

## From forests to fish: Mercury in mountain lake food webs influenced by factors at multiple scales

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### Abstract

Mountain lakes, while seemingly pristine, have been subjected to historical fish stocking practices and exposure to atmospherically deposited contaminants like mercury. Mercury bioaccumulation in these ecosystems varies widely due to strong environmental gradients, and there are complex, hierarchical factors that affect mercury transport and loading, methylmercury production, and food web biomagnification. We sought to assess how representative variables associated with watershed, lake, and food web-scale processes—specifically, catchment tree cover, lake benthic primary production, and fish diet, respectively—are associated with mercury concentrations in mountain lake fish. Mean fish mercury concentrations varied threefold between lakes, with nearshore tree cover and fish diet accounting for the most variance in fish mercury. Tree cover was likely positively correlated to fish Hg due to its contributions to local deposition and its effect on lake biogeochemistry. Fish with benthic diets tended to have higher mercury concentrations, illustrating that food web processes are an important consideration when investigating drivers of contaminant bioaccumulation. Our results suggest that both landscape and ecological factors are determinants of fish mercury bioaccumulation, and thus variables at multiple scales should be considered when managing mountain lake food webs for mercury exposure risk.

Remote montane lakes are important habitats in temperate regions because they help preserve aquatic biodiversity and water quality in the face of downstream urbanization and development. Substantial fish stocking efforts in the United States during the 19<sup>th</sup> and 20<sup>th</sup> centuries have also made mountain lakes a popular destination for anglers because of the cultural value placed on these ecosystems (Chiapella et al. 2018), and the perceived opportunity to catch fish free from industrial pollution common in more developed watersheds. Despite their isolation from many direct anthropogenic impacts, mountain lake watersheds are exposed to a variety of environmental contaminants via atmospheric deposition (Fitzgerald et al. 1998; Kallenborn 2006; Landers et al. 2008). Mercury (Hg) is of particular concern because it is globally prevalent, and when converted to the more toxic and bioavailable form, methylmercury (MeHg), it biomagnifies through food webs to concentrations that can pose health risks to

wildlife and humans. Importantly, the amount of inorganic Hg transported to ecosystems is generally a poor predictor of MeHg concentrations and risk (Heim et al. 2007) because of the complex factors driving MeHg biogeochemical cycling and accumulation through food webs (Eagles-Smith et al. 2016a). Thus, understanding the relative importance of various drivers controlling MeHg risk can inform potential management of human and ecological health. As a result, there have been substantial research efforts to understand the factors that influence MeHg accumulation in aquatic food webs (McIntyre and Beauchamp 2007; Lavoie et al. 2013; Eagles-Smith et al. 2016a). In mountain lakes, many of these factors span large gradients—often representing extremes—yet many of these lakes are difficult to access. As a result, much less is known about which factors predict fish Hg concentrations in mountain lakes specifically, leaving a large knowledge gap with potential repercussions for human health.

Methylmercury bioaccumulation is controlled by a complex and hierarchical suite of factors (Ullrich et al. 2001) that can be characterized by three broad categories at the catchment, lake, and food web scale: (1) factors influencing inorganic Hg transport and loading (Jaffe and Strode 2008; Sackett et al. 2010), (2) biogeochemical and morphological factors

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influencing MeHg production (Kainz and Lucotte 2006; Clayden et al. 2013; Klapstein and O'Driscoll 2018), and (3) ecological factors influencing MeHg bioaccumulation and biomagnification through aquatic food webs (Eagles-Smith et al. 2016a). However, the relative importance of these categories and the factors comprising them can vary across different habitat types (Eagles-Smith et al. 2016a), especially in mountain lakes, which occupy a landscape with strong environmental gradients (Loewen et al. 2018), often in relatively small geographic areas. While previous studies typically focus on individual factors that influence Hg bioaccumulation, we sought to identify representative variables at each scale that are relatively easy to measure, and could serve as indicators of Hg bioaccumulation.

Watersheds with high precipitation, dense coniferous forests, and abundant wetlands are typically conducive to higher mercury bioaccumulation in associated lake food webs. Precipitation and forest cover integrate processes associated with both inorganic loading and biogeochemical drivers of MeHg production. Precipitation is the primary pathway for direct wet deposition of Hg (Prestbo and Gay 2009), and forests can enhance Hg delivery to watersheds via increased deposition under canopies as a result of throughfall and litterfall, because leaves and needles can scavenge atmospheric Hg (Graydon et al. 2008; Drenner et al. 2013; Eagles-Smith et al. 2016b). Forest cover and basin morphology also influence Hg biogeochemistry. Heavily forested watersheds and low-slope wetland areas are often associated with higher Hg concentrations than barren and steep gradient catchments, due to interactions with dissolved organic carbon (DOC) and microbial processes (St. Louis et al. 1994; Ravichandran 2004; Winn et al. 2009; Jardine et al. 2012; Klapstein and O'Driscoll 2018).

Lake primary productivity and fish foraging habitat are also well-documented drivers of food web effects on Hg bioaccumulation. Benthic production plays an important role in temperate and montane lake food webs (Hecky and Hesselein 1995; Vadeboncoeur et al. 2002; Vander Zanden 2006; Sadro et al. 2011), and many of the factors that influence net MeHg production and bioavailability are also associated with benthic primary production, such as lake morphometry (Vadeboncoeur et al. 2008). Evidence suggests foraging habitat can have an outsized influence on Hg exposure, although the relative importance of different habitats can vary across the landscape (Cabana and Rasmussen 1994; Wong et al. 1997; Eagles-Smith et al. 2008). In oligotrophic systems, pelagic pathways tend to be more efficient for Hg accumulation (Power et al. 2002; Gorski et al. 2003; Karimi et al. 2016).

Remote mountain lakes provide a valuable setting to examine the mechanisms that influence Hg concentration in fish because they are situated in a landscape with strong gradients of precipitation, air temperature, forest cover, catchment morphometry, water temperature and chemistry, and food web structure. The goal of this study was to identify factors that are representative of the processes influencing Hg

bioaccumulation at the landscape, lake, and food web scale. To do so, we assessed Hg bioaccumulation in 19 mountain lakes in the Olympic and Cascade Mountains, Washington, USA. Study sites ranged from alpine (no tree cover), subalpine (sparse tree cover), and high elevation forest (high tree cover), with a wide range of morphometric attributes (Table 1). We hypothesized that: (1) tree cover would be the most important landscape-level predictor of fish Hg, due the promotion of Hg throughfall by conifers; (2) model-derived estimates of benthic primary production would be a key indicator of limnological effects on Hg bioaccumulation, and would be negatively correlated with fish Hg concentrations, because of reduced methylation potential from low redox (high oxygen) conditions at the sediment–water interface; and (3) habitat-specific foraging by fish would be an important food web factor of fish Hg because zooplankton typically have higher Hg than benthic invertebrates in oligotrophic lakes (Power et al. 2002; Karimi et al. 2016).

