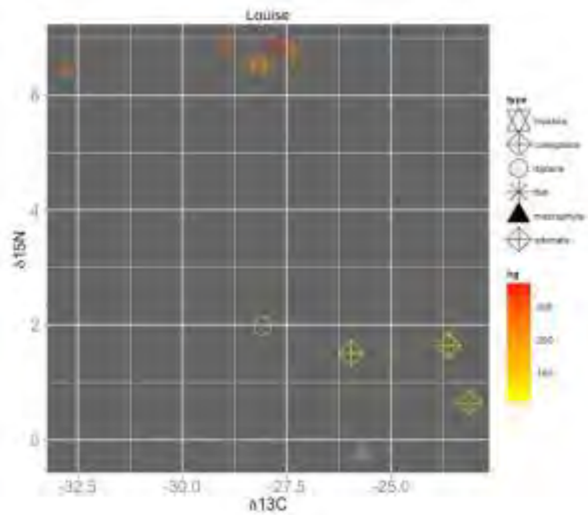
Appendix 1: Isotope & THg biplots for each lake

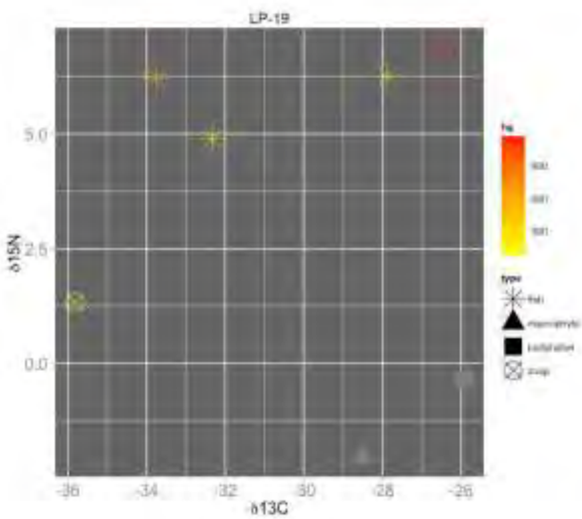
North Cascades National Park

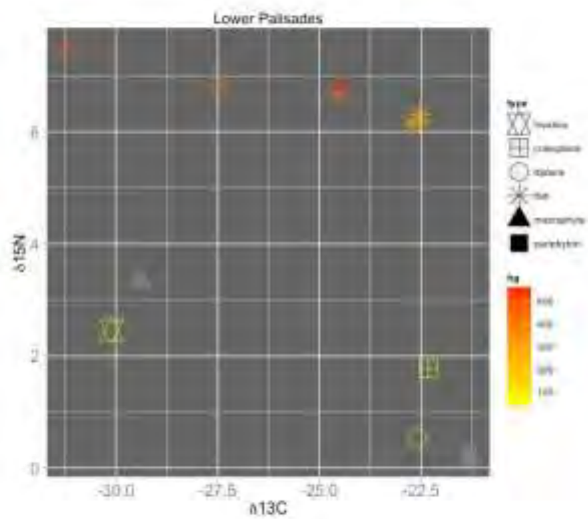


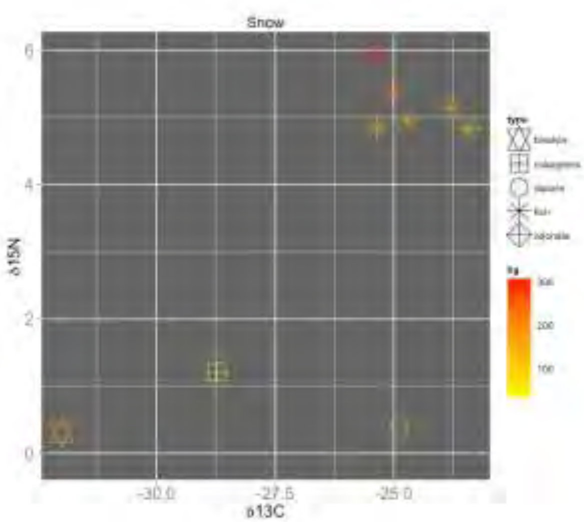
Mount Rainier National Park

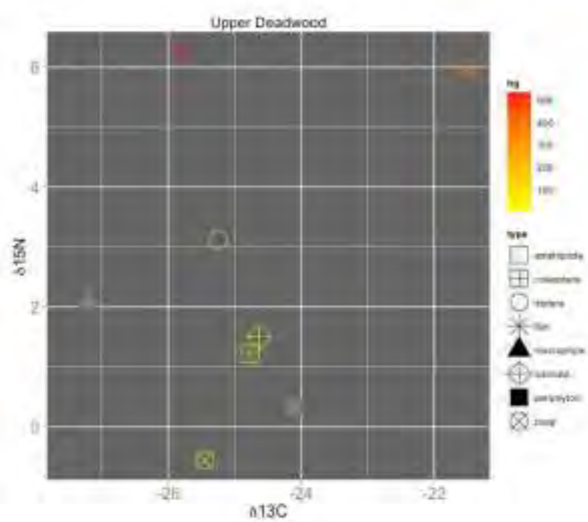












Olympic National Park