

School of Graduate Studies
Bemidji State University
1500 Birchmont Dr NE, #48
Bemidji, MN 56601-2699
218-755-2027

**TROPHIC STRUCTURE AND MERCURY BIOACCUMULATION IN WALLEYE AND
YELLOW PERCH IN THE UPPER AND LOWER RED LAKE BASINS**

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Marissa Pribyl

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APPROVAL BY THESIS ADVISOR

THIS THESIS HAS BEEN APPROVED ON THE DATE SHOWN BELOW:

Andrew W. Hafs

Andrew W. Hafs, Ph.D.
Committee Chair
Professor of Biology

20 Mar 2026

Date

[Signature]
Dean, College of Sciences and Health

23 March 2026

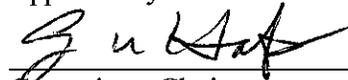
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Marissa Pribyl

Mercury is a persistent global contaminant that biomagnifies through aquatic food webs, with dietary and environmental factors serving as the primary drivers of accumulation in fishes. Trophic structure and methylmercury dynamics in Walleye (ogaa; *Sander vitreus*) and Yellow Perch (asaawens; *Perca flavescens*) were investigated in the Upper and Lower Red Lake basins in Red Lake, Minnesota, during 2024–2025. Diets of Walleye and Yellow Perch were assessed through stomach dissections, and tissue samples from both species were analyzed for total mercury concentrations. Additional analyses included shiners (giigoozens; *Notropis* spp., *Hudsonius* spp.) along with a variety of freshwater fish and invertebrates to evaluate mercury concentrations across prey sources. Previous research identified significant basin-specific differences in Walleye mercury concentrations, indicating that lake basin is a critical factor influencing mercury burdens (Orgon et al. 2023). Results from the 2024–2025 study support this conclusion, showing that Walleye, Yellow Perch, and prey items consistently exhibited higher mercury concentrations in Upper Red Lake compared to Lower Red Lake. Emerald Shiners (*Notropis atherinoides*) and Spottail Shiners (*Hudsonius hudsonius*) were the most abundant prey species in the Red Lakes and a key component of the Walleye diet. Invertebrates such as Trichoptera, Chironomidae, and Amphipoda were targeted for mercury analysis due to their importance in Yellow Perch diets. These findings highlight the importance of understanding system-specific trophic structures when examining mercury dynamics in large freshwater systems. Such knowledge provides valuable context for interpreting diet-related variation in tissue mercury concentrations and supports the development of fish consumption advisories to better protect the Red Lake community.

Approved by:



Committee Chair

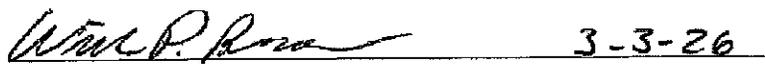
20 Mar 2026

Date



Committee Member

3 March 2026



Committee Member

3-3-26



Committee Member

20-March-2026



Graduate Faculty Representative

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It is important to acknowledge that these research efforts took place on the ancestral and unceded territory of the Red Lake Anishinaabe. Acknowledging research was additionally conducted on lands that were taken from the Red Lake Anishinaabe is important. The Indigenous peoples in this area have a deep connection to the land and the fisheries and wildlife within it. It is important to educate ourselves on the history and the steps taken by the Red lake Anishinaabe to keep their connection to this land over many centuries. The Tribe maintains its Tribal sovereignty and remains committed to the stewardship of this land for future generations. I am honored and grateful I was able to conduct my research on these traditional lands and learn about Red Lake Nation culture, history and language through knowledge from Tribal members and members of the community. I have gained valued relationships throughout this process, and I plan on continuing to grow those relationships and educate myself further on Red Lake Anishinaabe language, culture and history. I hope this encourages other researchers to take the time to educate themselves on the Indigenous presence within their research study area.