## Methods

### Study area

The Cascade and Olympic Mountain ranges extend through the state of Washington, U.S.A. The Olympic Mountains are coastal, and occupy the Olympic Peninsula, whereas the Cascade Range is further inland, and stretches from northern California to the U.S.-Canada border. National forest, national park, and designated wilderness areas make up much of the land area in the Olympic and Cascade Mountains. Study sites included lakes in North Cascades National Park and Mt. Baker-Snoqualmie National Forest, hereafter collectively referred to as North Cascades National Park (2014–2015), Mount Rainier National Park (2015), and Olympic National Park (2015) (Table 1). Data from North Cascades National Park and Mt. Baker-Snoqualmie National Forest were pooled for later analyses because of the close proximity of sites. Our sites, while all mountainous, encompass a heterogeneous landscape with lake basins that have a wide range of sizes and include a mix of high forest, subalpine and alpine scrub vegetation, and variable annual temperatures. Although historically fishless, all sample lakes were at one point stocked with fish, and now retain reproducing populations of either Cutthroat Trout (*Oncorhynchus clarkii*), Rainbow Trout (*Oncorhynchus mykiss*), or Eastern Brook Trout (*Salvelinus fontinalis*). We sampled 19 lakes in 2014 and 2015 from late July to September to ensure complete ice-off. We selected lakes based on elevation, vegetation zone (high forest, subalpine, alpine), and surface area to ensure we captured a wide environmental gradient.

We measured water temperature ( $^{\circ}\text{C}$ ), specific conductance ( $\mu\text{S cm}^{-1}$ ), and dissolved oxygen ( $\text{mg L}^{-1}$ ) using a YSI ProPlus at 1-m intervals at a deep area of each lake. pH was measured in situ using an Extech EC500. Water samples for total phosphorus (TP) and nitrogen were collected as an integrated

**Table 1.** Summary of measured and modeled environmental variables for all lakes.

Variable	Median	Min	Max	Usage
Mean depth (m)	4.90	1.40	17.70	Bathymetric models
Max depth (m)	8.90	2.80	79.00	Bathymetric models
Surface area (ha)	4.0	0.5	25.0	Bathymetric models
Slope (%; 100 m buffer)	46.88	12.90	86.89	Bathymetric models, tree cover estimation
Slope (%; 25 m buffer)	22.59	5.13	99.80	Bathymetric models, tree cover estimation
Elevation (m)	1387	1250	1747	Site selection
Elevation change units (m; 25% buffer)	1406.5	1254.6	1780.3	Bathymetric models, tree cover estimation
Clarity (% max depth)	75.8	15.1	100.0	Benthic primary production model
Hypolimnetic temperature* (°C)	9.80	5.04	16.50	Benthic primary production model
Mean annual air temperature (5-yr; °C)†	5.42	2.03	6.09	Mercury models
Mean annual watershed precipitation (5-year; mm)†	3114.6	1820.8	4326.4	Mercury models
Hypolimnetic dissolved oxygen saturation (%)*	87.11	16.40	124.40	Mercury biogeochemistry inference, not used in models
Specific conductance ( $\mu\text{S cm}^{-1}$ )	41.6	8.8	138.8	Mercury biogeochemistry inference, not used in models
pH	6.80	6.18	7.83	Mercury biogeochemistry inference, not used in models
Phosphorus ( $\mu\text{g L}^{-1}$ )‡	5	2	22	Benthic primary production model
Edible Ch <i>a</i> ( $\mu\text{g L}^{-1}$ )‡	0.37	0.03	2.77	Benthic primary production model
Benthic primary production (%)*	39	19	57	Benthic primary production model output
Nearshore tree cover (%)	26.1	2.2	93.0	Tree cover estimation

\*Hypolimnetic temperature and oxygen were only measured in lakes with maximum depth < 30 m ( $n = 17$ ).

†Modeled values.

‡Values obtained either from sampling or from Williams and Labou (2017).

epilimnetic sample using a 2.5-cm diameter Tygon tube, kept on snow or ice, and frozen until analysis. We did not include nitrogen in our analyses, because analytical problems led to erroneous values; additionally, the benthic primary production model (below) only required TP as a nutrient input. Clarity was estimated by measuring Secchi depth, then calculating the proportion of maximum depth that was represented by Secchi depth. While some of these limnological variables were not included in our models of fish mercury, they were collected to provide us with contextual understanding of each lake ecosystem with respect to mercury biogeochemistry and the extent of environmental gradients exhibited by our study sites (Table 1).

### Food web collection

At each lake, zooplankton were collected with a 30-cm diameter 80- $\mu\text{m}$  mesh conical net via horizontal tows to aggregate enough biomass for analyses. Benthic macroinvertebrates were collected from the littoral area of the lake using a D-net with 500- $\mu\text{m}$  mesh until enough biomass was collected for analyses (~ 500 mg of biomass per taxon). All invertebrates were coarsely sorted to taxonomic order in the field and placed into respective amber glass jars with a Teflon lid and stored in either a final concentration of 70% HPLC-grade etha-nol (2014) or kept cold until frozen in the lab (2015). To ensure methodological differences did not confound our results, we collected and preserved zooplankton at varying time intervals to test whether ethanol preservation influenced

Hg concentrations compared to frozen samples. We found long-term storage of 180 d resulted in mean MeHg concentrations that were not significantly different than frozen samples (Tukey's Honest Significant Difference [HSD]:  $p = 0.999$ ; Fig. S1). All of our invertebrate samples were stored for > 180 d.

Fish were collected via a monofilament gill net at each site (36 m long  $\times$  1.8 m wide; mesh sizes: 12.5 mm, 18.5 mm, 25 mm, 33 mm; Lundgrens Fiskredskap AB). Nets were deployed perpendicular to shoreline, with the small mesh sizes closer to shore. Nets were checked and cleared of any captures approximately every 30–60 min, and nets were generally retrieved after 5–6 fish were caught. Captured fish were removed and immediately euthanized via stunning force to the head followed by pithing of the brain (IACUC protocol #36, Portland State University). We identified fish to species, recorded for weight and total length, and then wrapped them in solvent-washed aluminum foil and kept cool on snow or ice until frozen in the lab, where they were stored at  $-20^\circ\text{C}$  until processing.

Total chlorophyll *a* (Chl *a*) was sampled by collecting 1 liter of water from the epilimnion with a 2.5-cm diameter tube sampler and concentrating it directly onto the collection filter, while a second liter was first passed through a 35- $\mu\text{m}$  filter to exclude the larger, more inedible algal fraction (Cyr and Curtis 1999). Each fraction (all sizes; < 35  $\mu\text{m}$ ) was filtered onto a 1.2- $\mu\text{m}$  pore size glass fiber filter. At each lake, periphyton samples for stable isotope analyses were collected by placing

3–4 cobbles in a bin with water, scrubbing them with a brush, and then filtering the water through a 1.2- $\mu\text{m}$  pore size glass fiber filter. All filters were wrapped in aluminum foil, then kept on ice or snow until frozen in the lab. If present, macrophytes were collected and wrapped in foil for isotope analysis.

### Sample processing and analysis

Chl *a* samples were analyzed using acetone to extract chlorophyll from filters, and concentrations were measured using U.S. Environmental Protection Agency (EPA) Method 445 (Arar and Collins 1997) with a TD-7200 fluorometer using the Trilogy Chl *a* NA Module (Turner Designs). TP was analyzed at the University of Washington's Marine Chemistry Laboratory, using methods from Valderrama (1981). For some lakes, water samples were lost due to adverse weather conditions or transportation complications. In an attempt to fill data gaps, Chl *a* and phosphorus values, which were required for the benthic primary production models, were gathered from Williams and Labou's (2017) database. For our lakes, the database typically had a single value of either TP or total dissolved phosphorus (TDP); for the few lakes with multiple reported values, standard deviations were low (0–0.002  $\mu\text{g L}^{-1}$ ), suggesting that temporal variability in P is low and therefore, that single samples are representative. In some cases, only TDP data were available from the database for our lakes with missing TP values. In most cases, TDP and TP concentrations were similar for the subset of lakes that had both values: the range of values was small (0.001–0.01  $\text{mg L}^{-1}$ ), negating the utility of a correlation analysis, but using a paired *t*-test, we found no significant difference between TDP and TP ( $t = -1.20$ ,  $p = 0.260$ ,  $n = 12$ ) across published and measured concentrations. Thus, we used whatever concentration was available for a given lake (TP or TDP). For lakes where both measures were available, we used our TP measurements. Unless otherwise stated, all total and dissolved phosphorus values are henceforth referred to as phosphorus.

