Mercury Bioaccumulation in Dragonfly Larvae and its Impact on Aquatic Invertebrate Community Structure in Black Rock Forest, NY

By

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ABSTRACT:

Mercury (Hg) is a neurotoxin found in aquatic systems worldwide. Dragonfly larvae are often used to assess potential environmental health risks due to mercury because they are easy to sample and often correlate to fish Hg exposure. This study investigates mercury levels in larval dragonflies from four different wetlands in Black Rock Forest (Cornwall, NY USA), including Jim's Pond (n = 6), Sutherland Pond (n = 4), Aleck Meadow (n =3), and Sphagnum Bog (n=3). Jim's Pond and Sutherland Pond were sampled on July 6th, 2023 and September 9th, 2023 while Aleck Meadow and Sphagnum Bog were only sampled on September 9th, 2023. In the lab,

dragonflies were composited by family (Aeshnidae, Corduliidae, Libellulidae, and Macromiidae) before being tested for Hg (range = 1.54 to 6.15 ng and range = 81.88 to 258.52 ppb, dry weight). We found that Sutherland Pond, which was the only natural site, had the lowest dragonfly mercury concentrations.

At each of these sites and sampling dates, we also collected other parts of the aquatic invertebrate community, including Tetragnatha (Stretch Spiders), Ranatra (Water Scorpions), Dolomedes triton (Six-Spotted Fishing Spider), Hesperocorixa (member of Water Boatmen in family Corixidae), Mesovelia mulsanti (Mulsant's Water Treader), Buenoa (member of Typical Backswimmers), Gyrinus (Whirligig Beetle), and Laccophilus and Neoporus (Predaceous Diving Beetles). Our goal is to compare Hg concentrations in larval dragonflies among the sites and see how the overall invertebrate community could impact Hg bioaccumulation. This area has relatively little research on Hg cycling and this pilot study helps us understand which water bodies may be at highest risk to Hg.

KEY WORDS: Mercury, Bioaccumulation, Biomagnification, Ecosystem, Wetland, Dragonfly Larvae, Aquatic Invertebrate, Community Structure, Trophic Levels

INTRODUCTION:

Mercury Hg is a metal and can be a toxic pollutant. Mercury contamination in aquatic ecosystems poses significant risks to wildlife and human health. Dragonfly larvae are particularly valuable as biosentinels for monitoring methylmercury contamination in wetlands due to several aspects of their ecology. Not only are they widespread across the country in most freshwater environments, but they have long larval stages and are able to bioaccumulate the toxin very effectively which serves as a great benchmark to compare mercury levels over large areas of land (Nelson et al. 2020). Biosentinels are organisms used to detect risks to humans and other animals and provide advance warning due to their sensitivity to changes in the environment, pollutants, or pathogens. They inhabit such a variety of aquatic environments such as ponds, lakes, streams, and vernal pools which makes them ideal for studying methylmercury contamination on a broad scale. The prolonged larval stages of dragonflies can enhance their utility as bioindicators as well, because they can remain larva in their aquatic environment for several months and up to 5 years depending on the species. Their extended exposure period as larva enables researchers to explore temporal and spatial trends and variations in mercury concentrations (Zong et al. 2024).

Dragonfly larvae are recognized as biovectors of mercury, playing a significant role in the bioaccumulation and biomagnification of this toxic element within aquatic ecosystems

(Buckland-Nicks et al. 2014). As predators, these larvae accumulate mercury through their diet, primarily from consuming contaminated prey. This mercury is retained and can be transferred to higher trophic levels when dragonfly larvae are predated upon by fish, birds, and other wildlife, effectively bridging the gap between aquatic and terrestrial food webs. The presence of mercury in dragonfly larvae thus poses substantial ecological risks, as it can lead to elevated mercury levels in riparian communities and impact the health of diverse species, including those at the top of the food chain.

While we did not asess MeHg we did measure THg (total Hg) which has proven to be correlated with MeHg in lake water (Nelson et al. 2020). Total Mercury refers to the sum of all forms of mercury present in the sample including inorganic and organic mercury compounds and elemental mercury. Methylmercury MeHg is mercury in its organic form after methylation has occurred. When inorganic mercury enters aquatic systems, anaerobic bacteria may take it up, metabolize it and add a methyl group (CH_3) to the mercury, converting it into methylmercury MeHg which is highly toxic. In its organic form (MeHg), mercury is more soluble in the water and more bioavailable which means more opportunity for the toxin to bioaccumulate in organisms and biomagnify in the food web.

While dragonflies are the focus of much Hg research, dragonflies are just one part of the larger freshwater food web. Each water body has a unique community of aquatic invertebrates and vertebrates, which can affect the movement of mercury. Aquatic invertebrate community surveys play a vital role in assessing the health and biodiversity of freshwater ecosystems. These surveys involve collecting and identifying various species of invertebrates that inhabit these water bodies and examining the composition and abundance of these species. In this way we can gain insights into the overall ecological integrity of the site, as well as the potential impact of pollutants like mercury. By comparing the ecology of the most prominent species found in each site, a greater understanding of how well the ecosystem functions was revealed.