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Trophic Structure and Mercury Bioaccumulation in Walleye and Yellow Perch in the Upper and Lower Red Lake Basins

Abstract

Mercury is a persistent global contaminant that biomagnifies through aquatic food webs, with dietary and environmental factors serving as the primary drivers of accumulation in fishes. Trophic structure and methylmercury dynamics in Walleye (ogaa; *Sander vitreus*) and Yellow Perch (asaawens; *Perca flavescens*) were investigated in the Upper and Lower Red Lake basins in Red Lake, Minnesota, during 2024–2025. Diets of Walleye and Yellow Perch were assessed through stomach dissections, and tissue samples from both species were analyzed for total mercury concentrations. Additional analyses included shiners (giigoozens; *Notropis* spp.) along with a variety of freshwater fish and invertebrates to evaluate mercury concentrations across prey sources. Previous research identified significant basin-specific differences in Walleye mercury concentrations, indicating that lake basin is a critical factor influencing mercury burdens (Orgon et al. 2023). Results from the 2024–2025 study support this conclusion, showing that Walleye, Yellow Perch, and prey items consistently exhibited higher mercury concentrations in Upper Red Lake compared to Lower Red Lake. Emerald Shiners (*Notropis atherinoides*) and Spottail Shiners (*Hudsonius hudsonius*) were the most abundant prey species in the Red Lakes and a key component of the Walleye diet. Invertebrates such as Trichoptera, Chironomidae, and Amphipoda were targeted for mercury analysis due to their importance in Yellow Perch diets. These findings highlight the importance of understanding system-specific trophic structures when examining mercury dynamics in large freshwater systems. Such knowledge provides valuable context for interpreting diet-related variation in tissue mercury concentrations and supports the development of fish consumption advisories to better protect the Red Lake community.

Introduction

For centuries, The Red Lake Band of Ojibwe, located in north-central Minnesota, have relied on subsistence and traditional fishing, with Walleye and Yellow Perch being the species of greatest production and harvest in the Red Lakes (National Wildlife Federation 2006). As a part of tradition

and culture, the Anishinaabe used birch bark canoes to collect and transport fish along river highways (National Wildlife Federation 2006). Historically, the Band would harvest Walleye (ogaa; *Sander vitreus*), Northern Pike (ginoozhe; *Esox lucius*), Goldeye (waapagesii; *Hiodon alosoides*), Sturgeon (nome; *Acipenser fulvescens*), Black Crappie (gidag-agwadaashi; *Pomoxis nigromaculatus*), Freshwater Drum (mannashigan; *Aplodinotus grunniens*), Yellow Perch (asaawens; *Perca flavescens*), and Whitefish (adikameg; *Coregonus clupeaformis*). Such fish were brought to fish camps along the shore of Upper and Lower Red Lake where they would be cleaned, smoked, and dried out in the sun (A. Pemberton, personal communication, February 11, 2025). Allen Pemberton, Red Lake Band Member, Redby District Representative and Red Lake Department of Natural Resources Director grew up going to fish camps where particular fish like Goldeye and Whitefish were smoked inside of a smoke house or over a fire (A. Pemberton, personal communication, February 11, 2025). Walleye, Yellow Perch, and other species that are culturally and economically important are still harvested today by the Band using techniques like netting and angling (National Wildlife Federation 2006). The Red Lake Fisheries Association was formed in 1917 to help supply food for the World War 1 effort (Mittelholtz 1957). After the end of the war, the Red Lake commercial fishery continued to harvest fish using nets and wooden fish boxes (Brill 1974). Then, in 1929, commercial fishing harvest officially began to solely benefit the Tribe and soon became one of the greatest sustaining assets of the Red Lake Band (Mittelholtz 1957). Allen Pemberton recalls his grandpa and uncle netting for fish in the morning hours for the commercial fishery and being involved in the egg take procedures for Walleye (A. Pemberton, personal communication, February 11, 2025). Numerous Tribal families can be traced back to working for or with the Red Lake commercial fishery operation (A. Pemberton, personal communication, February 11, 2025). Later on, around 1996, the Walleye population experienced a crash, and Walleye harvest was prohibited throughout the Red Lakes (Glade et al. 2023). After management collaboration with Tribal, state, and federal agency recovery plans, the Walleye population rebounded in 2006 (Orgon et al. 2023). Subsequently, the commercial fishery continues today with Tribal fisherman using gill nets and aluminum boats, harvesting fish to feed Tribal families, and benefiting the economy of the Nation (Brill 1974). To further protect and conserve these resources for Tribal members, the Red Lake DNR collected tissue from Walleye to analyze mercury concentrations to provide safe eating guidelines for fish caught in Upper and Lower Red Lake (National Wildlife Federation 2006). This is important for the health of the Red Lake

community due to higher fish intake from Tribal traditional fishing and cultural practices that they maintain today (Xue et al. 2015).