Fish samples were thawed to room temperature, and dorsal muscle tissue plugs were excised from each fish. Fish tissue, zooplankton, and macroinvertebrates were freeze-dried then homogenized using a mortar and pestle. Small subsets of each sample were placed into tin capsules for stable isotope analysis. Fish stomach contents were examined; however, little diet inference could be made because stomachs were either empty or food was nearly fully digested. Periphyton filters were oven-dried at 60°C for 12–24 h, and then periphyton was scraped off filters and placed into tin capsules for stable isotope analysis. Capsules were analyzed for stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes at the Yale University Isotope Lab (2014) and UC Davis Stable Isotope Facility (2015) on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd.) with reference material Vienna PeeDee Belemnite and air for carbon

and nitrogen, respectively (standard deviations of reference material were  $< 0.2$  for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  at both labs).

We analyzed total Hg (THg) concentrations in the remaining fish tissue homogenate via combustion on a Milestone DMA 80 tri-cell Hg analyzer (Milestone) following EPA Method 7473 (1998). Macroinvertebrate and zooplankton homogenates were analyzed for MeHg via cold-vapor atomic fluorescence following EPA Method 1631 (2002) at the U.S. Geological Survey contaminant ecology research lab in Corvallis, Oregon. We analyzed total mercury (THg) in fish because most (90–95%) Hg in fish muscle is in the form of MeHg (Bloom 1992). Invertebrates were analyzed for MeHg because ratios of MeHg:THg in invertebrates are less consistent than in fish (Tremblay et al. 1996). All Hg values are reported on a dry mass basis (unless stated otherwise), and quality assurance protocols including matrix blanks, duplicates, and spikes were used. Recoveries for calibration verification standards were 102.7% ( $n = 12$ ) for THg and 104.4% ( $n = 16$ ) for MeHg. Certified reference material recoveries were 101.73% ( $n = 12$ ) for THg and 98.8% ( $n = 10$ ) for MeHg (standard deviation = 6.48). Relative percent difference averaged 1.26% for all THg duplicates and 4.52% for MeHg. Matrix spike recoveries for MeHg averaged 111%.

### Spatial and modeled variables

Lake and watershed morphometry were estimated using the models outlined in Heathcote et al. (2015) and Messenger et al. (2016) (Eqs. 1 and 2). Briefly, we measured lake elevation, surface area, bathymetry, and watershed size and slope using ArcMap 10 (ESRI). Each lake perimeter was outlined using 1:24,000 USGS topographic maps and satellite photography. Using the National Hydrologic Dataset, we delineated watersheds by identifying flow direction, water accumulation, and catchment pour points within 10-m digital elevation models (U.S. Geological Survey 2017). We created two buffers around each lake, one that was 25% of the lake's area, and one that extended 100 m from shoreline. We calculated mean elevation change within the 25% buffer and mean slope within the 100-m buffer (Heathcote et al. 2015; Messenger et al. 2016). These parameters were then used to model volume (Eq. 1, Heathcote et al. 2015) and mean depth (Eq. 2, Model 5 from Messenger et al. 2016) for each lake:

$$\log_{10}V = \log_{10}SA \times 0.96 + \log_{10}(\Delta\text{elevation}_{25}) \times 0.77 \quad (1)$$

$$\begin{aligned} \text{Surface areas } 0.1 - 1 \text{ km}^2 : \log_{10}D = & 0.3826 \\ & + 0.1512 \times \log_{10}SA + 0.4820 \times \log_{10}S_{100} \end{aligned} \quad (2a)$$

$$\begin{aligned} \text{Surface areas } 1 - 10 \text{ km}^2 : \log_{10}D = & 0.1801 \\ & + 0.2985 \times \log_{10}SA + 0.8473 \times \log_{10}S_{100} \end{aligned} \quad (2b)$$

$$\begin{aligned} \text{Surface areas } 10 - 100 \text{ km}^2 : \log_{10}D = & 0.0379 \\ & + 0.2445 \times \log_{10}SA + 0.1517 \times \log_{10}S_{100} \end{aligned} \quad (2c)$$

where  $V$  = volume ( $\text{m}^3$ ),  $SA$  = surface area (ha),  $\Delta\text{elevation}_{25}$  = elevation change within 25% buffer,  $D$  = mean depth (m),  $S_{100}$  = slope within a 100 m buffer.

We used the volume calculated from existing bathymetric maps to compare with volume estimates from the models of Heathcote et al. (2015) and Messenger et al. (2016). The models had varying accuracy, depending on the lake. Therefore, measured or preexisting values of mean depth and volume were selected for use when available, while maximum depth and observational knowledge of basin morphometry were used to select which modeled value to use in lakes that did not have measured values available (Table S1).

Water temperature was also an important variable to consider in our models, because of its strong relationship to lake biogeochemistry and productivity. Because water temperature is dynamic, especially in shallow lakes, yet lakes could only be sampled once per season, we used data from PRISM models to estimate the mean annual air temperature in each catchment in lieu of using water temperature (PRISM Climate Group 2018). There exists some evidence to assume that changes in surface water temperature are concurrent with changes in air temperature during ice-out, which we expected to roughly correlate to average annual air temperature (O'Reilly et al. 2015). Although air temperature is not a perfect replacement for water temperature due to differences in lake depth, aspect, and snow pack, we also anticipate air temperature to be important due to its relationship to many other physiochemical properties in lakes and their catchments. Additionally, we obtained mean annual precipitation values for each lake catchment (PRISM Climate Group 2018). We used 5-yr averages (2011–2015) of both annual precipitation and mean annual temperature to account for any uncharacteristically hot/cold or wet/dry years.

We estimated benthic primary production (as a proportion of whole-lake production) using the model from Vadeboncoeur et al. (2008), following modifications by Vander Zanden et al. (2011). The model uses established relationships between light (Secchi depth), morphometry (lake area, mean depth, maximum depth), nutrients (TP), and primary algal production for benthic and pelagic habitats. While the model does not explicitly include macrophytes in its estimation of primary production, most sites had negligible or no macrophyte production. For those lakes ( $n = 4$ ) with macrophytes, the models are likely a conservative estimate of benthic primary production. The model uses pelagic Chl  $a$  to estimate pelagic primary producer biomass, and sums this value over the depth of the photic zone ( $> 1\%$  incident light) to yield total planktonic primary production per square meter of lake surface area. The model estimates benthic primary production at discrete depth intervals, as a function of light availability at depth. As in Vander Zanden et al. (2011), we set the maximum, light-saturated rate of primary productivity to  $30 \text{ mg C}^{-1} \text{ m}^{-2} \text{ h}^{-1}$  because it represents a value typical of oligotrophic to moderately productive lakes (Table 1). The model

uses this value to scale relative contributions of benthic and primary production to the whole-lake level using lake area, mean depth, and maximum depth (Vadeboncoeur et al. 2008). Although this approach does not explicitly include a value for a deep chlorophyll maxima, the majority of our sample sites did not appear to have one (we observed evidence of deep chlorophyll layers in 3 of the 19 lakes we sampled, Fig. S3).