One aspect of these surveys is the calculation of Shannon diversity, used to quantify the diversity within a community by considering both the number of species present and their relative abundance. By calculating Shannon diversity for each site, ecological richness and evenness across different locations were compared. Higher Shannon diversity values typically indicate a more balanced and varied invertebrate community, which can suggest a healthier and more resilient ecosystem. These surveys, therefore, provide crucial data for understanding how different communities of aquatic invertebrates influence the movement and bioaccumulation of mercury within freshwater environments.

This study aimed to investigate mercury concentrations in dragonflies and to assess the broader community structures of aquatic invertebrates across four distinct sites in Black Rock Forest in Cornwall, New York. The first section of this project focused on measuring mercury levels in dragonfly larvae, which serve as effective bioindicators due to their position in the food

web and their sensitivity to environmental changes. The second portion of this study was to examine the general community aquatic invertebrate community at the same locations to evaluate biodiversity and community composition/structure. Another goal of this study was to determine whether there is a correlation between the observed community structures and the associated mercury concentrations in the dragonfly larvae. In this way, future studies could have a baseline insight into water quality and overall ecosystem quality.

STUDY AREA:

Four sites were sampled for this study including Jim's Pond (JP), Sutherland Pond (SP), Sphagnum Bog (SB), and Aleck Meadow (AM). Jim's Pond and Sutherland were sampled twice; once on July 6th, 2023 and September 9th, 2023. Sphagnum Bog and Aleck Meadow were only sampled once on September 9th, 2023. Jim's Pond and Aleck Meadow are man-made water bodies, Sutherland is a natural lake, and Sphagnum Bog is a small natural wetland downstream of the man-made Sphagnum Pond. For the sake of this study, Sutherland Pond is the only site considered natural.

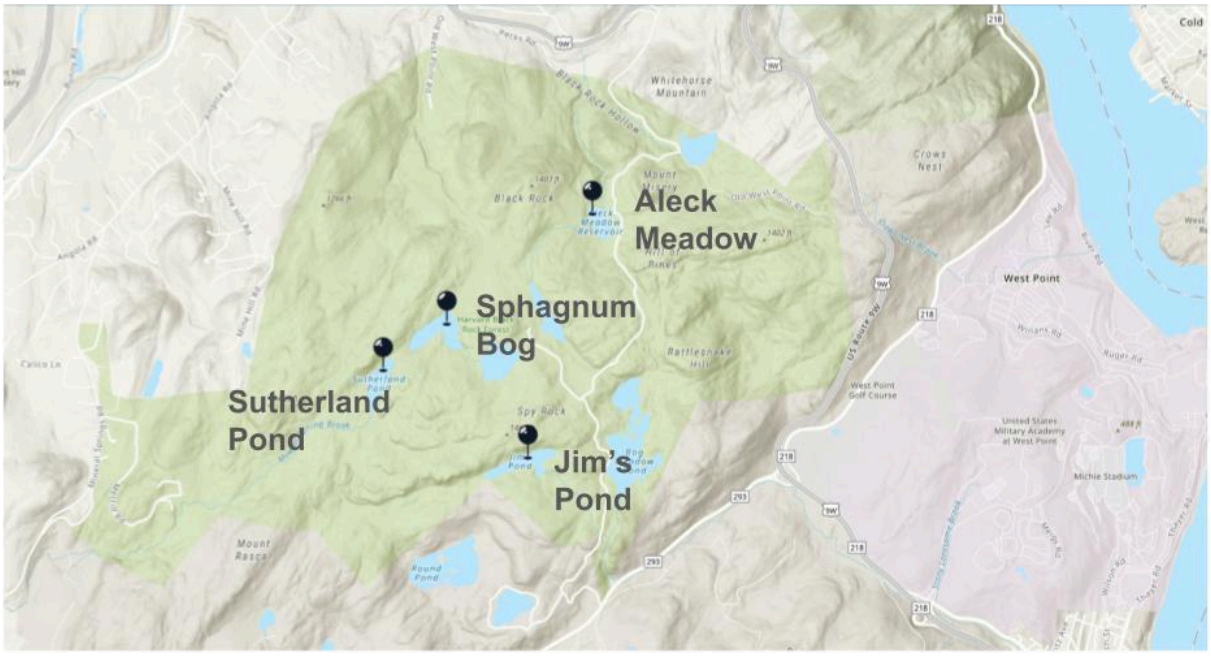


Figure 1. This map was created using ArcGIS and displays the study area with the four distinct wetlands pinned. The pinned regions represent: Jim's Pond (JP), Sutherland Pond (SP), Sphagnum Bog (SB), and Aleck Meadow (AM).

