Mercury Transfers in the Aquatic Food Web of a High Alpine Lake Ecosystem, Green Lakes Valley, Colorado

An Honors Thesis by Phillip Thornton

Department of Environmental Studies, University of Colorado Boulder

Defense Date: April 5, 2022

Thesis Advisor:

Dr. Eve-Lyn S. Hinckley, Assistant Professor, Department of Environmental Studies

Defense Committee:

Dr. Eve-Lyn S. Hinckley, Department of Environmental Studies, Dr. Valerie McKenzie, Department of Ecology and Evolutionary Biology, Dr. Colleen Scanlon-Lyons, Department of Environmental Studies

Table of Contents

Acknowledgments

Without my incredible advisor Eve Hinckley this project could never have been possible. Eve was paramount in my decision to conduct this research, and I am fortunate to have been able to work so closely with her. She supported not only my work but was always there for me as a mentor and friend. Eve, thank you for putting up with me and my constant antics. Without your support, I would have never been able to accomplish what I have in my college career. A special thank you to Hannah Miller. Without your constant support, this research would not have been possible. Thank you for always being willing to deal with my lack of experience and for being such an excellent teacher. Thank you, especially, for helping me fumble around in R studio and conducting the mercury analysis for this project; without your guidance, this research would never have been completed. A special thanks to Sammy Yevak, Cliff Adamchak, and Stacie DeSousa, who helped conduct field sampling with me. I would also like to thank Kathryn Snell, who was kind enough to allow us to use her lab for sampling! Lastly, I would like to thank the Environmental Biogeochemistry Group at the University of Colorado at Boulder. Thank you all for taking me in and sharing your expertise with me as I went through this process. Being around you all inspired and motivated me to complete this work. Thank you, especially to Michelle Leung, who embarked on this process alongside me with her own thesis and showed me the standard for great undergraduate work!

The research was funded by the Niwot Ridge LTER program (NSF DEB−1637686) and a University of Colorado Undergraduate Research Opportunities Program (UROP) fellowship.

Abstract

Mercury (Hg) is a global pollutant whose cycling has been dramatically influenced by mobilization of elemental Hg (II) from anthropogenic sources. Mercury mobilization has led to a significant increase in atmospheric deposition of inorganic Hg, which can cycle within terrestrial and aquatic ecosystems. Prior research has documented the long-distance transport of Hg, thus allowing for deposition in remote regions. High elevation areas have been disproportionately affected due to increased deposition and retention of Hg in these areas. There remains, however, a lack of knowledge about the Hg cycle in these areas, particularly as it pertains to the mobilization of Hg into the food chain. Under anoxic conditions, usually in aquatic systems, Hg can be methylated by sulfate and iron-reducing bacteria into methylmercury (MeHg), a neurotoxin that bioaccumulates at each successive trophic level. Prior research at Niwot Ridge in the Colorado Rocky Mountains, U.S., showed elevated concentrations of MeHg in weasels (*M. frenata*), with low concentrations in its terrestrial food sources, pointing to aquatic systems of the area as a potential source of MeHg. In order to investigate the movement of MeHg into the alpine aquatic food web, I sampled fish, zooplankton, water, and lake sediments in the Green Lakes Valley, Colorado. I present total mercury (THg), methylmercury (MeHg), and stable isotope data for each sample type to determine the flows of MeHg between lakes within the Green Lakes Valley and the factors that affect the bioaccumulation of MeHg in the aquatic food web. The data suggest that MeHg production and bioaccumulation in the Green Lakes Valley increased with elevation, likely due to their proximity to Arikaree rock glacier. Results showed clear rates of MeHg bioaccumulation at each succesive trophic level. Furthermore, fish within the Green Lakes Valley showed no MeHg concentrations over set limits of 200 ng MeHg g^{-1} ww at which trout show no effect from MeHg concentrations. This project provides the first look into the movement of MeHg and bioaccumulation within an aquatic food web of the Green Lakes Valley. In combination with prior studies on the terrestrial food web in the area, it provides more knowledge of the Hg cycle in high elevation mountain ecosystems.

Introduction

Mercury (Hg) is a naturally occurring, geologically sourced element. However, human activities, such as fossil fuel combustion and industrial processes, have increased the atmospheric cycling of Hg by a factor of three to five (Selin et al., 2009). Fossil fuel emissions release Hg in its reactive form, Hg (II), through combustion. Natural sources of Hg on the other hand, release Hg in its elemental form Hg (0) (Driscoll et al, 2013). Hg (0) has a residence time of one year once in the atmosphere, at which point it is oxidized into Hg (II) (Selin et al., 2009). Mercury is then transported around the world through atmospheric processes. Mercury enters terrestrial and aquatic ecosystems via wet and dry deposition of inorganic Hg (II) (Selin et al., 2009). Direct anthropogenic sources account for 29-33% of total Hg emissions on a global scale. Industrial and energy facilities in the Western U.S., Mexico, and Western Canada release 11,000 kg of Hg alone, accounting for roughly one fifth of the continent's direct anthropogenic emissions (Eagles-Smith et al., 2016).

Land use also has a major impact on the Hg cycle. Mining is a notable source of Hg pollution across the Western U.S., including the Colorado Rocky Mountains, accounting for significant changes to the transport and cycling of Hg (Eagles-Smith et al., 2016). Numerous studies have shown the effects of gold, silver, and Hg mining on point-source Hg contamination (Porcella et al., 1997; Bishop et al., 2020). Mercury is also a common pollutant in industrial wastewater, which is likely attributed to its use as a preservative in biological and pharmaceutical applications (Wagner-Döbler et al., 2003). Increased wildfire frequency also influences the release of Hg into the atmosphere, increasing atmospheric Hg deposition to the biosphere (Bishop et al., 2020). According to Bishop et al. (2020), Hg deposition from wildfires will increase 28% by the year 2050. As Hg is deposited to ecosystems, it can then be converted to more toxic forms that may be harmful to wildlife.

Under anoxic conditions—often in aquatic ecosystems—inorganic Hg can be converted to methylmercury (MeHg), a neurotoxin that bioaccumulates in food webs. Methylation is carried out by bacteria and archaea under anoxic conditions, elevated sulfate concentrations, and high organic matter content (Selin et al., 2009). Sulfate and iron-reducing bacteria play a large role in Hg methylation. However, recent studies point to a wide range of methylating organisms, including fermenters, syntrophs, and methanogens that can be involved in Hg methylation (Peterson et al., 2020). The common feature of anaerobic microorganisms that are capable of Hg

methylation are the hgcAB genes. Multicellular organisms take up MeHg as MeHg- cysteinate (MeHgCys), which bioaccumulates ~10-fold at each successive trophic level (Hammerschmidt et al., 2006). MeHgCys is known to demethylate Hg by breaking down into HgSe and Hg(S, Se) granules (Manceau et al., 2021). In upper aquatic tophic levels MeHg accounts for nearly all total Hg (THg) levels (Bloom et al., 1992). There are still many unknowns about MeHg, but research on the subject remains essential, based on the reported effects that the neurotoxin has on animal physiology (Chételat et al., 2020).

The importance of MeHg bioaccumulation in animals stems from the adverse effects on cognitive and reproductive functions with higher concentrations. Methylmercury is harmful, particularly for top predators, causing, for example, cognitive decline in humans and softening of eggs in avian species. By the time MeHg ascends the aquatic food web to piscivorous fish, concentrations can be 10^7-fold greater than those of water (Eagles-Smith et al., 2016). In addition to trophic position, other factors that can influence MeHg bioaccumulation are: 1) the daily rate and MeHg concentration of food consumed by the fish, and 2) the energy content of the food as well as the allocation of the energy to metabolism or biomass production. Thus, dietary intake from MeHg is controlled by trophic position; ontogenetic shifts in diet, migration, and movement; habitat specific feeding; and diet subsidies from other ecosystems (Chételat et al., 2020).