We estimated percent tree cover within a buffer of each lake to evaluate the importance of nearshore terrestrial processes—such as Hg throughfall from conifers—for Hg bioaccumulation. Buffers were scaled to 25% of lake area (as in Heathcote et al. 2015) to compensate for large differences in lake size because the influence of the terrestrial landscape on a lake should be proportional to lake size. Estimates of percent tree cover were calculated by hand from Landsat 8 satellite imagery in ArcMap (TerraColor 2013–2018). Within the 25% lake buffers, polygons were drawn around tree stands and then cut away from the buffer. The area of the clipped buffer was then divided by the area of the original buffer to estimate what percent of the buffer area was occupied by trees.

### Statistical approach

To account for the multiple interacting factors that influence THg bioaccumulation in fish, we used a tiered information-theoretic approach, which we outline here, followed by a more detailed description below. We first characterized the overarching patterns of Hg concentrations in mountain lake food webs by comparing mean Hg concentrations between each park, and between different invertebrate and fish taxonomic groups. We used nitrogen stable isotopes to estimate fish trophic positions, and carbon stable isotopes in mixing models to quantify fish foraging habitat reliance. We then used a two-tiered statistical approach to determine the effectiveness of our indicator variables—benthic reliance, benthic primary production, and tree cover—while statistically correcting for individual level-factors that affect fish THg. We first standardized fish THg concentrations by trophic position by conducting an analysis of covariance and then calculating least-squares means for each lake. Lastly, we evaluated how watershed precipitation and air temperature, catchment tree cover, benthic primary production, and mean benthic reliance influenced these lake-specific THg values using multiple linear regression models with Akaike Information Criterion model selection for small sample sizes ( $\text{AIC}_c$ ).

First, we investigated overarching trends to determine the general distribution of THg concentrations in fish among species and across study sites. The low sample size and moderate level of replication precluded the use of multilevel models, therefore to understand how much THg concentrations differed across North Cascades ( $n = 8$ ), Mount Rainier ( $n = 5$ ), and Olympic ( $n = 6$ ) National Park lakes, we used ANOVA followed by a Tukey's HSD test. Each of these parks occupies a distinctive region that could lead to differences in Hg

deposition; lakes in Olympic National Park represent coastal mountain lakes on a marine peninsula, whereas lakes in Mount Rainier and North Cascades National Park represent northern and southern bounds of an inland range, each with distinctive positioning relative to a major urban area (Moran et al. 2007). We also tested how much mean THg concentrations differed among fish species in lakes using an ANOVA and a Tukey's HSD test.

We then compared MeHg concentrations across invertebrate taxonomic groups, and between pooled pelagic zooplankton and littoral macroinvertebrate groups within each lake, to investigate variation in MeHg concentrations in fish prey. Zooplankton MeHg values for each lake were obtained from multiple horizontal tows, and were thus representative of the range of zooplankton MeHg concentrations in each lake. Mean lake benthic invertebrate MeHg values included all taxa from each lake to capture a representative average of MeHg concentrations which assumes fish do not discriminate between taxa, and that the taxonomic distribution we collected was representative of a fish's diet. Because macroinvertebrate sampling was opportunistic due to low abundances, means were calculated with equal taxonomic weight, and could not be weighted by relative abundance. Macroinvertebrates included amphipods (Amphipoda), beetles (Coleoptera), dipteran flies (Diptera), mayflies (Ephemeroptera), dragonflies (Odonata), and caddisflies (Trichoptera). An ANOVA was used to determine if there were differences in mean MeHg for each macroinvertebrate group within each park region (with lake as the sample unit), followed by a Tukey's HSD test. Lastly, we tested for a relationship between fish THg and prey MeHg (mean MeHg in pelagic primary consumers, i.e., pooled zooplankton; mean MeHg in benthic primary consumers, i.e., pooled macroinvertebrates) in each lake using linear regression models.

We used nitrogen stable isotopes ( $\delta^{15}\text{N}$ ) to calculate invertebrate and fish trophic position, and stable carbon isotopes ( $\delta^{13}\text{C}$ ) to estimate percent benthic reliance of fish. We calculated trophic position using the  $\delta^{15}\text{N}$  of fish and baseline-adjusted  $\delta^{15}\text{N}$  of either a primary consumer (typically chironomid larvae) or primary producer (typically periphyton) using the equation for trophic position from Post (2002). We calculated fish benthic reliance using a two end-member mixing model (Eq. 3; from Vander Zanden et al. 2011), because the lack of replicates of each taxa precluded usage of probabilistic models.

$$\text{Benthic reliance} = \frac{(\delta^{13}\text{C}_{\text{fish}} - \delta^{13}\text{C}_{\text{pelagic primary consumer}})}{(\delta^{13}\text{C}_{\text{benthic primary consumer}} - \delta^{13}\text{C}_{\text{pelagic primary consumer}})} \quad (3)$$

We used the  $\delta^{13}\text{C}$  of composite zooplankton samples as the pelagic end-member of the model for each lake, and we used the organism with the most enriched  $\delta^{13}\text{C}$  value—either

periphyton, a macrophyte, or a macroinvertebrate—as the littoral-benthic end-member (Table S1). We used the organism with the most enriched  $\delta^{13}\text{C}$  value for the littoral end-member, because the littoral community composition of each lake was variable, and no single taxon was represented across all lakes. Additionally, logistical challenges with sampling resulted in the loss of certain samples. Although primary producer  $\delta^{13}\text{C}$  is often temporally variable, we used periphyton when appropriate (i.e., when it had the most enriched  $\delta^{13}\text{C}$ ), because mountain lakes have a short growing season, and the values of periphyton and primary consumer  $\delta^{13}\text{C}$  in a given lake were typically within 2‰ of each other (Table S1; Supplemental Data 3). For some fish (9.6%), the mixing model produced values greater than 1 or less than 0, in which case the values were set to either 1 or 0, respectively (Vander Zanden et al. 2011).

To account for within-lake variation of Hg, we normalized mean fish THg concentrations for each lake to trophic position with an analysis of covariance, because trophic position influences Hg (Kidd et al. 1995), and because it had a stronger relationship with THg than length (Pearson's  $r = 0.41$  vs.  $r = 0.32$ , respectively). Additionally, trophic position integrates differences in diet (and thus Hg) that are a function of fish size and age. We used an analysis of covariance (with lake as the grouping factor and trophic position as the interaction term) to estimate the average THg concentration of fish adjusted to identical trophic positions in each lake (Williams et al. 2002). We used this approach because the number of fish we caught in each lake was variable; most lakes only had one fish species present, and our variables were at very different scales. As a result, mixed effects models were not appropriate.