SAMPLE COLLECTION FOR DRAGONFLY MERCURY:

We used dip nets to collect our samples from the littoral areas of the wetlands and kept them on ice until later identification and analysis in the lab. We chose the largest larva for

mercury testing (n=15). All specimens were rinsed with deionized water and identified down to family or genus using a microscope, scope camera, dichotomous key, and iNaturalist for assistance. After they were rinsed and cleaned they were labeled and weighed in a weigh boat (>5mg, preferred >10-15mg). The specimens were then dried in an oven at 50℃ for approximately 48 hours. After being dried, samples were placed in vials based on site and family, and their dry weights were recorded to measure moisture content. Individuals or multiples, depending on their weight, were then ground to a fine powder for a portion of it to be tested for total mercury concentrations THg.

SAMPLE COLLECTION FOR AQUATIC INVERTEBRATE COMMUNITY:

Using dip nets in the littoral parts of the wetlands, all present aquatic invertebrates were collected and kept in vials of ethanol and on ice. Using a Northeast aquatic invertebrates guide book, a microscope, scope camera, and iNaturalist specimens were sorted, organized, and labeled by site, identification, and date collected. Shannon Diversity was calculated using the following formula:

$$Shannon\ Diversity\ (H) = \sum_{i=1}^{s} p_i \ln p_i$$

RESULTS:

The results of the study revealed notable differences in the distribution and presence of dragonfly families across the four sites (Fig. 2). Aleck Meadow was unique in hosting the Macromiidae family, which was not found at any other location. The Libellulidae family was the most widespread, being present at every site and during both sampling periods. Sutherland Pond had a limited diversity, with only Corduliidae and Libellulidae observed, and was the only site where Aeshnidae were absent. Corduliidae and Libellulidae emerged as the most consistently prominent families overall, except at Jim's Pond in September, where Aeshnidae dominated the larval population.

The mercury analysis revealed distinct differences in contamination levels across the four sites. Sutherland Pond exhibited the lowest mercury levels compared to the other locations (Fig. 3). Jim's Pond and sphagnum Bog had similar ranges of mercury concentration, indicating comparable levels of contamination. Aleck Meadow, while showing a broader range of mercury levels, had lower concentrations overall.

The temporal analysis of mercury levels revealed subtle changes between the July and September sample collections (Fig. 4). At Jim's Pond, there was a slight increase in the median mercury level, accompanied by a marginally wider range of concentrations over this period.

Sutherland Pond, on the other hand, exhibited a more notable change, with a narrower range and a lower median mercury level in September compared to July. Despite these variations, the overall changes at both sites were minimal, suggesting that the time of year did not significantly impact mercury level (statistical difference at sutherland pond tho so I think this will change.)

The analysis of mercury concentrations among dragonfly families across the sites revealed that Corduliidae and Libellulidae were the most prevalent families throughout the sites (Fig. 5). At Sphagnum Bog, there was a notable difference of 55.5 ng/g between the mercury levels in these two families. Aeshnidae exhibited the highest mercury levels at Jim's Pond, averaging 247.60 ng/g. Libellulidae also showed high concentrations at Sphagnum Bog, with a level of 236.63 ng/g. Although Corduliidae was absent from Aleck Meadow, it displayed relatively consistent mercury concentrations across the other three sites, ranging from 88.94 ng/g to 181.13 ng/g. Macromiidae, exclusively found at Aleck Meadow, had the highest mercury concentration of any family at that site, with a level of 186.53 ng/g.

The larger aquatic invertebrate community (beyond dragonflies) varied among sites (Fig. 6 and Table 1). Looking at approximately distributions, Aleck Meadow and Jim's Pond appeared to have similar community composition, while Sutherland and Sphagnum Bog appeared to differ. After calculating Shannon diversity for each site, it was revealed that Sutherland pond had the highest diversity with a value of 1.77 followed closely by Jim's Pond which had a value of 1.76. Sphagnum bog had the lowest Shannon diversity value of 0.97, and Aleck Meadow was in the middle at 1.21.