Methylmercury elimination rates are typically slow and range from one to three years in freshwater fish. Once MeHg is ingested it circulates throughout the body and can be expelled through digestive processes as well as passed down to offspring through maternal transfer (Chételat et al., 2020). However, even with these possible elimination methods MeHg is typically stored for many years in fishes' muscle and fatty tissues. Benchmarks have been set for MeHg concentrations in fish to monitor the risk to predators, as well as the fish themselves (Chételat et al., 2020). In a study of brook trout (*Salvelinus fontanalis*) cutthroat trout (*Oncorhynchus clarkii*), rainbow trout (*Oncorhynchus mykiss),* and lake trout in 21 national parks, researchers used a "no observed effects residue" of 200 ng/g ww and a "lowest observed effects residue" of 300 ng/g ww as benchmarks to assess the potential risk to the fish. The study cited that 300 ng/g ww indicates the threshold at which fish may be affected by the MeHg exposure (Eagles-Smith et al., 2014)

Trout species, particularly brook trout (*S. fontanalis*) and cutthroat trout (*O. clarkii*), are present in subalpine lakes throughout the Colorado Rocky Mountains. These species represent a top predator in subalpine aquatic ecosystems, and are at risk of MeHg bioaccumulation due to their trophic position. Because MeHg is more abundant in aquatic ecosystems and fish are mostly consuming prey in these ecosystems, they are more likely to have a higher concentration of MeHg than animals feeding primarily in terrestrial food systems. Trophic position can also be discerned by looking at the stable isotopes of C and N (Bartrons et al., 2015). The stable isotopes of nitrogen ($^{15}N/^{14}N$) can be used to determine the trophic position of animals, while the stable isotopes of carbon ($^{13}C/^{12}C$) can be used to determine the major energy sources for a particular organism in the food web, as described by Braaten et al. (2014).

High elevation ecosystems are particularly important to investigate as they often show higher atmospheric Hg deposition rates than lowland areas, and there is some indication that MeHg accumulates in the food web (Adamchak, 2021). Higher atmospheric Hg deposition rates occur in high elevation mountainous areas due to greater photochemical transformations of Hg (0) into Hg (II) and increased rates of atmospheric Hg deposition (Murphy et al. 2006). These factors are compounded by greater precipitation and cold condensation, which adds to the total atmospheric Hg load (Mast et al., 2005). Prior research at Rocky Mountain National Park shows that alpine ecosystems are a sink for up to 80% of atmospherically deposited Hg (Mast et all., 2005). A study that took sediment cores from alpine lakes near Rocky Mountain National Park also shows that Hg accumulation in the area is four-fold higher than in the pre-Industrial age (Mast et all., 2005). The availability of inorganic Hg in high alpine areas creates the potential for MeHg production to occur, particularly in wetter parts of the landscape, which may lead to MeHg accumulation in aquatic food webs (Adamchak, 2021). This evidence, along with history of mining and dam development, makes the Colorado Rocky Mountains a particularly important region to investigate the Hg cascade through alpine ecosystems.

Previous studies at Niwot Ridge Long-term Ecological Research (LTER) site in the Colorado Rocky Mountains have focused on aspects of the Hg cycle in high alpine ecosystems (Miller, unpublished data; Adamchak, 2021). A previous study of MeHg bioaccumulation in the alpine terrestrial food web found low rates of bioaccumulation from dust particles to pika (*Ochotona princeps*) that feed on herbaceous plants in the area. However, this study did find bioaccumulation of MeHg in weasels (*M. frenata*). Weasels feed on both terrestrial and aquatic

food sources. The work of Adamchak (2021) pointed to the organisms of alpine aquatic ecosystems as a particularly important next step in determining the flows of Hg through the food web, as well as their implications for the health of high elevation ecosystems.

Futhermore, a study by Crawford et al., found sulfate export in the area has increased over 200% between 1984 and 2015 (Crawford et al., 2019). This is likely caused by the weathering of the Arikaree rock glacier caused by warmer air temperatures (Crawford et al., 2019). Because sulfate reducing bacteria are essential to the methylation process, there is likely an increase in reducing conditions within the lakes of the Green Lakes Valley, which may be causing increased mobilization of MeHg into the local food web. Thus, Arikaree rock glacier may be the key driver of elevated MeHg concentrations within the main drainage of the Green Lakes Valley, and account for elevated concentrations within aquatic organisms.

To date, there has not been a study to quantify MeHg accumulation in the fish populations primarily brook trout (*S. fontanalis*) and cutthroat trout (*O. clarkii*) in the Green Lakes Valley, proximal to the Niwot Ridge LTER. Therefore, the goal of this honors thesis project was to determine the flows of MeHg through the aquatic food web. To address this unknown, I collected phytoplankton, zooplankton, cutthroat trout (*O. clarkii*), and brook trout (*S. fontinalis*), in order to determine the effects of atmospheric Hg deposition on MeHg bioaccumulation within the aquatic food web. I addressed the following questions in my research related to MeHg cycling in high alpine lake environments.

Research Questions and Hypotheses

Q1: Which of the lakes in the Green Lakes Valley has the greatest average concentration of MeHg in trout?

H1: Lake Albion will have the greatest MeHg concentrations, while Green Lake 1 will have the lowest because all lakes in the valley drain into Albion, allowing for a larger amount of MeHg to enter the lake ecosystem through atmospheric deposition, lake drainage, and runoff.

Q2: What is the relationship between fish age/size and MeHg concentrations in fish tissues?

H2: Concentrations of MeHg will directly correlate with the age and size of trout due to increased consumption of MeHg-containing food sources.

Q3: Do different diets of trout affect their MeHg concentrations?

H3: Trout that feed at a higher trophic level will correlate positively with increased concentrations of MeHg

Study site

The Green Lakes Valley (GLV) (**Figure 1**) is a subalpine watershed in the Colorado Rocky Mountains and is adjacent to the Indian Peaks Wilderness Area. The GLV is part of the Boulder Creek Watershed and is not open to the public. The area is a part of Niwot Ridge LTER. The GLV contains 6 lakes: Lake Albion, Green Lake 2, Green Lake 1, Green Lake 3, Green Lake 4, and Arikaree, in order from lowest to highest elevation. These lakes range from 3,355 m to 3,633 m of elevation. Green Lakes 2, 3, 4, and Lake Albion differed only by elevation but are in the same drainage (connected by North Boulder Creek). Green Lake 1, however, is fed by runoff and lies adjacent to the main drainage. This positioning is important to this study due to the presence of Arikaree rock glacier at the headwaters of North Boulder Creek, with Green Lake 1 immediately downstream. Based on climate data from the D1 weather station located directly adjacent to the drainage (40° 3' 36" N, -105° 37' 12" W, elevation 3739 m), the area has an annual average mean temperature of -4.1°C, and reports ~ 1,000 mm of precipitation, 80% of which is deposited as snow (Kittel et al. 2015; Williams et al. 1996; Greenland and Losleben, 2001). Approximately 70% of runoff from the area comes from snowmelt, which peaks in the spring, from April to mid-July, and provides a primary water source for the City of Boulder (Badger et al., 2021). Populations of *S. fontanalis* and *O. clarkii* have been found in Green Lake 1, Green Lake 2, Green Lake 3, and Lake Albion. Green Lake 4 has had a fish population in the past but was fished out within the last 10 years.

Methods

Field Methods

I sampled phytoplankton and zooplankton from the deepest point in each lake. Phytoplankton were collected using a Vandoren (a pipe with a door on each side that traps water at a designated depth) to trap a bulk sample at 3m. I collected zooplankton using a plankton net to filter out any phytoplankton. Once I collected these samples, I took them to the lab, where the phytoplankton were refrigerated in Nalgene containers. I filtered zooplankton once more through an 80 μm mesh and put the samples into 1oz Whirlpaks

with a scoopula (rinsed with deionized water). I then froze zooplankton samples until analysis. Phytoplankton samples were not used within this research but were archived to be used for further study.