In our second tier of analysis, we used multiple linear regression models in an information theoretic framework (i.e., AIC; Burnham and Anderson 2002) to evaluate the influence of percent tree cover, foraging habitat (represented as benthic reliance), benthic primary production, air temperature (5-yr mean), precipitation (5-yr mean), and sampling date on fish THg concentrations within a lake. Although isotopic turnover is seasonal, and thus at a mismatch with the temporal scale of tree cover, precipitation, and temperature data, we included these landscape variables as way of establishing baseline climatic conditions for each lake, as opposed to understanding how they covary with lake-level factors like fish diet. Additionally, mercury bioaccumulation occurs across the lifetime of the fish, and thus is on a similar temporal scale to the temperature and precipitation data. By using trophic position corrected mean fish THg as our response variable, we were able to account for the individual-level factors (e.g., trophic position) that influence fish THg concentrations, and use more comparable estimates of Hg exposure across lakes. Due to the significant negative orographic relationship between precipitation (5-yr mean) and tree cover ( $r^2 = 0.37$ ,  $p = 0.003$ ), we used the residuals from this relationship as our precipitation variable in our fish THg models (Fig. S2).



Because we only caught one fish in some lakes, we ran two sets of models. The first model included all 19 lakes, while the second model included the 13 lakes with larger samples sizes of fish (ranging from 2 to 10 fish). We used AIC<sub>c</sub> to calculate model Akaike weights ( $\omega_i$ ) and variable weights ( $\omega_\beta$ ) for model selection, and variance inflation factors (VIF) to identify if variables had high multicollinearity, retaining variables with VIF < 3. Collinear predictors with the highest VIF were removed first; if a difference between two factors was not large, the one with greater assumed biological significance was retained. To ensure a balanced design for allowing interpretation of variable weights, we included all possible combinations of variables in our candidate model set ( $n = 40$ ). However, to accommodate the low sample sizes of lakes, we only allowed a maximum of four variables to appear in any given model (as in Eagles-Smith et al. 2016b). All analyses were completed using R version 3.2.1 (R Core Team, 2016). Statistical analyses were conducted with packages MASS (Ripley et al. 2019), MuMIn (Bartoń 2019), and lsmeans (Lenth 2018).

## Results

### Mercury in lake food webs and across the landscape

Lakes had a wide range of morphometry, tree cover, benthic primary production, and annual precipitation (5-yr mean) (Table 1; Supplemental Data 1). Water quality was also varied, with relatively large ranges in hypolimnetic dissolved oxygen, water clarity, conductance, and Chl *a*, while phosphorus concentrations were consistently low across sites (Table 1; Supplemental Data 1).

We collected 100 fish from 19 lakes across the three study areas. Fish ranged from juvenile to adult, and the most common species caught was Rainbow Trout (46%), followed by Eastern Brook Trout (38%) and Cutthroat Trout (16%) (Table 2). Fish THg concentrations are known to vary with fish size, age, and trophic position, which are often correlated with one another. To avoid issues of collinearity, we chose to use trophic position to control for THg because it is generally the most important driver of fish THg concentrations (because Hg bioaccumulates), all else being equal. We were unable to

**Table 2.** Summary of fish variables for all lakes.

Variable	Median	Min	Max
Geometric mean THg (ng g <sup>-1</sup> dm)	272	109	549
Average THg (dm; ng g <sup>-1</sup> dm)	318	114	612
Weight (g)	118	12	360
Length (mm)	177	20	339
Trophic position*	2.9	2.3	3.5
Benthic reliance (%)*	67.16	0.00	100.00

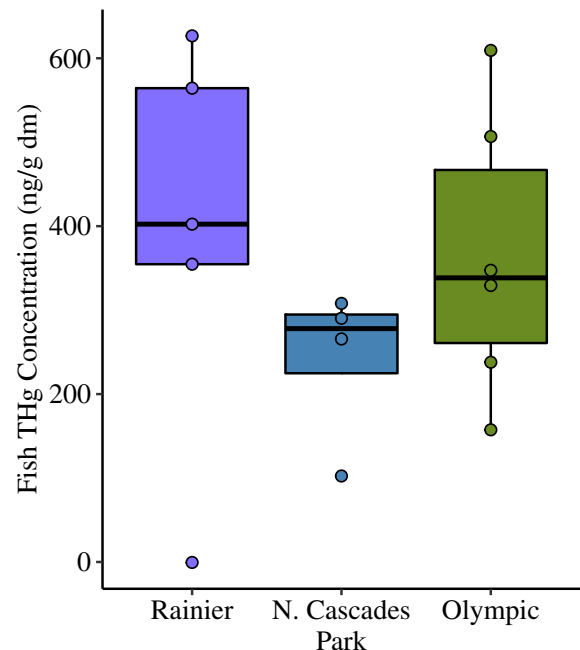
dm, dry mass.

\*Modeled values.

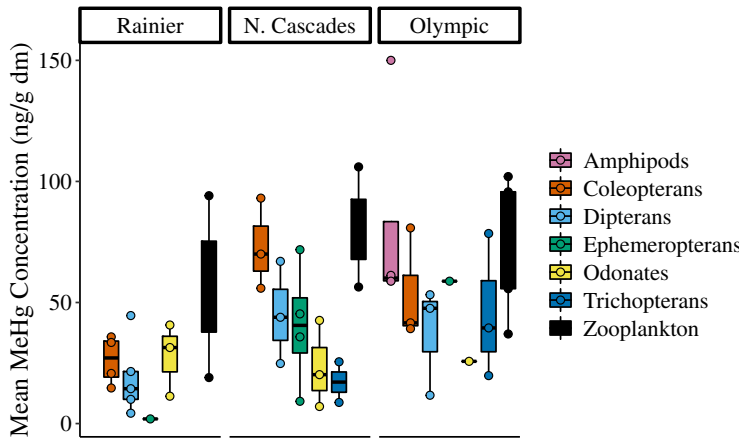
estimate fish age, as scales are not reliable for long-lived fish in alpine systems (Thaulow et al. 2017), and otolith extraction was largely unsuccessful.

The geometric mean fish THg concentration across all lakes, fish sizes, and species was 243.2 ng g<sup>-1</sup> dry mass (dm) ( $\pm 186.06$  SD), and ranged more than three-fold across lakes (Table 2; Supplemental Data 2). When converted to wet mass concentrations, all fish were below the EPA fish tissue MeHg criterion value of 300 ng g<sup>-1</sup> wet mass (wm) for the protection of human health, but generally above the EPA recommendation for subsistence consumption (50 ng g<sup>-1</sup> wm) (Borum et al. 2001; Fig. S4). Across all sample lakes, we did not find significant differences in fish THg concentrations among parks (ANOVA:  $F_{2,16} = 1.25$ ,  $p = 0.323$ ), although mean concentrations were 90 ng g<sup>-1</sup> dm lower in North Cascades ( $241 \pm 107$  ng g<sup>-1</sup> dm) lakes relative to Olympic ( $329 \pm 126$  ng g<sup>-1</sup> dm) and Mount Rainier ( $332 \pm 144$  ng g<sup>-1</sup> dm) lakes (Fig. 1). THg concentrations differed among species (ANOVA:  $F_{2,97} = 7.66$ ,  $p = 0.001$ ); Rainbow Trout had significantly lower THg than Cutthroat in North Cascades National Park (Tukey's HSD:  $p = 0.024$ ), and Eastern Brook Trout in Olympic National Park (Tukey's HSD:  $p = 0.002$ ; Fig. S4).

Mean invertebrate MeHg concentrations ranged approximately fivefold among taxonomic groups (31.2–157.0 ng g<sup>-1</sup> dm; Supplemental Data 2). In all parks, zooplankton had significantly higher MeHg than dipteran and



**Fig. 1.** Differences in fish THg ( $n = 101$ ), between Mount Rainier ( $n = 25$ ), North Cascades ( $n = 51$ ), and Olympic National Park ( $n = 25$ ). Individual points represent trophic position-corrected mean fish THg concentrations (ng g<sup>-1</sup> dm [dry mass]) for each lake. There were no significant differences between parks.