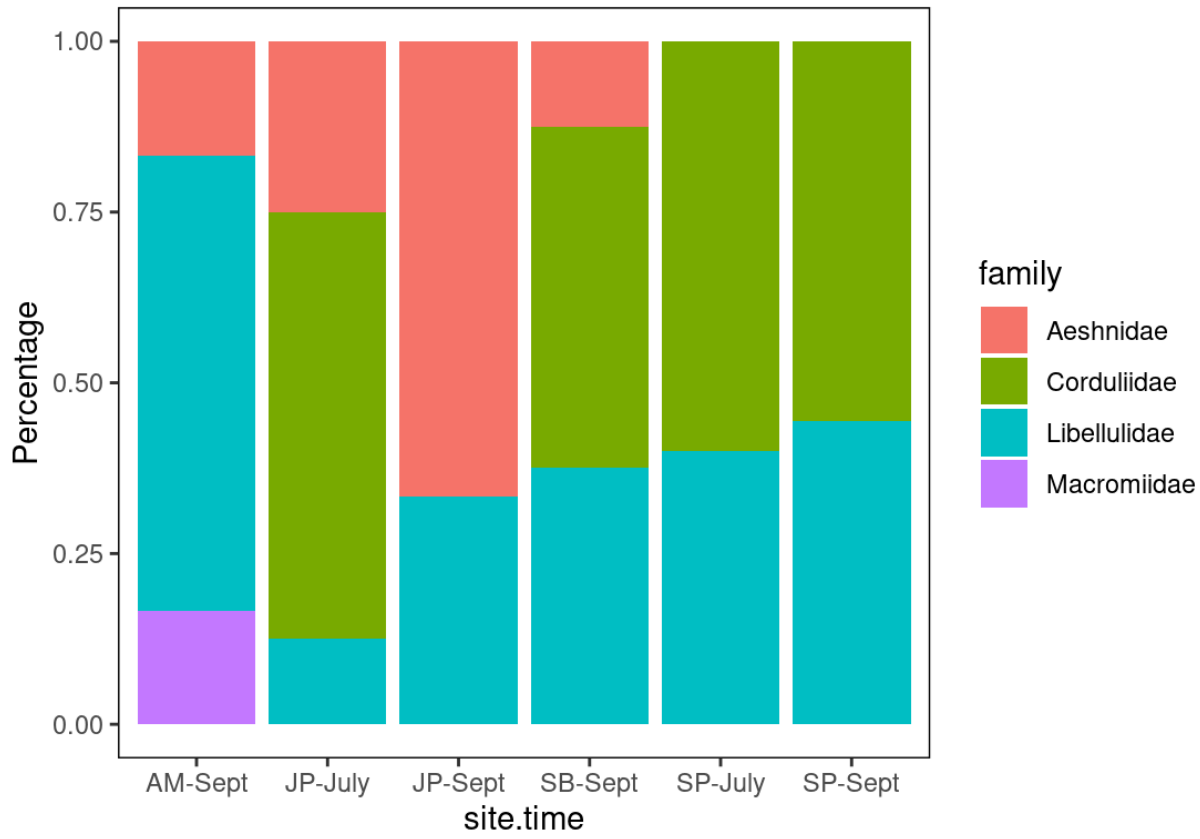


Figure 2. This bar plot was created using R to compare the distribution of dragonfly families found at each study site with two additional bars to account for the repeated sites Jim's Pond and Sutherland Pond. These bars are scaled to sum 100% for more accurate comparison.