I collected trout using hook and line. This sampling took place over 1-2 days to collect 2-7 fish per lake. Sampling started at Green Lake 1 and continued until the limit of 6-7 fish were caught or a period of 90 min had passed. The sampling proceeded to Green Lake 3, Green Lake 2, and Albion. When I caught a fish, I netted it and immediately killed it in accordance with the IACUC permit. I weighed the fish (to the nearest 0.1g) and measured (to the nearest 1 mm). After recording the measurements, I gutted each fish and placed it in a plastic bag filled with ice. Organs collected during the gutting process were bagged with ice. I hiked these samples to the vehicle parked at Green Lake 1 and transported them on ice to the laboratory.

Laboratory Methods

In the lab, I thawed fish to room temperature (20°C). I then sub-sampled 5-10 g of skinless axial muscle tissue from the upper fillet with a clean scalpel for THg, MeHg, total carbon (TOC) and nitrogen (TN), and the stable isotopes of C ($^{13}C/^{12}C$) and N ($^{15}N/^{14}N$). Each muscle sample was rinsed with deionized water and placed in a 1 oz Whirlpak. To determine the age of the fish, I removed the otoliths and placed them in Whirlpak bags. The stomach contents of the fish were placed in ethanol until analysis to determine the trophic position, primary source of food, and the relationship of these factors with fish age, according to Eagles-Smith et al. (2014).

The ear bones or otoliths in each fish were analyzed to estimate age. As trout age each winter, their otolith develops a dark ring, which can then be counted under a microscope to determine the estimated age in years. I placed each otolith on the microscope and captured a picture of the area with the most visible bands, which I subsequently counted.

The stomach contents of three fish from each lake were prepared for C and N stable isotope analysis, as well as determination of THg and MeHg concentrations. During examination of stomach contents, I recorded notes and pictures of each fish's stomach. Contents were then packed into a Whirlpak and placed in a freeze drier. Once freeze-dried, I subsampled each sample for C and N stable isotope analysis in the Earth Systems Stable Isotopes Laboratory at the

University of Colorado, Boulder. The remaining sample was then sent the the USGS Mercury Research lab in Madison, WI for THg and MeHg analysis.

MeHg Extraction and Analysis

Each biological sample was weighed into Teflon vials and then digested in 4.5 M nitric acid for 8 hr at 60°C. The sample extract was then added to reagent water in 42 ml vials. The sample was titrated with 5M Potassium Hydroxide and buffered with Sodium Acetate to a pH of 4.5-5. Sodium tetraethyl borate was then added to the sample. This resulted in the ethylation of the Hg species. Argon gas was then used to eliminate the ethylated species and Hg (0), retain the samples on Tenex traps, desorb thermally back into the sample stream, and separate them by mass. The ethylated species and Hg (0) were then released from the column into the sample stream, oxidized into Hg (II), and detected by cold vapor atomic fluorescence spectrometry (CVAFS). Analysis was then conducted using the Brooks-Rand "MERX" Hg analytical system.

Total Hg Extraction and Analysis

Each biological sample was weighed into Teflon vials and digested in 4.5M nitric acid for 8 hr at 60°C. The samples were then treated with ultraviolet light for three to five days to destroy dissolved organic matter. Bromine monochloride (BrCl) was added to the sample and heated to 50°C for five days. This process oxidized all forms of Hg to Hg (II). The digest was added to an analytical vial and neutralized the BrCl by adding hydroxylamine hydrochloride (NH_2OOH*HCl). Stannous chloride was then used to reduce the Hg (II) to Hg (0). The volatile Hg (0) was then purged from the sample and captured by a gold sand trap. The sample was then desorbed and detected by cold vapor atomic fluorescence spectrometry (CVAFS). Sample analysis was then conducted using the Brooks-Rand "MERX" automated Hg analytical system.

Data Analysis

My data violated the assumptions of parametric tests due to non-normality. Thus, I conducted a Kruskal-Wallis test in R studio with the "kruskal.test" function to compare the concentrations of Hg species in fish tissues across lakes. In addition, a post hoc Dunn test was used in R studio to determine significant differences among lakes in the Green Lakes Valley with the "dunnTest" function. I used linear regression to determine the relationships between fish

characteristics (i.e., age, size, and length) and MeHg concentrations in fish tissues. To do this, I used the "lm_temp" function in R studio.

Results

Methylmercury concentrations in fish tissues from the Green Lakes Valley revealed that Green Lake 3—the lake at the highest elevation—had the highest MeHg concentration in fish tissues (466.5 ± 112.4 ng MeHg g^{-1}). Conversely, Green Lake 1, a lake fed directly from runoff and adjacent to the main drainage, had the lowest concentration of MeHg in fish tissues (144.8 ± 89.35 ng MeHg g^{-1}) (**Table 1**, **Figure 2**). A Kruskal-Wallis test revealed significance between MeHg and THg in the Green Lakes Valley ($p = 0.04$). However, when a post hoc Dunn test was applied to the data, it showed no significant difference between the tested lakes. Figure 3, however, shows a low mean MeHg concentration in zooplankton in Green Lake 3 (13.5 ng MeHg g^{-1}) and the highest mean concentration in Lake Albion (27.5 ng MeHg g^{-1}).

MeHg wet weight results all fell within the range of a USGS study on the Big Thompson River drainage (0.01-0.167 ww ppm (Mast et al,. 2005). Figure 4 shows MeHg concentrations in the Green Lakes Valley are within those taken in the previous study. However, Green Lakes 2 and 3 show MeHg wet weight on the higher end of the of the lakes tested within this study and comparable to unpublished USGS data.

A linear regression model was applied to test the relationship between MeHg concerntrations in fish and the elevation of each lake within the Green Lakes Valley, as well as the unpublished USGS dataset. The model revealed no significant relationship between elevation and the MeHg concentration of fish within these lakes ($p > 0.05$) (**Figure 5**).

A linear regression model was applied to test the relationship between fish characteristics and MeHg concentrations. The model revealed a significant relationship between fish age and THg concentration ($p = 0.007$), as well as fish age and MeHg concentration ($p = 0.002$) (**Figure 6**). Fish length was found to have less effect on both THg concentration ($p = 0.296$) and MeHg concentration ($p = 0.251$) and results were not statistically significant.

Results for carbon (C) and nitrogen (N) stable isotopes in fish, zooplankton, vegetative and lake sediment samples have not yet returned from the stable isotope lab at the University of Wyoming by the time that I defended and filed this thesis. However, results for C ($^{13}C/^{12}C$) and N ($^{15}N/^{14}N$) stable isotopes in fish stomach contents have been analyzed. Mean stable isotope

data for $\delta^{13}C$ in fish stomach contents was -25.88 ± 0.35‰. Mean stable isotope data for $\delta^{15}N$ in fish stomach contents was 4.18 ± 0.39‰. Green Lake 3 had mean $\delta^{13}C$ and $\delta^{15}N$ values at -26.35 ± 0.23‰ and 4.29 ± 0.05‰ followed by Green Lake 2 (-25.87 ± 0.50‰, 4.18 ± 0.76‰), Lake Albion (-25.79 ± 0.04‰, 4.19 ± 0.22‰), and Green Lake 1 (-25.66 ± 0.15‰, 4.11 ± 0.35‰).

Discussion

This study addressed the flow of Hg through an alpine aquatic food web of the Green Lakes Valley. A prior study by Adamchak (2021) found low levels of MeHg in alpine herbaceous plants and *O. princeps*, but elevated MeHg concentrations in *M. frenata* tissues. Concentrations of MeHg were as follows: dust (7.03 ± 1.66 ng MeHg g^{-1}), lichen (1.39 ng MeHg g^{-1}), litter (0.152 ng MeHg g^{-1}), forbs (0.014 ng MeHg g^{-1}), and graminoids (0.0243 ng MeHg g^{-1}). This prior evidence suggested that rather than the terrestrial ecosystem, aquatic ecosystems, including lakes, wetlands, streams, and stream sediments may be a source of MeHg. My study found levels of THg in lake water to be 0.74 ± 0.19 ng THg L^{-1} (MeHg concentrations were below detection limit) and MeHg in lake sediments to be 6.83 ng MeHg g^{-1}. These findings indicate MeHg production in the aquatic ecosystems of the alpine zone.