The presence of mercury contamination results from natural and anthropogenic sources. Cinnabar, an important mercury ore, has been mined since 415 BCE (Eisler 2000). Mercury has been used in gold extraction, electrical instruments, pharmaceuticals, fungicides, medical antiseptics, and other products (Eisler 2000). Mercury poisoning in humans causes damaging effects on the nervous system, immune-system, and organs, and, in severe cases, death (Madenjian et al. 2016). Fetuses, nursing infants, and children under the age of 15 are most at risk for mercury poisoning (Madenjian et al. 2016). There is no known treatment to mitigate mercury poisoning symptoms (Eisler 2000). In Northern Minnesota, atmospheric deposition is the primary distribution source of mercury, found in even the most remote lakes (Eisler 2000). Consequences of this have resulted in bans on sport and commercial fishing, issued health warnings, and restrictions on fish consumption (Eisler 2000). As the mercury discharges into bodies of water from the atmosphere and other sources, the inorganic (Hg) mercury converts into methylmercury (MeHg) by using forms of bacteria like, sulfate-reducing bacteria and other natural processes (St. Louis et al. 2004). This process makes it possible for methylmercury to biomagnify in food chains and become a substantial health threat to humans and wildlife.

Toxic metals like mercury can only accumulate in aquatic organisms after it has been converted into the chemical compound methylmercury (Hosseini et al. 2013). Prey selection and diet significantly alter trace metals accumulated in fish (Qiu 2015). Fish absorb methylmercury through prey selection in their diet, where MeHg tightly bonds to proteins in their tissue and muscle (Winner 2010). Biomagnification starts at the base of the food chain, with invertebrates, phytoplankton, and zooplankton that are consumed by smaller fish (Marziali et al. 2021). As mercury biomagnifies through the food web, it accumulates, reaching prominent levels in apex predators (Johnson et al. 2015, Burger et al. 2000). Larger sport fish like Walleye have increased mercury concentrations in their tissue due to them being piscivorous, placing them higher in the food chain (Hosseini et al. 2013). Yellow Perch are a consistent prey component for Walleye in the Red Lakes, making them equally important for gathering mercury concentrations (Orgon et al. 2023). More knowledge of mercury concentrations on Walleye is present compared to Yellow

Perch in Upper and Lower Red Lake (Orgon et al. 2023). Yellow Perch are opportunistic foragers, feeding on zooplankton, benthic invertebrates, and smaller fish, placing them lower in the food chain (Happel et al. 2015). Species of shiners like, Spottail Shiners and Emerald Shiners are the most abundant minnow in the Red Lakes, and are a significant food component for Walleye (Orgon et al. 2023). Shiners are a foraging species feeding primarily on zooplankton and benthic invertebrates (Lloyd et al. 1957). Many benthic invertebrates are present in the Red Lakes, including Gastropods, Bivalves, Amphipods, Trichoptera, Anisoptera, Zygoptera and Chironomidae. A majority of these invertebrates feed on algae, bacteria, leaves and organic matter that enters the water (Marziali et al. 2021). Other invertebrates like Trichoptera, Anisoptera and Zygoptera are considered filter feeders (Marziali et al. 2021). Additionally, invertebrates live near sediments and are directly exposed to methylmercury-contaminated sediments (Marziali et al. 2021). Invertebrates serve as food for multiple fish species in aquatic environments, including Yellow Perch and Spottail Shiners (Marziali et al. 2021). Little is known about mercury concentrations in fish and invertebrates at the base of the food chain in the Red Lake system.

In 2019 and 2020, a study done by Orgon (2023) discovered that total mercury concentrations for Walleye in the Upper Red Lake Basin were elevated compared to Walleye collected in the Lower Red Lake Basin (\bar{x} = 0.215 ± 0.117 and 0.144 ± 0.077 mg/kg, respectively). These findings highlight lake basin as an important factor governing mercury accumulation in fish (Orgon et al. 2023). Upper Red Lake has an abundance of wetlands, primarily peatlands, associated with multiple factors that increase mercury production (Orgon et al. 2023). Wetlands exhibit high dissolved organic carbon, low pH, high acid-volatile sulfides in sediment, low dissolved oxygen, and sulfate-reducing bacteria, aiding in methylmercury production (Jardine et al. 2013). The Tamarac River is connected to most of the wetlands in the Red Lake ecosystem, which discharges into Upper Red Lake, making it possible for mercury to enter the lake (Orgon et al. 2023). Additionally, Upper Red Lake is also influenced by numerous drainage ditches that channel water from the northern peatlands directly into the Upper Basin, originally created for agricultural purposes (Orgon et al. 2023). In comparison, Lower Red Lake has an increased area of upland forests, which can accumulate and retain mercury at an increased efficiency compared to other natural landscapes (Demers et al. 2013). Additionally, Lower Red Lake provides more extensive fish habitat than Upper Red Lake, leading to greater prey abundance in the lower basin (P. Brown,