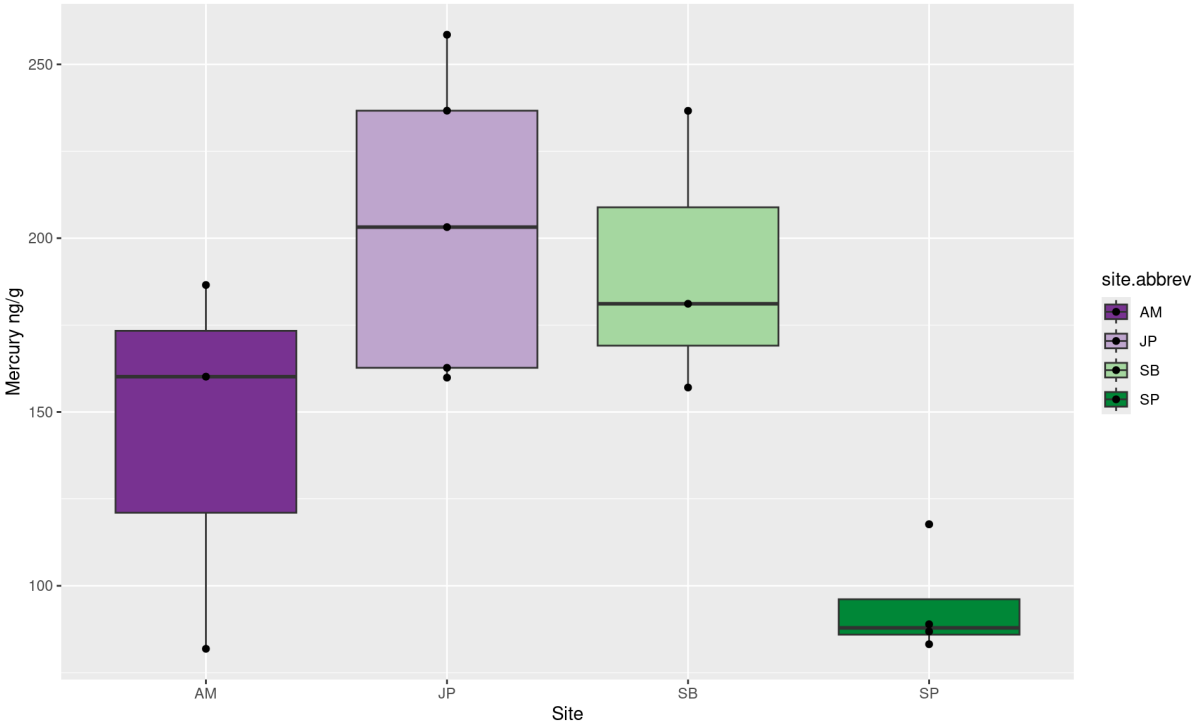


Figure 3. This box and whisker plot was created using R to illustrate the distribution of mercury concentrations (in ng/g) across four survey sites (AM, JP, SB, SP). Each site is also coordinated with a single color.

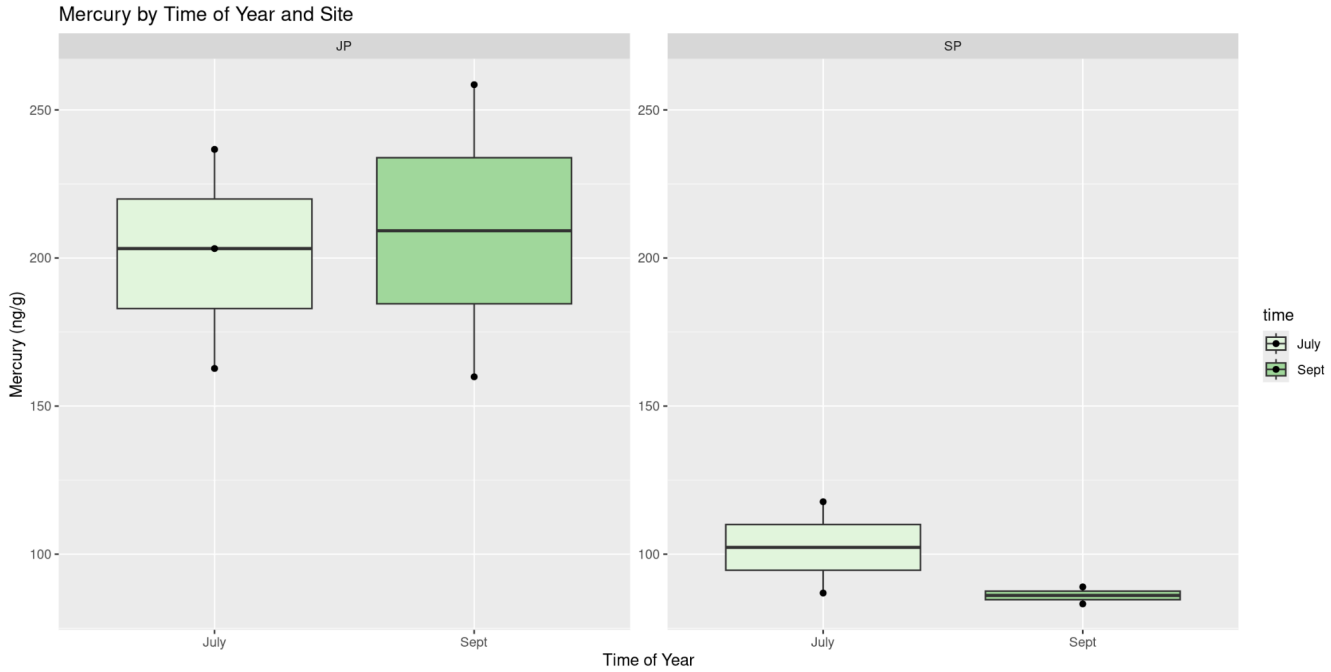


Figure 4. This box and whisker plot was created using R to show mercury levels at the repeated sites Jim's Pond and Sutherland Pond at the distinct times of year that they were sampled. The plot used light green to represent data from July and dark green to represent data collected in September.