**Fig. 2.** Boxplots of mean invertebrate MeHg concentrations ( $\text{ng g}^{-1} \text{dm}$ ) in each lake for each region, grouped by order (with the exception of zooplankton, which are a composite sample). Points represent mean MeHg for each group within each lake. Letters denote significant differences (Tukey's HSD,  $p < 0.05$ ) in invertebrate MeHg between taxa within each park.

odonate larvae; in North Cascades and Olympic lakes, zooplankton also had significantly higher MeHg than caddisfly larvae and in Olympic lakes, amphipods had significantly higher MeHg than dipteran and odonate larvae (Fig. 2; ANOVA with Tukey HSD test:  $p < 0.05$  for all pairs). No other significant differences existed between other taxa. We did not observe a relationship between fish THg and pooled zooplankton MeHg ( $R^2 = 0.05$ ,  $p = 0.530$ ,  $df = 8$ ) or pooled macroinvertebrate MeHg concentrations ( $R^2 = -0.08$ ,  $p = 0.802$ ,  $df = 12$ ); therefore, we did not consider these variables in subsequent models.

**Indicators of fish mercury concentrations**

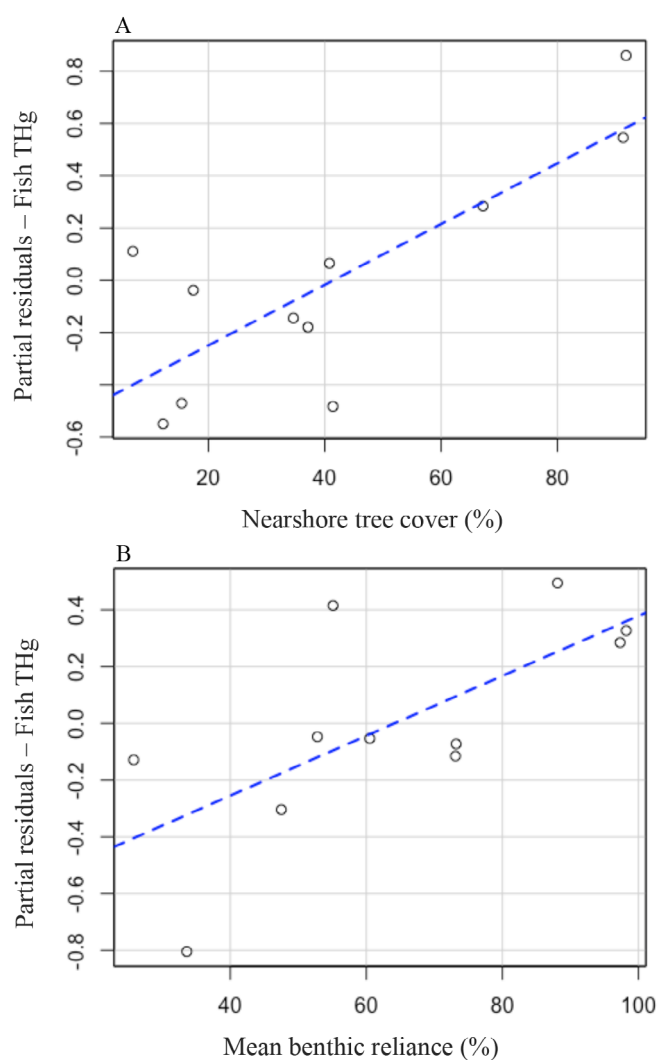
We examined the factors influencing fish THg concentrations across lakes with multiple linear regression using two datasets: one that included all lakes ( $n = 19$ ), and one that included only lakes where  $> 1$  fish was caught ( $n = 13$ ). In our top models for both datasets, the 80% confidence intervals of the beta coefficient for percent tree cover and mean benthic reliance were greater than zero, indicating they explained the majority of variability in fish THg ( $\Delta\text{AIC}_c < 2$ ; Table 3; Supplemental Data 3). In contrast, the confidence interval overlapped zero for models including benthic primary production, suggesting it had little influence on fish THg concentrations. The second best models included only percent tree cover; this model was equally competitive with the top model for the dataset including lakes with  $> 1$  fish ( $\Delta\text{AIC}_c < 2$ ; Table 3), but the  $\Delta\text{AIC}_c$  was  $> 2$  for the dataset including all lakes ( $\Delta\text{AIC}_c = 2.8$ ; Supplemental Data 3). Percent tree cover and mean benthic reliance were both positively related to fish THg concentrations (partial residual  $R^2 = 0.65$  and  $R^2 = 0.47$ , respectively; Fig. 3). When we summed the  $\text{AIC}_c$  weights for each variable (Burnham and Anderson 2002), tree cover ( $\omega_\beta = 0.93$  for all lakes;  $\omega_\beta = 0.87$  for lakes with  $> 1$  fish) was more important than benthic reliance ( $\omega_\beta = 0.69$  for all lakes;  $\omega_\beta = 0.61$  for lakes with  $> 1$  fish), but both were strongly supported model variables. In contrast, there was little support for all other model variables ( $\omega_\beta < 0.1$ ; Supplemental Data 3). Benthic primary production was not an important variable in the top models for either of our datasets, however it was included as a variable—in addition to precipitation—in the next best models, indicating that while benthic primary

**Table 3.** Comparison of top model results (all models with  $\Delta\text{AIC}_c < 2$ ) between (a) all lakes and (b) lakes from which more than one fish was caught.

<b>(a) All lakes</b>													
<b>Model 1</b>													
	$p$	$R^2_{\text{adj}}$	$F$	$df$	$\Delta\text{AIC}_c$	$\omega_i$							
	$<0.01$	0.55	8.89	11	0	0.52							
	$\beta \pm \text{SE}$		$t$	$p$		$\omega_\beta$							
Intercept	4.28±0.38		11.14	$<0.01$									
Percent tree cover	0.013±0.003		3.73	$<0.01$		0.93							
Mean benthic reliance	0.013±0.005		2.63	0.02		0.69							
<b>(b) Lakes with &gt; 1 fish</b>													
<b>Model 2</b>							<b>Model 3</b>						
	$p$	$R^2_{\text{adj}}$	$F$	$df$	$\Delta\text{AIC}_c$	$\omega_i$	$p$	$R^2_{\text{adj}}$	$F$	$df$	$\Delta\text{AIC}_c$	$\omega_i$	
	0.01	0.61	8.66	8	0	0.54	0.03	0.34	6.16	9	1.70	0.23	
	$\beta \pm \text{SE}$		$t$	$p$			$\beta \pm \text{SE}$		$t$	$p$	$\omega_\beta$		
Intercept	4.38±0.32		13.66	$<0.01$			5.11±0.21		24.77	$<0.01$			
Percent tree cover	0.012±0.003		3.61	$<0.01$			0.010±0.004		2.48	0.03	0.87		
Mean benthic reliance	0.011±0.004		2.65	0.03			—		—	—	0.61		

Akaike weights ( $\omega_i$ ) are presented for each model, while variable weights ( $\omega_\beta$ ) are provided for each model set.