personal communication, December 9, 2024). As a result, fish in Lower Red Lake may have access to more abundant and higher quality prey, which could contribute to differences in mercury concentrations between the two basins (P. Brown, personal communication, December 9, 2024). This variation may be explained by somatic growth dilution, a process where fish accumulate less mercury relative to their body mass when consuming highly nutritious prey (Chetelat et al. 2020). Enhanced somatic growth dilution is often associated with improved habitat conditions that support increased prey availability. This highlights the importance of understanding what fish are consuming within the food web in each basin, as diet plays a key role in influencing mercury accumulation (Qiu 2015). Therefore the objectives of this study are to (1) explore prey assemblage and mercury concentrations in Walleye and Yellow Perch to determine trophic structure in Upper and Lower Red Lake and how it relates to the trophic transfer of mercury; (2) determine if changes in diet composition is one of the factors explaining the difference in Walleye mercury concentrations observed between the Upper and Lower Red Lake basins in 2019. Completing these objectives will give the Red Lake DNR and Minnesota DNR baseline knowledge on dietary methylmercury exposure in the Red Lake ecosystem. This background knowledge could provide Red Lake Nation with potential solutions to mitigate mercury concentrations in Walleye and Yellow Perch for safer consumption.

Methods

Study area

Red Lake Nation is located in north-central Minnesota within the boundaries of Beltrami, Clearwater and other counties and is home to the Red Lake Anishinaabe (Figure 1). Red Lake Nation is Minnesota's only contiguous, un-allotted reservation, and is one of few un-allotted reservations in the United States. The Nation practices the traditional political structure of hereditary representative chiefs with a modernized Tribal council, maintaining their Tribal sovereignty. The Tribe holds all land in common for its members' benefit, with the right to hunt, fish and gather for harvest. To ensure long-term success for its members, Red Lake Nation retains complete and exclusive control and has preserved communal ownership of Tribal lands, making it a closed reservation. In addition to other treaties, some of the sample area is located within the

ceded lands of the 1889 Treaty, where Red Lake ceded 44515 km² to the U.S. government. The total lake area for Upper and Lower Red Lake within the Nations boundary is 963 km², while the remaining 185.7 km² of Upper Red Lake lies within the management of the state of Minnesota. Upper and Lower Red Lake make up two large basins that are connected by a 1 km strait, referred to as the Narrows. Upper and Lower Red Lake are within the Upper/Lower Red Lake Watershed consisting primarily of lakes, streams, rivers, wetlands, peatlands and mixed forests, making it a diverse ecosystem. The Red Lake River is the only outlet of the two lakes, flowing west out of Lower Red Lake. There are 12 other tributaries that discharge into Upper and Lower Red Lake. The Upper and Lower Red Lake basins are the remnants of glacial Lake Agassiz and make up Minnesota's largest inland lake. The two basins are classified as eutrophic, with Upper Red Lake having a max depth of around 5.5 meters, and Lower Red Lake having a max depth of around 9.1 meters respectively.

Species collection

Walleye and Yellow Perch were collected during the spring, summer, and fall of 2024 in selected sites in Upper and Lower Red Lake. Samples during the spring and summer were collected with the help of the Red Lake DNR and Red Lake Commercial Fishery using standard gill nets, experimental gill nets and angling practices. In the fall, Walleye and Yellow Perch were collected by collaborating with the Red Lake DNR and MN DNR's experimental surveys for annual population assessments. Specific freshwater fish species and invertebrates that were determined as prey items for Walleye and Yellow Perch were collected during spring, summer and fall in selected sites. Prey fish species included Spottail Shiners, Emerald Shiners, Trout Perch (*Percopsis omiscomaycus*), Walleye and Yellow Perch. These species were collected by the Red Lake DNR and MN DNR during their annual population assessments using seine haul procedures along the shores of Upper and Lower Red Lake. Composite samples were collected depending on total or average length of the fish species. If only one fish within a fish species was present in the seine nets they would be analyzed individually. Fish that were collected together and within the same species were collected in composite samples varying between 2 - 12 fish in each sample in order to have enough tissue for mercury analysis purposes. Trichoptera, Zygoptera, Amphipods, Chironomidae, Gastropods, Bivalves, Anisoptera, Hirudinea and Notonectidae were the primary

invertebrates collected in proximity to the rivers and tributaries surrounding Upper and Lower Red Lake. Invertebrates were collected using Ekman dredges and aquatic dip nets; multiple samples were taken to ensure sufficient tissue amounts for mercury analysis.