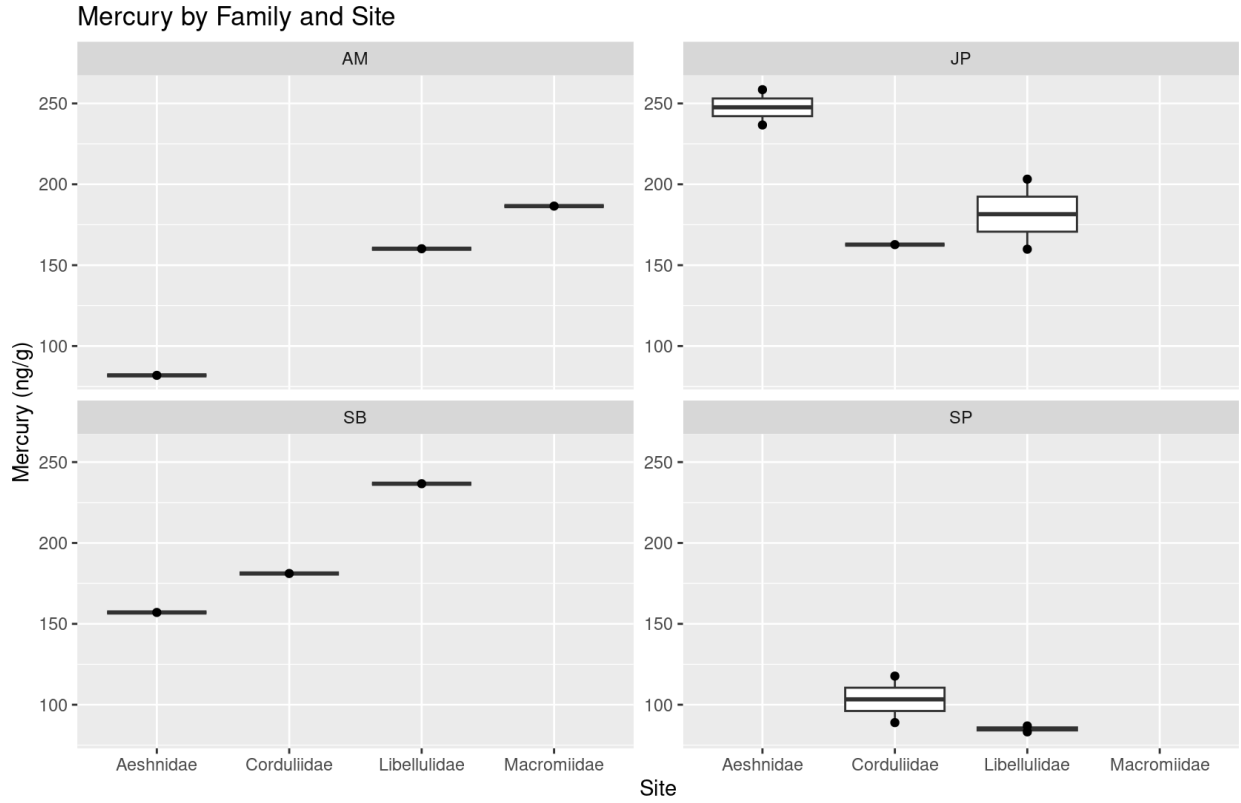


Figure 5. These four box and whisker plots were created using R, one plot for each site, each showing the mercury levels (measured in ng/g) found in each family found there.