Furthermore, I found evidence of MeHg mobilization into the aquatic food web. While Adamchak (2021) found that *O. princeps*, which feed exclusively on terrestrial food sources, had a mean MeHg concentration of 62.83 ng MeHg g^{-1}, and *M. frenata*, which feed on both terrestrial and aquatic food sources, had a mean MeHg concentration of 625 ng MeHg g^{-1}, I found mean MeHg concentrations in *O. clarkii* and *S. fontinalis* to be 247.78 ng MeHg g^{-1} ± 165.58 ng MeHg g^{-1}. These results point to elevated rates of MeHg production in aquatic environments, which allows for a greater rate of bioaccumulation in fish, eventually affecting top predators (Mast et al., 2006).

Contrary to H1, the results of this study showed a positive correlation in the main drainage (GL2, GL3, Lake Albion) with MeHg concentrations in fish, and the proximity to the Arikaree rock glacier. Green Lake 3 showing the highest mean concentration of MeHg in fish (466.5 ng MeHg g^{-1}). Green Lake 1 showed the lowest concentrations of MeHg and is fed solely by runoff and snowmelt. These results point to sulfate export from Arikaree rock glacier as a possible driver of MeHg concentrations within the Green Lakes Valley. Another possibility is elevation. A study on Hg concentrations in arctic char (*Salvelinus alpinus*) in the French Alps

found similar results and cited elevation as having the greatest effect on Hg concentrations (Blais et al., 2006). Investigators found a positive correlation between elevation and MeHg concentrations in fish, citing that enhanced atmospheric deposition and retention of Hg in high alpine ecosystems may be the cause. However, my results did not show a significant relationship between elevation and MeHg concentration in fish when looking at sites both in Green Lakes Valley, and those tested in an unpublished USGS study (**Figure 5**).

Data on zooplankton samples contradicted the results of MeHg concentrations in fish (**Figure 3**). These findings may also be a result of greater biomass in Lake Albion and Green Lake 2, which can naturally cause less bioaccumulation through "bloom dilution". Bloom dilution is a process in which concentrations of MeHg and other bioaccumulating substances may be diluted by a population feeding within a confined pool of the substance. Thus, with decreased biomass, concentrations of MeHg cannot be diluted throughout the system (Ward et al., 2010). An important factor to note is the mining and dam building activity that took place in the 1800s and early 1900s. Green Lake 3 is located near the opening of an abandoned mine shaft. This is interesting to note, due to possible point source pollution (e.g., Suchanek et al., 2008). Future research should investigate this potential connection. However, if the Green Lakes Valley is affected by point source mobilization of Hg from historic mining activity, it would be logical that MeHg concentrations decrease with downstream distance from the mine.

Compared to unpublished USGS dataset on fish MeHg wet weight concentrations in Rocky Mountain National Park (RMNP) (**Figure 4**), the lakes in the Green Lakes Valley exhibited similar values. However, MeHg concentrations in the Green Lakes Valley varied significantly compared to those tested in RMNP. This finding is surprising considering that the lakes in the Green Lakes Valley are all within the same drainage and within 3,352m to 3,505m elevation. However, the USGS sample sites are much more variable, and while the lakes all originate from the same region, there is a significant difference in elevation and distance between each lake compared to the Green Lakes Valley. Lakes sampled in the USGS data ranged from 2,133m to 3,505m and were spread over the entirety of RMNP. Therefore, it is interesting that the range in MeHg values from the Green Lakes Valley was similar to those measured across RMNP. These results suggest that other factors such as watershed characteristics (i.e., trophic productivity, tree catchment area) are a more significant influence on THg and MeHg concentrations. Thus, it appears that lakes within the same drainage can vary highly in their

flows of Hg, and MeHg concentrations within the lakes may be dependent on the environmental factors specific to each lake.

Results on the relationship between fish age and MeHg concentrations showed a positive correlation, supporting **H2**. A linear regression showed statistical significance for the effect of age on MeHg and THg concentrations at ($p < 0.01$) for both sets of data (**Figure 6**). I also found a positive correlation between length and weight and MeHg concentrations ($p < 0.05$). These data support previous research on Hg in fish showing that THg and MeHg concentrations were affected by the fish size and weight (Eagles-Smith et al., 2014). Thus, it is likely that age is the most significant factor to determine MeHg and THg concentrations on fish, followed by their food intake and diet, which likely regulate how large they grow over time. Stomach contents tested for THg concentrations found the highest mean THg concentrations in Green Lake 3 (33.89 ng THg g^{-1}), followed by Green Lake 1 (29.43 ng THg g^{-1}), Green Lake 2 (26.37 ng THg g^{-1}), and Lake Albion (17.18 ng THg g^{-1}). These data support the hypothesis and previous research from Blais et al. (2006) on arctic char, that lakes at higher elevations are likely to have higher MeHg concentrations in fish, but more investigation is needed to determine causality.

Mean MeHg concentrations at each site exhibited no concentrations above-set standards of 200 ng MeHg g^{-1} ww for "no observed effects residue," (the MeHg concentration at which a fish would not be affected negatively) (Beckvar et al,. 2005) and 300 ng MeHg g^{-1} ww for "lowest observed effects residue" (the lowest concentration of MeHg at which a fish would be negatively affected) (Sandhiendrich et al., 2011; Eagles-Smith et al., 2014). Thus, my data indicate no concern for MeHg effects on the fish themselves, as the greatest concentration in any fish sampled was 167.43 ng MeHg g^{-1} ww. However, four of the fish sampled in Green Lakes 2 and 3 showed MeHg concentrations that could pose a risk to predatory birds with high Hg sensitivity (> 90 ng MeHg g^{-1} ww) (Eagles-Smith et al., 2014). Yet these results did fall short of 270 ng MeHg g^{-1} ww, the benchmark for risk to predatory birds with low sensitivity to Hg. All samples were below EPA regulations for human health of 300 ng MeHg g^{-1} ww and were far from the Great Lakes Advisory Group (GLAG) recommendations (i.e., no fish should be consumed) at 950 ng MeHg g^{-1} ww.

Although the stable isotope dataset for C ($^{13}C/^{12}C$) and N ($^{15}N/^{14}N$) is not yet complete, making it difficult to reconstruct the food web-based on $\delta^{15}N$ and $\delta^{13}C$ values, MeHg concentrations increase at successive trophic levels. Thus, it is possible to infer the position of

each sample in the trophic pyramid. Figure 8 indicates bioaccumulation of MeHg in successive trophic levels of Green Lake 1 and Lake Albion. Green Lake 1 shows lower levels of MeHg in fish (144.8 ± 89.35 ng MeHg g^{-1}), zooplankton (17.5 ng MeHg g^{-1}) and THg in lake water (0.57 ± 0.0 ng THg L^{-1}). Conversely, Lake Albion shows higher levels of MeHg in fish (198.75 ± 82.16 ng MeHg g^{-1}), zooplankton (27.5 ng MeHg g^{-1}) and THg in lake water (0.92 ± 0.19 ng THg L^{-1}). These results also suggest proximity to Arikaree rock glacier is the driver of MeHg concentrations in the Green Lakes Valley.

Figure 6 shows the MeHg concentrations found in this study in the context of values for the Green Lakes Valley. These data suggest that MeHg bioaccumulates from lake sediments (6.83 ng MeHg g^{-1}) and lake water (below detection limits for MeHg) to zooplankton (20.06 ng MeHg g^{-1}) and subsequently fish (247.78 ng MeHg g^{-1}) in the aquatic ecosystem. Data collected on caddisflies in the nearby alpine wet meadow shows low levels of MeHg (4.23 ng MeHg g^{-1}), suggesting that small wetland areas may not be a significant source of MeHg. Prior research by Adamchak 2021 shows weasel (*M. frenata*) MeHg concentrations of 625 ng MeHg g^{-1}, suggesting that these terrestrial animals source a large portion of their diet from alpine aquatic ecosystems. While these data begin to paint a picture of the trophic pyramid of the Niwot Ridge LTER, forthcoming stable isotope data will yield a much more in-depth look into the food chain.