**Fig. 3.** Partial residual plots of fish THg and top model variables of (A) percent nearshore tree cover and (B) mean benthic reliance for lakes with > 1 fish. Model variables are on the x-axes, and component-specific partial residuals of ln trophic-position corrected fish THg are on the y-axes. Dashed lines represent increases in fish mercury concentrations with greater nearshore tree cover and a higher degree of benthic reliance.

production explains some variability, it is not as effective of an indicator as benthic reliance or tree cover ( $\Delta AIC_c > 4$ ; Supplemental Data 3).

## Discussion

We observed a wide range of mercury bioaccumulation in invertebrates and fish, both within and among lakes. Our models suggested that both catchment and ecological processes were important for explaining differences in Hg bioaccumulation across our sample lakes. However, benthic primary production, which we used as an indicator variable for limnological and morphometric drivers of Hg

bioaccumulation, was not an important predictor of fish THg, likely due to the high uncertainty introduced by modeling this variable, instead of measuring it directly. Mercury concentrations in mountain lake fish from all of our study lakes were below the EPA fish tissue MeHg criterion value, indicating relatively low risk of health effects from mercury in comparison to top predator or longer-lived fish in ecosystems with higher mercury levels (Table 4; Eagles-Smith et al. 2016a).

Although there were no significant differences in mean fish THg concentrations among parks, concentrations were lower in Rainbow Trout compared to Cutthroat and Brook Trout, despite a consistent trophic position among species. The difference in mercury concentrations among species could be the result of size differences, or differences in consumption rates (Hilderbrand and Kershner 2004). Foraging preferences may also play a role; for example, Brook Trout have been observed to more aggressively prey upon amphibian eggs and larvae relative to Rainbow Trout in mountain lakes (Bull and Marx 2002; Murphy 2002). Therefore, species identity may be an important factor to consider in the management of mountain lake fisheries and potential Hg exposure to anglers.

Tree cover was positively correlated to fish THg in our models, which agrees with previous studies that found relationships between conifer cover and fish THg (Drenner et al. 2013; Eagles-Smith et al. 2016b), as the forests in our study lakes were all conifer dominated. Conifers contribute to lake Hg pools via throughfall and litterfall (e.g., in the form of needles and cones that have accumulated atmospheric Hg; Graydon et al. 2008; Drenner et al. 2013). Contrary to past studies, our calculations of tree cover were within a buffer around each lake, instead of at the catchment scale. We chose this approach because the mechanisms by which tree cover leads to higher Hg bioaccumulation are likely most pronounced at the lake edge, and proportional to lake size (i.e., the scaled buffer assumes larger lakes will be influenced by a larger proportion of their surrounding landscape than small lakes, whereas catchment size does not always correlate to lake size). Additionally, strong elevation gradients exist within a single lake catchment in these regions, leading to large differences in nearshore vs. catchment-scale tree density. Based on the consistent importance of tree cover for Hg bioaccumulation in our study and others, and the implications climate change has for expansion of tree cover to higher elevations (Theurillat and Guisan 2001), future studies should investigate the possible implications for mercury bioaccumulation in high elevation waterbodies. For example, using estimates from our more conservative model (lakes with > 1 fish), fish THg concentrations increased by 52% between the 25<sup>th</sup> to 75<sup>th</sup> percentile range of tree cover we observed (a change of  $149 \text{ ng g}^{-1} \text{ dm}$  between 12.6% and 67.2% tree cover).

Because diet is the primary route of MeHg exposure in fishes (Hall et al. 1997), variation in prey items is often a key determinant influencing fish THg concentration, particularly

**Table 4.** Comparison of mean mercury concentrations in fish from this study (bolded) to mean concentrations in salmonids in select high elevation lakes in other regions globally. Species include Arctic Char (AC), Kokanee (Ko), Brook (Bk), Brown (Br), Bull (Bu), Cutthroat (Ct), Golden (Go), and Rainbow (Rb) Trout. Lake is the unit of replication for range unless otherwise stated. Data are organized from highest to lowest mean mercury concentrations within each continent. Most studies report mercury in wet mass (wm), therefore, to avoid introducing uncertainty from the broad use of conversion factors, we report all mercury concentrations in wet mass.

Area/region	Species	Altitude (m)	Mean Hg (ng g <sup>-1</sup> wm)	Range Hg (ng g <sup>-1</sup> wm)
North America				
Lassen Volcanic NP*	Bk	1984–2038	96.4	28.6–493.4
Yellowstone NP*	Br, Ct, Rb	2261–3189	95.1	44.6–312.2
Olympic NP*	Bk, Ct, Rb	1227–1646	85.0	34.9–208.8
Yosemite NP*	Br, Rb	2916–3189	82.1	24.9–1108.6
Rainier NP*	Ct, Rb	974–1682	78.1	12.5–384.5
<b>Rainier NP</b>	<b>Bk, Rb</b>	<b>1372–1664</b>	<b>77†</b>	19.8–252.0
<b>Olympic NP</b>	<b>Bk, Rb</b>	<b>1357–1642</b>	<b>75†</b>	19.6–195.0
Grand Canyon NP*	Br, Rb	621–887	76.0	31.8–241.2
Rocky Mountain NP*	Br, Ct, Rb	2590–3378	59.3	9.9–528.3
North Cascades NP*	Bk, Ct	1529–1569	54.9	22.4–209.3
<b>North Cascades NP</b>	<b>Ct, Rb</b>	<b>1250–1747</b>	<b>54†</b>	14.0–128.0
Wrangell-St. Elias NP*	Ko, Rb	860	53.3	27.8–1108.6
Great Sand Dunes NP*	Bk, Ct	3365–3513	52.7	29.1–136.6
Wallowa-Whitman NF‡	Bk, Ct, Rb	1558–2725	45.4†§	5.4–97.0
Sequoia-Kings Canyon NP*	Bk, Go, Rb	3324–3517	43.8	13.3–124.5
Great Basin NP*	Bk	2281–3243	40.0	19.0–85.5
Cascades – Crater Lake*	Ko Bu, Rb	1502–1649	38.0	16.8–76.0
Tetons – Grant Teton NP*	Ct	2564–2814	32.6	16.2–99.0
South America				
Andes – Los Alerces NP¶	Bk, Br, Rb	517–527	112.5†	6.8–539
Andes – Nahuel Huapi NP¶	Bk, Br, Rb	770–975	100.7†	10.8–324.3
Europe				
Pyrenees#	AC, Bk, Br	1163–2492	303	219.6–750.3
Pyrenees – Ariège**	Br	1653–1676	271.8	60.2–780
Pyrenees – Spain††	Br	2240	68	15–158
Pyrenees – France††	AC	2091	55	37–78
Norwegian alpine	Br	1053	44	21–79
Alps – Switzerland††	Br	2519	37	18–56
Alps – Austria††	Br	2417	25	16–39
Norwegian alpine††	Br	728	21	14–31

NP, National Park; NF, National Forest.

\*Eagles-Smith et al. (2014).

†For studies that reported dry mass only ( $n = 3$ ), a conversion factor of 0.212 was used to estimate wet mass, under the assumption that dry mass of most trout species is approximately 78.8% of dry mass.

‡Eagles-Smith et al. (2016b).

§Geometric mean.

||Unit of replication = lake.

¶Rizzo et al. (2011).

#Blais et al. (2006).

\*\*Hansson et al. (2017).