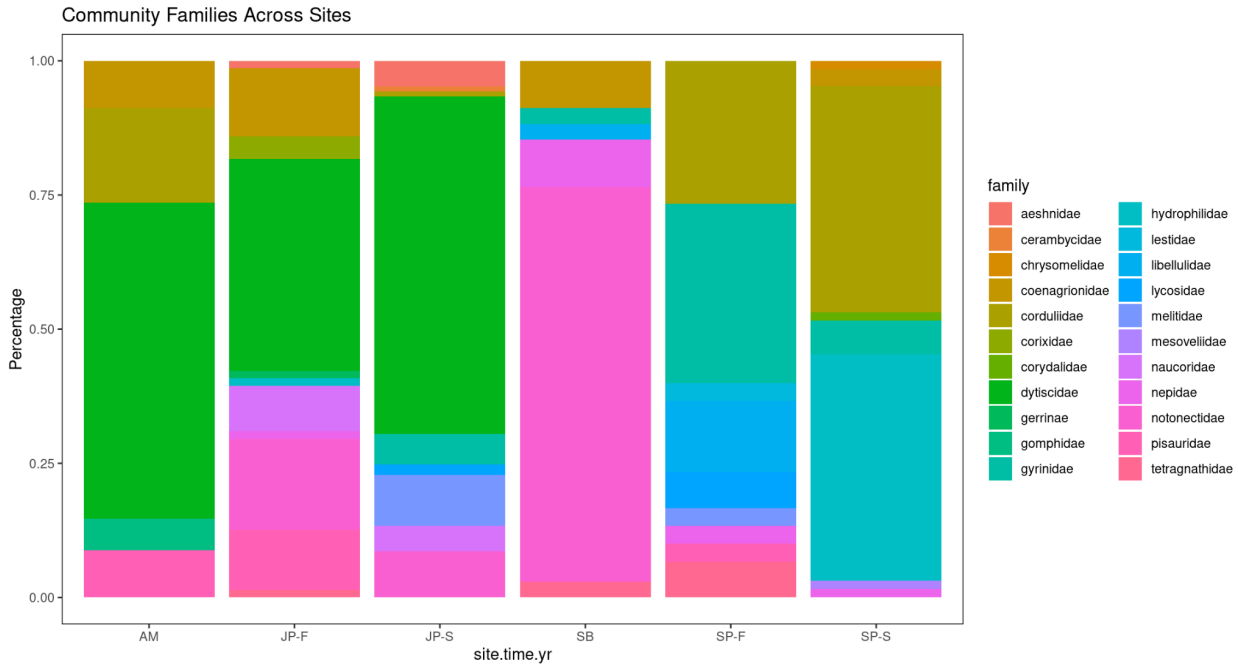


Figure 6. This bar plot was created using R to represent the distribution of aquatic invertebrate families sampled for the community data across four survey sites (AM, JP, SB, SP) with "-F" representing the Fall collection and "-S" representing the summer collection. Each family is associated with a specific color and each bar was scaled to sum 100% for accurate comparison of community structure.

Phyla Order	Family	Site			
		AM	JP	SB	SP
Amphipoda	Melitidae	-	*10	-	*1
Araneae	Lycosidae	-	*2	-	*2
	Pisauridae	*3	*8	-	*1
	Tetragnathidae	-	*1	*1	*2
Coleoptera	Cerambycidae	-	*1	-	-
	Chrysomelidae	-	-	-	*1
	Dystiscidae	*20	*94	-	-
	Gyrinidae	-	*6	*1	*14
	Hydrophilidae	-	*1	-	*27
Hemiptera	Corixidae	-	*3	-	-
	Gerrinae	-	*1	-	-
	Mesoveliidae	-	-	-	*1
	Naucoridae	-	*11	-	-
	Nepidae	-	*1	*3	*2
	Notonectidae	-	*21	*25	-
Megaloptera	Corydalidae	-	-	-	*1
Odonata	Aeshnidae	-	*6	-	-
	Coenagrionidae	*3	*9	*3	*2
	Corduliidae	*6	*2	-	*34
	Gomphidae	*2	-	-	-
	Lestidae	-	-	-	*1
	Libellulidae	-	-	*1	*4

Figure 7. This table displays the survey data of aquatic invertebrate communities across four sites. The table includes phyla order and family name along with their corresponding abundances at each site (AM, JP, SB, SP). This data set highlights the species richness and distribution, providing insight into the diversity and ecological health of the surveyed aquatic environments. The “*” denotes the presence of that species.

DISCUSSION:

The study found that mercury (Hg) levels were consistent across all sites except for Sutherland Pond, which exhibited the lowest levels and was the only natural wetland in the study. This suggests that natural wetlands may have lower mercury concentrations due to more established ecological processes and better developed communities that reduce or counter

mercury methylation more effectively than man made sites. Sutherland Pond had the highest Shannon diversity, supporting this idea. Additionally, community structure varied between sites, with Aleck Meadow and Jim's Pond displaying similar characteristics, while Sphagnum Bog and Sutherland emerged as distinct.

Reasons for Sutherland's low Hg concentrations?

There are many potential explanations for why a particular waterbody would have higher or lower Hg levels including atmospheric, biological, hydrological, ecological, geochemical, and anthropogenic processes (Hsu-Kim et al. 2018). Lake size, lake type (reservoir or natural), forest cover, the amount of organic matter, type of Hg deposition, and community composition are all factors in determining why a site may harbor more Hg than others. As Sutherland Pond was the only natural lake site in this study, it is possible that Hg cycles differently than in the reservoirs and other sites in this study, leading to a lower baseline Hg exposure in Sutherland. The absence of fluctuating water levels in Sutherland Pond reduces the potential for sediment erosion and resuspension, which are key processes that can increase the availability of mercury for methylation. In reservoirs, the rise and fall of water levels can enhance the breakdown of organic matter leading to higher concentrations of dissolved organic carbon (DOC) (Ahn et al. 2010). This DOC plays a crucial role in facilitating mercury methylation, a process that converts inorganic mercury into its more toxic form, methylmercury (Janssen et al. 2024). Therefore, in limiting the extent of sediment wetting and drying cycles there is a better chance of minimizing the release of mercury into the water column, in turn also reducing the chances of methylation. Furthermore, the natural organic matter in Sutherland Pond might play a role in reducing mercury mobilization by coating particles and limiting clustering, thus decreasing the rate at which mercury-containing particles settle (Hsu-Kim et al. 2018).

In contrast, research over the past four decades has consistently shown that reservoirs tend to have elevated mercury levels compared to natural lakes and rivers which can be seen in this study as well. The artificial manipulation of water levels in reservoirs not only increases the mobilization of mercury from sediments but also creates conditions that are more conducive to mercury methylation (Hsu-Kim et al. 2018). The combination of increased sediment erosion, enhanced DOC production, and water-level fluctuations in reservoirs contributes to higher methylmercury concentrations, which are not as prevalent in natural settings like Sutherland Pond. This likely supports the findings that the altered or artificial sites, including Jim's Pond, Aleck Meadow, and Sphagnum Bog would have higher mercury levels than that of Sutherland Pond.