Limitations of the Study and Future research

Due to limited time and high costs of Hg analysis on samples, the sample sizes in each lake were small. However, the number of individual fish sampled was close to the upper allowable limit of 30 trout per our IACUC permit. A more extensive sampling of lake water, lake sediments, phytoplankton, and zooplankton would give the aquatic ecosystem a more comprehensive view of MeHg transfers. Given the surprising results in MeHg flows across lakes, it would be helpful to collect data on fish populations at each lake. These data would provide more information regarding how MeHg and THg concentrations range across populations.

The study area is distinctly remote, but it has a mining and dam construction history. While these are abandoned operations, there is still the possibility of lasting effects from Hg mobilized during these activities. Furthermore, more research is needed to evaluate the effects of historical mining in the area and provide data on a possible point source of Hg pollution that continues to affect downstream locations.

Due to limited time, we could not get the results for stable isotopes back from the stable isotope lab in Wyoming. These results are vital to understanding the trophic position of each of the tested samples in this study. Future research will be heavily focused on reviewing data on C ($^{13}C/^{12}C$) and N ($^{15}N/^{14}N$) to understand where each organism is in the trophic pyramid and its primary food sources. This data will provide insight into the Green Lakes Valley ecosystem by providing critical information on how MeHg is mobilized throughout the food system.

Research on predators feeding in the aquatic ecosystem (e.g., piscivorous birds and mammals), as well as any parasites feeding on fish, would yield missing information regarding total MeHg bioaccumulation. In order to complete such research, samples of muscle tissue and stomach contents of each organism in the area would be taken, and Hg species, as well as the stable isotopes of C and N, would be analyzed. This research would yield insight into what animals at each trophic position are eating, where they are eating it (aquatic/terrestrial), and how their MeHg concentrations are affected.

Lastly, it is important to note that results pointed to Arikaree rock glacier as the driver of increased production of MeHg in the Green Lakes Valley, due to increased sulfate export from climate-induced weathering. My results showed increasing MeHg concentrations of fish in the main drainage of the Green Lakes Valley with proximity to the Arikaree rock glacier. Further research is needed to determine the relationship between changes to sulfate export and stimulation of MeHg production in the Green Lakes Valley, including measurements of sulfate reduction and sulfate reducing bacteria in lake sediments.

References

Adamchak, C. R. (2021). “Quicksilver in the Alpine: Mercury Cycling and its Implications for Ecosystem Health”, 1-5.

Badger, A. M., Bjarke, N., Molotch, N. P., & Livneh, B. (2021). “The sensitivity of runoff generation to spatial snowpack uniformity in an Alpine watershed: Green Lakes Valley, Niwot Ridge long-term ecological research station”. *Hydrological Processes*, *35*(9).

Bartrons, M., Gratton, C., Spiesman, B. J., & Vander Zanden, M. J. (2015). “Taking the trophic bypass: Aquatic-terrestrial linkage reduces methylmercury in a terrestrial food web”. *Ecological Applications.*

Beckvar, N., Dillon, T. M., & Read, L. B. (2005). “Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds”. *Environmental Toxicology and Chemistry*.

Beyer, W. N., & Meador, J. P. (2011). “Environmental contaminants in biota: Interpreting tissue concentrations”. *CRC Press*.

Bishop, K., Shanley, J. B., Riscassi, A., de Wit, H. A., Eklöf, K., Meng, B., Mitchell, C., Osterwalder, S., Schuster, P. F., Webster, J., & Zhu, W. (2020). “Recent advances in understanding and measurement of mercury in the environment: Terrestrial Hg cycling”. *Science of The Total Environment*.

Blais, J. M., Charpentié, S., Pick, F., Kimpe, L. E., Amand, A. S., & Regnault-Roger, C. (2006). “Mercury, polybrominated diphenyl ether, organochlorine pesticide, and polychlorinated biphenyl concentrations in fish from lakes along an elevation transect in the French pyrénées”. *Ecotoxicology and Environmental Safety*.

Bloom, Nicolas S. 1992. “On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue.” *Canadian Journal of Fisheries and Aquatic Sciences*.

Braaten, H. F. V., Fjeld, E., Rognerud, S., Lund, E., & Larssen, T. (2014). “Seasonal and year-to-year variation of mercury concentration in perch (Perca fluviatilis) in boreal lakes”. *Environmental Toxicology and Chemistry*.

Chételat, J., Ackerman, J. T., Eagles-Smith, C. A., & Hebert, C. E. (2020). “Methylmercury exposure in wildlife: A review of the ecological and physiological processes affecting contaminant concentrations and their interpretation”. *Science of The Total Environment*.

Driscoll, C. T., Mason, R. P., Chan, H. M., Jacob, D. J., & Pirrone, N. (2013). "Mercury as a Global Pollutant: Sources, Pathways, and Effects". *Environmental Science & Technology*.

Eagles-Smith, C.A., Willacker, J.J., and Flanagan Pritz, C.M., (2014) , "Mercury in fishes from 21 national parks in the Western United States—Inter and intra-park variation in concentrations and ecological risk". *U.S. Geological Survey*.

Eagles-Smith, C. A., Wiener, J. G., Eckley, C. S., Willacker, J. J., Evers, D. C., Marvin-DiPasquale, M., Obrist, D., Fleck, J. A., Aiken, G. R., Lepak, J. M., Jackson, A. K., Webster, J. P., Stewart, A. R., Davis, J. A., Alpers, C. N., & Ackerman, J. T. (2016). "Mercury in western North America: A synthesis of environmental contamination, fluxes, bioaccumulation, and risk to fish and wildlife". *Science of The Total Environment*.

Fitzgerald, William F., and Gary A. Gill. 1979. "Subnanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis." *Analytical Chemistry* 51(11).

Greenland, D., and Losleben, M. (2001). Climate. In W. D. Bowman, and T. R. Seastedt (Eds.), *Structure and Function of an Alpine Ecosystem* (pp. 15–31). New York, NY: Oxford University Press.

Hammerschmidt, C. R., & Fitzgerald, W. F. (2006). Bioaccumulation and Trophic Transfer of Methylmercury in Long Island Sound. *Archives of Environmental Contamination and Toxicology*.

Herrmann, S. J., Nimmo, D. W., Carsella, J. S., Melnykov, I. V., Kennedy, C. M., Rogers, K. B., & Herrmann-Hoesing, L. M. (2020). "Differential bioaccumulation of mercury and selenium in stomach contents and tissues of three Colorado, USA, cutthroat trout populations". *Bulletin of Environmental Contamination and Toxicology*.

Kittel, Timothy G.F. et al. 2015. "Contrasting Long-Term Alpine and Subalpine Precipitation Trends in a Mid-Latitude North American Mountain System, Colorado Front Range, USA." *Plant Ecology and Diversity*.

Layman, C. A., & Post, D. M. (2008). "Can stable isotope ratios provide for community-wide measures of trophic structure?" *Ecology*.

Manceau, A., Brossier, R., Janssen, S. E., Rosera, T. J., Krabbenhoft, D. P., Cherel, Y., Bustamante, P., & Poulin, B. A. (2021). "Mercury isotope fractionation by internal

demethylation and biomineralization reactions in seabirds: Implications for environmental mercury science". *Environmental Science & Technology*.

Marusczak, N., Larose, C., Dommergue, A., Yumvihoze, E., Lean, D., Nedjai, R., & Ferrari, C. (2011). "Total Mercury and methylmercury in high altitude surface snow from the French Alps". *Science of The Total Environment*.

Mast, M. A., Campbell, D. H., Krabbenhoft, D. P., & Taylor, H. E. (2005). "Mercury Transport in a High-Elevation Watershed in Rocky Mountain National Park, Colorado". *Water, Air, and Soil Pollution*.

Mast, M. A., 2005. "Preliminary Results of Streamwater Mercury Concentrations in the Big Thompson Watershed, Colorado". *USGS: Science for a Changing World*

Murphy, D. M. et al. 2006. "Observations of Mercury-Containing Aerosols." *Environmental Science and Technology*.