††Rognerud et al. (2002).

if there are differences in prey MeHg concentrations. We found that zooplankton MeHg concentrations were generally higher than those of benthic invertebrates, thus it is surprising that benthic reliance was positively correlated with fish THg concentrations across our sample lakes. While the importance

of benthic reliance as a driver of THg may be due in part to the inherent variability around using isotopes to estimate diet, we did see strong isotopic separation between our end members in most of our lakes, and thus have reasonable confidence in our estimates (Table S1). Because mountain lakes are

typically oligotrophic, fish diet is likely dictated by prey availability, meaning lakes containing fish with high benthic reliance probably have macroinvertebrate-dominated food webs. In fact, benthic secondary production may be the most important energy pathway to fish in mountain lakes (Vander Zanden et al. 2006). Additionally, prey quality may be a factor influencing this result. For instance, fish with a benthic diet in New England lakes had less Hg than fish with a planktonic diet because benthic prey were higher quality, and fish need to consume fewer benthic invertebrates relative to zooplankton to meet their caloric demands (Karimi et al. 2016). Perhaps the opposite is true in mountain lakes: benthic prey may be of lower quality, and thus fish need to eat more—and consequently accumulate more Hg—to meet their caloric demands. Many of the common benthic invertebrates in the New England lakes were soft bodied, including amphipods, dragonfly larvae, and chironomids, whereas the more common benthic invertebrates found in our lakes included beetle larvae, adult aquatic beetles, and caddisflies, whose bodies contain more recalcitrant material (and a high C:N ratio). Indeed, Symons et al. (2019) determined that fish body condition was positively correlated to  $\delta^{13}\text{C}$  in Sierra Nevada mountain lakes, indicating that pelagic or terrestrial prey (which are depleted in  $\delta^{13}\text{C}$  relative to benthic invertebrates) were associated with better fish body condition than benthic prey. In addition to the issue of benthic invertebrate prey quality, terrestrial prey could be influencing our estimate of dietary important for fish mercury.

Terrestrial prey may be an important prey subsidy to fish in mountain lakes (Vander Zanden et al. 2006; Vander Zanden and Gratton 2011; Rola et al. 2018), but the stable carbon isotope ratios of pelagic and terrestrial organisms can often overlap (Pace et al. 2004). This overlap could be contributing to inflated estimates of pelagic reliance of our fish, especially considering that mountain lake consumers are highly omnivorous (Sánchez-Hernández et al. 2015). These overestimates could in turn be overshadowing biodilution of Hg in fish who rely on terrestrial prey as a significant part of their diet, because terrestrial insects have lower Hg than within-lake prey (Bartrons et al. 2015). Stomach content analysis revealed little insight to the role of terrestrial insects, as the majority of fish collected had empty, or nearly empty stomachs.

Additionally, terrestrial carbon subsidies to invertebrate diets may complicate our current understanding of mercury pathways to fish. For example, evidence of terrestrial carbon in invertebrates was present in Grand Lake; dragonfly and aquatic beetle larvae had more depleted  $\delta^{13}\text{C}$  than zooplankton (e.g.,  $-29\text{‰}$  vs.  $-24\text{‰}$ ), and more closely matched the  $\delta^{13}\text{C}$  of the nearshore vegetation, which in this case had a distinct carbon isotope ratio relative to lake primary producers ( $-31\text{‰}$  vs.  $-18\text{‰}$ , Supplemental Data 1). Interestingly, macrophytes often had an intermediate  $\delta^{13}\text{C}$  value relative to zooplankton and benthic primary consumers or periphyton.

Amphipods were the only invertebrate with  $\delta^{13}\text{C}$  values that matched well with macrophytes, however the lack of an obvious macrophyte signal for other benthic invertebrates does not necessarily mean other invertebrates avoid consuming macrophytes at all. As such, the presence of macrophytes likely adds further uncertainty both around our calculations of benthic reliance as well as mercury bioaccumulation in the food web at-large. Further investigation into the role of community structure, fish diet, and terrestrial subsidies will be important for gaining a better understanding of how food web structure and foraging habitat influence Hg bioaccumulation in mountain lakes.

There is likely much more to understand about how lake production interacts with Hg bioavailability and bioaccumulation that we could not learn from our modeled values of percent benthic primary production (and the compounding errors that may have led to difficulty in fitting this variable to a regression model). For example, we hypothesized that higher proportions of benthic production would lead to conditions antagonistic to MeHg production, and thus less bioaccumulation at the base of the food web. However, lakes with high benthic primary production also tend to have lower catchment slopes than lakes with lower benthic primary production (Vadeboncoeur et al. 2008). Therefore, lakes with high benthic production may also produce more labile DOC in the littoral zone than lakes with lower benthic production, as a result of wetland areas that are common in low-slope catchments (Winn et al. 2009). This higher labile DOC production by wetlands can facilitate increased MeHg production, while contributions of recalcitrant DOC may inhibit photodemethylation (Ravichandran 2004; Klapstein and O'Driscoll 2018). Thus, the relative importance of benthic primary production for Hg bioaccumulation likely varies in a context-dependent fashion, requiring more robust inquiries, including a larger number of lakes sampled. A deeper understanding of the role of benthic primary production is critical, because it can comprise a substantial portion of lake primary production, and affect fish diet availability via secondary production (Vander Zanden et al. 2006, 2011).

The complex and interacting roles of landscape, limnological, and food web processes clearly make it difficult to assess Hg bioaccumulation potential in mountain lakes, especially given constraints to sample size introduced by sampling access and a short open water season. Yet mercury concentrations in mountain lake fish vary widely around the globe and even within single mountain ranges (Table 4), further necessitating the need for effective indicators. For example, different regions within the Cascade, Rocky Mountain, and Pyrenees ranges can see two- to fivefold differences in fish Hg (Table 4). Fish Hg concentrations from our lakes represent an intermediate value in the range of concentrations observed in fish-stocked mountain lakes globally, and our lakes spanned a diverse range of sizes and catchment characteristics—from

high temperate rain forest in the Olympic mountains to arid alpine areas in the Northeastern Cascades. Thus, our lakes may be an effective venue for developing indicators that can be applied broadly, and we encourage future work to investigate the effectiveness of these indicators in other regions.

## Conclusion

Our results indicate that both landscape and food web factors are important determinants of mercury bioaccumulation in mountain lake food webs. Together, tree cover and benthic reliance (a function of prey availability) explained up to 61% of the variability in mean fish THg concentrations in mountain lakes across Washington. Therefore, it may be possible to identify lakes with the potential for higher Hg bioaccumulation in fish by surveying nearshore tree cover and invertebrate communities. Because tree cover was an important variable in our models, as well as in other studies of Hg bioaccumulation, future work should investigate how shifting tree lines as a result of climate change could influence Hg bioaccumulation. Our findings also have implications for the management of mountain lake fisheries globally. For example, in the Pyrenees, there is an ongoing effort to remove fish from high mountain lakes to conserve sensitive fauna, but angling is an important and culturally protected activity for native residents, and common in lakes accessible from villages and urban areas (Miró and Ventura 2013); information about mercury bioaccumulation potential could help inform decisions about prioritizing fishing bans in certain lakes. In the United States, existing annual invertebrate surveys that are conducted by the National Parks Service may be used to inform the decision-making process around fish stocking and/or fish removal. Lakes that have both high Hg bioaccumulation potential and are heavily impacted by non-native trout could be good candidates for fish removal. Such tools are important for justifying management decisions around the political issue of fish removal and stocking in lakes on public lands (Chiapella et al. 2018).

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**Conflict of Interest**

None declared.

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