As mentioned before, there are many ecological factors contributing to Hg and eventually MeHg levels, one of the most prevalent being food web dynamics (Eagles-Smith et al. 2018). It would also be expected that the site with the highest biodiversity would have the lowest mercury

levels since the pollutants can interrupt and harm the natural function of ecosystems. In this case, Sutherland Pond did have the highest biodiversity and the lowest mercury levels, supporting the idea that natural sites have a greater chance and ability to mitigate the effects of mercury deposition and methylation due to higher biodiversity and better ecosystem function.

These community compositional differences are likely driving this difference in Hg exposure, as the higher abundance of Hydrophilidae, which are detritivores, could be attributed to more and more diverse vegetation and decaying material. The abundance of Corduliidae could possibly be attributed to greater vegetation as well since they prefer hunting near water surfaces and vegetation and require this environment for laying eggs. They capture their prey on their wings and typically prefer small flying insects which would be well supported in a natural wetland. The stability that comes with natural wetlands also supports Corduliidae through their larval stages which can last from one to three years. The absence of Dytiscidae at Sutherland Pond could be due to a greater population of fish and other predators like amphibians or other larger insects (Liao et al. 2022). This greater abundance of predators could have also contributed to the small sizes of the dragonfly specimens collected compared to their size at other sites. Dytiscidae or predaceous diving beetles are known for their aggressive hunting abilities, proficient swimming capabilities, and with all of that the ability to keep other aquatic invertebrate populations down (Liao et al. 2022). Dytiscidae were in fact found prominently in Aleck Meadow and Jim's Pond during the summer and fall, as seen by the large blocks of green. In each case, no other family's population was nearly as abundant as dytiscidae. Notonectidae are known as backswimmers and are also predators but tend to be much smaller and therefore could be prey to families like dytiscidae (Griffith and Gillett-Kaufman 2021). Notonectidae was the prominent family found in Sphagnum Bog which could be due to the greater density of vegetation, shallower waters, and lower predation pressures potentially from acidity.

What do these mercury levels mean?

When considering the full spectrum of mercury levels found in dragonflies, the sites we studied appear to have relatively low and non-problematic concentrations. The data suggests that the mercury concentrations in these dragonflies are not high enough to trigger significant ecological or health concerns, indicating that these environments are less impacted by mercury contamination than others. According to the Dragonfly Mercury Project's Integrated Impairment Index, these mercury levels at all four sites fall within the range 100 to 299ppb which poses moderate risk to both human and animal health. This index equates 1ng/g to 1ppb. The moderate effect range risks are posed to people who eat fish, fish-eating birds, insect-eating fish, and fish-eating fish. The site of least concern, Sutherland Pond, with an average of 100.99ng/g falls at the very bottom of the moderate effect range, Sphagnum Bog with an average of 191.6ng/g, Aleck Meadow with an average of 149.02, and the site of most concern Jim's Pond with an average of 204.19ng/g. (Eagles-Smith et al., 2020; Chumchal & Seymour et al., 2023)

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APPENDIX I
SAMPLE COLLECTION FOR DRAGONFLY MERCURY PROJECT (DMP):

We collected samples from Jim's Pond and Sutherland Pond on July 7th, 2023 to be analyzed for the National Dragonfly Mercury Project. We used dip nets to collect our samples from the littoral areas of the wetlands. We collected as many as possible and then chose the largest 20 to send for sampling. Using ice cube trays with water to keep the larvae in, we measured and recorded their lengths, and recorded their identification using a dichotomous key down to the family level. Only families Libellulidae and Cordullidae were found at these sites with lengths ranging from 12mm-24mm at Jim's Pond and 12mm-18mm at Sutherland Pond. Samples were double bagged, labeled, and kept on ice.

DOC for DMP:

Using gloves we used a syringe with filters to test the water for dissolved organic carbon for the Dragonfly Mercury Project. We did this prior to our dragonfly larvae collection so that we collected undisturbed water. We rinsed the syringes three times without a filter and expelled that water on land so as to not disturb the wetland for an ideal sample. Once the syringe was rinsed we attached the filter to the end and used filtered water to rinse the vial three times as well. Then we filled the vial until it was full with filtered water, about 40mL. Then we disposed of the filter for each sample, and we took two samples for each site.

PFAS for DMP:

Using gloves we also collected water samples to test for PFAS. We used one bottle and everything was rinsed three times as described in the DOC protocol. We only filled these vials ¾ of the way full as opposed to completely full.

Dragonfly and water samples were sent on September 11, 2023 and arrived in Oregon on September 12th, 2023.