Peterson, B. D., McDaniel, E. A., Schmidt, A. G., Lepak, R. F., Janssen, S. E., Tran, P. Q., Marick, R. A., Ogorek, J. M., DeWild, J. F., Krabbenhoft, D. P., & McMahon, K. D. (2020). "Mercury methylation genes identified across diverse anaerobic microbial guilds in a eutrophic sulfate-enriched lake". *Environmental Science & Technology*.

Porcella, D. B., Ramel, C., & Jernelov, A. (1997). "Global Mercury Pollution and the role of Gold Mining": An overview. *Water, Air, & Soil Pollution*.

Suchanek, T. H., Richerson, P. J., Zierenberg, R. A., Eagles-Smith, C. A., Slotton, D. G., Harner, E. J., Osleger, D. A., Anderson, D. W., Cech, J. J., Schladow, S. G., Colwell, A. E., Mount, J. F., King, P. S., Adam, D. P., & McElroy, K. J. (2008). "The legacy of Mercury Cycling from mining sources in an aquatic ecosystem: From ore to organism". *Ecological Applications*.

Selin, N. E. (2009). "Global Biogeochemical Cycling of Mercury: A Review". *Annual Review of Environment and Resources*.

Ward, D. M., Nislow, K. H., & Folt, C. L. (2010). "Bioaccumulation syndrome: Identifying factors that make some stream food webs prone to elevated Mercury bioaccumulation". *Annals of the New York Academy of Sciences*.

Wagner-Döbler, I. 2003. "Pilot Plant for Bioremediation of Mercury-Containing Industrial Wastewater." *Applied Microbiology and Biotechnology* 62(2–3).

Williams, Mark W., Mark Losleben, Nel Caine, and David Greenland. 1996. “Changes in Climate and Hydrochemical Responses in a High-Elevation Catchment in the Rocky Mountains, USA.” *Limnology and Oceanography*.

Tables and Figures

Table 1: MeHg Concentrations of Each Sample Type by Lake.

Site	Elevation (ft)	Zoo Thg (ng g^{-1})	Zoo MeHg (ng g^{-1})	Fish Thg (ng g^{-1})	Fish MeHg (ng g^{-1})	MeHg ww (ppm)
Albion	3,356	104	27.5	218.75	198.75	0.044
Green Lake 2	3,431	67.3	24.8	344.29	344.29	0.034
Green Lake 1	3,438	58.3	17.5	160.24	144.8	0.08
Green Lake 3	3,454	38	24.8	452.5	466.5	0.12
Green Lake 4	3,563	46.2	13.5			

Figure 1

Figure 1. Study area includes the Green Lakes Valley, Colorado. Names of each lake that were sampled are shown.

Figure 2

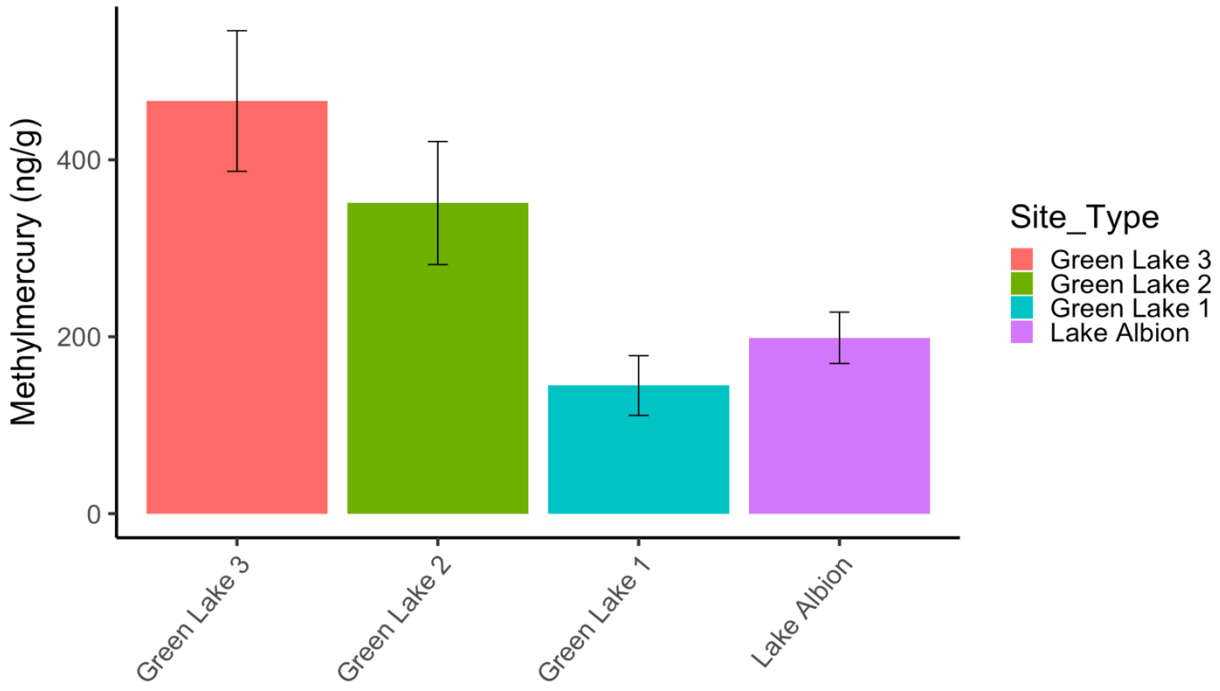

Figure 2. Mean MeHg (± 1SE) concentrations in fish for each lake in the Green Lakes Valley. A Kruskal Wallis test revealed statistically significant differences in mean MeHg among lakes ($p < 0.05$), however, a post hoc Dunn test showed no significant difference among individual lakes. Green Lake 3 n = 2, Green Lake 2 n = 8, Green Lake 1 n = 7, and Lake Albion n = 8.

Figure 3

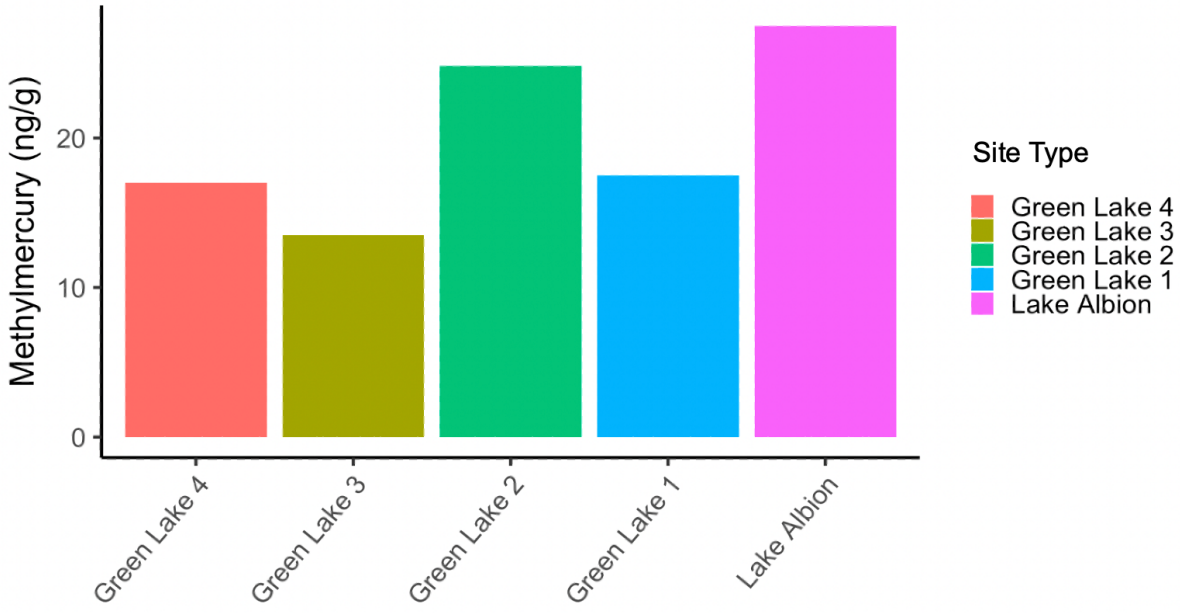

Figure 3. Mean (± 1SE) methylmercury concentrations of zooplankton across lakes. N = 1

Figure 4

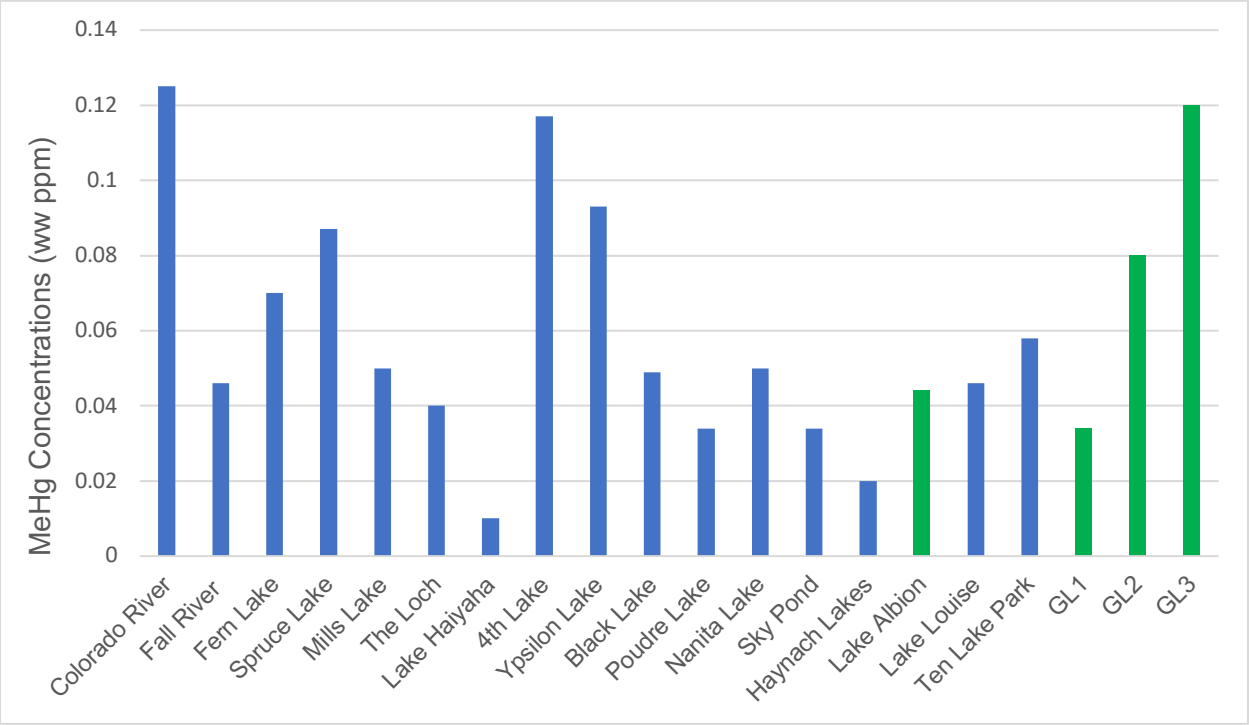

Figure 4. Methylmercury concentrations in fish from Green Lakes Valley compared to unpublished USGS data in the Big Thompson Drainage, Colorado. Lakes tested in this study are highlighted in green. Data is ordered from lowest to highest elevation (left to right).

Figure 5

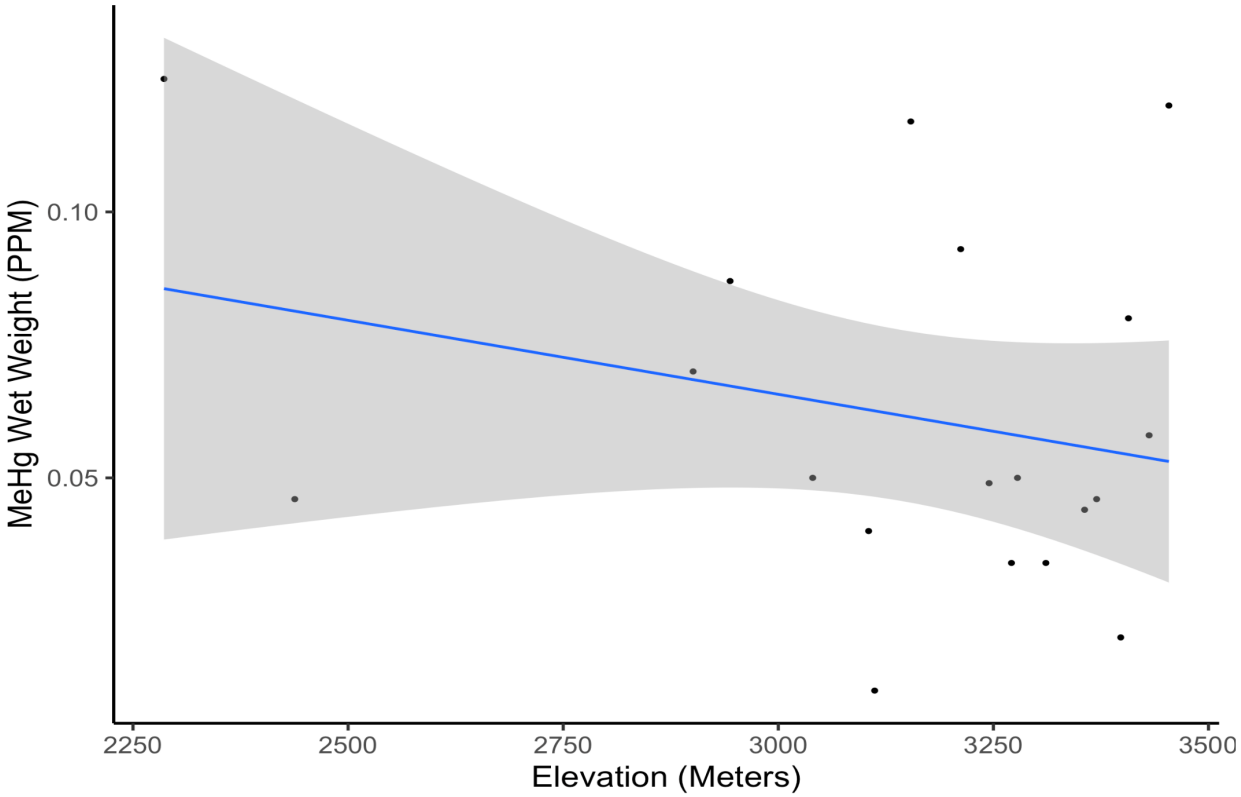

Figure 5. Linear regression model comparing elevation and fish MeHg concentrations (ng/g wet weight). The relationship between fish MeHg concentrations and elevation was not statistically significant ($p > 0.05$). $R^2 = -0.058$

Figure 6

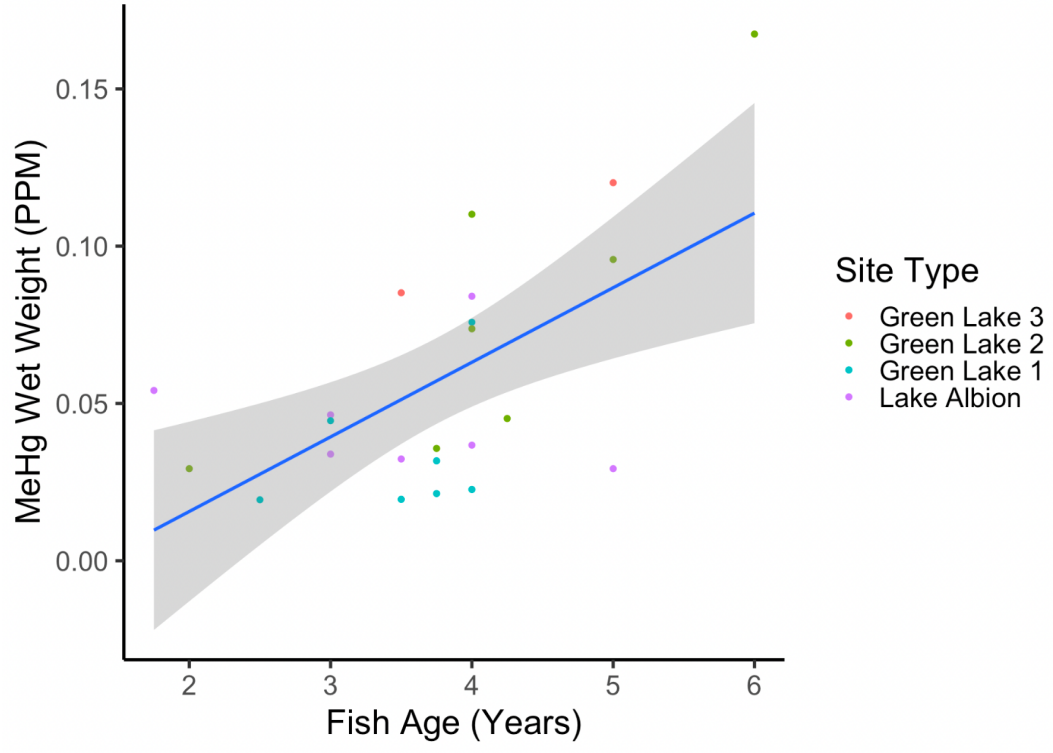

Figure 6. Linear regression model comparing fish age and MeHg concentrations (ng/g wet weight). Colors correspond to sites. The relationship between fish age and MeHg concentrations was statistically significant ($p < 0.01$). Multiple $R^2 = 0.35$, Adjusted $R^2 = 0.32$.

Figure 7

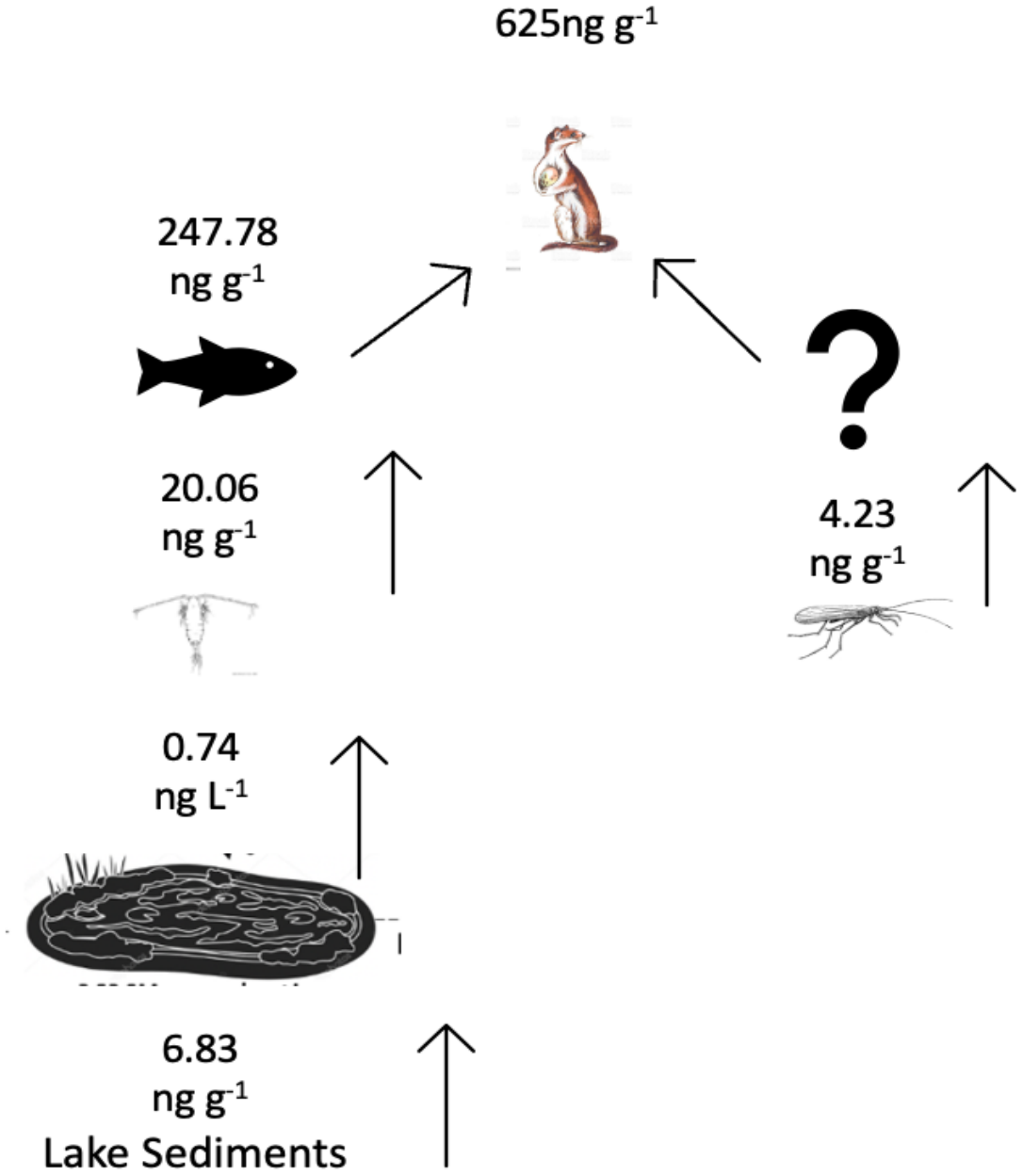

Figure 7. Approximate reconstruction of the trophic pyramid in the Green Lakes Valley based on MeHg concentration levels. Lake water represented in THg due to MeHg levels below detection limits.

Figure 8

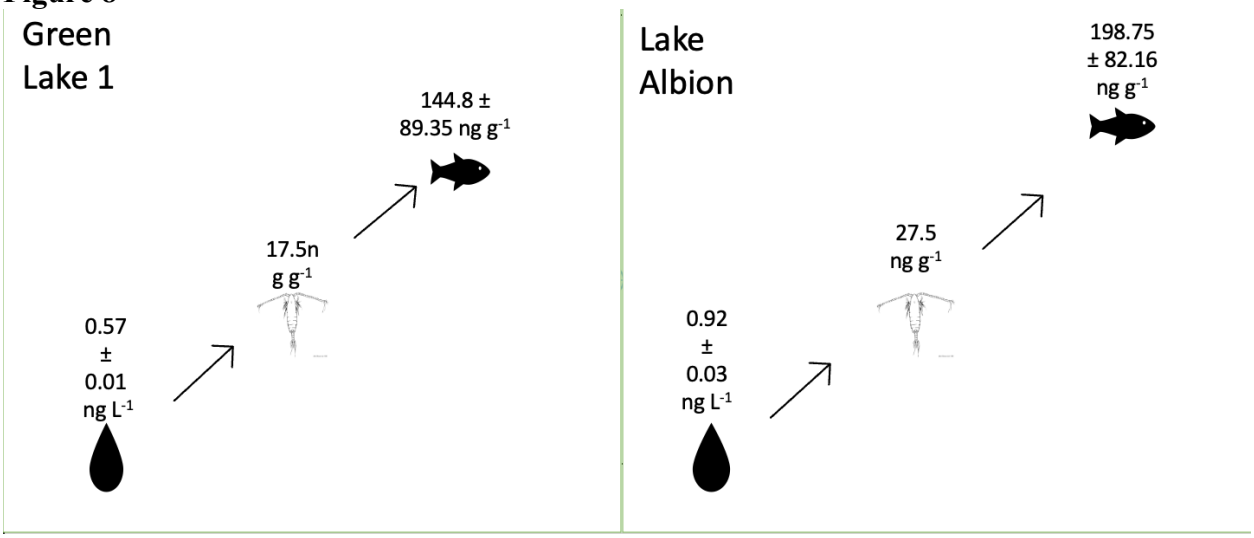

Figure 8. Methylmercury concentrations in successive trophic levels at 2 different lakes. Lake water represented in THg due to MeHg levels below detection limits.