Sample collection

Before tissue samples could be extracted from Walleye and Yellow Perch, length (mm), biomass (g), sex, maturity (immature male, mature male, immature female, and mature female), and otoliths for aging were collected. Using a fillet knife, tissue with the skin off were collected from Walleye and Yellow Perch on the left side anterior to the dorsal fin. To avoid contaminating tissue samples for mercury analysis, work surfaces were kept clean, field personnel wore nitrile gloves, and the fillet knife was cleaned with deionized water between each tissue extraction. Tissue samples were cleaned with deionized water, and wet biomass (g) was collected. The wet weight of the tissue ranged from 1.98 to 21.79 grams depending on the size of the Walleye or Yellow Perch. Each tissue sample was placed into a Whirl-Pak with serial numbers relating to the species ID. During tissue extraction, diets were collected through stomach dissection in Walleye and Yellow Perch. Stomachs were extracted using a sharp fillet knife, cutting as close to the esophagus as possible to prevent the diet contents from escaping. Each stomach was placed into a Whirl-Pak with 95 % ethanol for preservation and labeled with the corresponding fish species ID. Once the smaller fish species were collected they were separated out by species. Spottail Shiners, Emerald Shiners, Trout Perch, Walleye and Yellow Perch were the main species collected as they were present in both Walleye and Yellow Perch diets. The fish were cleaned with deionized water and separated into composite or individual samples. Individual lengths (mm) for each fish was recorded and bulk or individual biomass (g) was collected, depending on whether it was composite or individual sample. Composite sample fish lengths were averaged to facilitate subsequent statistical analyses. Invertebrate samples were separated out by family or species and cleaned with deionized water. Bulk wet biomass (g) was recorded which varied based on the number of invertebrates separated out within that sample. Both fish and invertebrate prey samples were placed into Whirl-Pak's with serial numbers to be lyophilized later.

Sample analysis

Walleye, Yellow Perch, smaller prey fish and invertebrate samples were lyophilized using a Harvest Right stainless-steel freeze dryer and then homogenized using a mortar and pestle. Dry biomass (g) was collected from each homogenized tissue sample and put into a vial to be analyzed. Samples were analyzed for mercury using a Milestone TriCell Dual Beam Direct Mercury Analyzer (DMA-80evo). Before homogenized tissue samples were analyzed, calibration standard samples were analyzed at 0.5 ng, 5.0ng, and 50 ng of Hg to check the calibration curve. All samples were weighed out to 0.025 grams +/- 0.005 grams and put into a nickel sample boat. A total of 20 boats with homogenized tissue were placed in the Direct Mercury Analyzer to analyze for the detection of mercury. DORM-4, matrix spikes, and matrix spike duplicates were analyzed to evaluate the accuracy of DMA-80evo. Diet contents in Walleye and Yellow Perch were observed and identified down to family or species if anatomy permitted, using taxonomic keys and a dissecting scope. It was important to analyze the diet contents before collecting prey species, to ensure the correct species were sampled independently for mercury concentration analysis. Frequency of occurrence in the diets was essential to get a promising idea of the trophic structure within the Red Lakes.

Data analysis

For diet data analysis comparisons between basin and season for Walleye and Yellow Perch, one-way permutational multivariate ANOVAs (PERMANOVAs; Anderson 2001) were performed using the “adonis2” function from the vegan package within R (Oksanen et al. 2025). Diet comparisons between the seasons in each basin were conducted using count and biomass data of the species found in the diets of Walleye and Yellow Perch. When PERMANOVA results were significant ($P < 0.05$), a permutation test for multivariate dispersion was conducted using the function “betadisper”, to evaluate whether the significant differences were attributable to variation in diet composition among groups or to differences in group dispersion. If the permutation test for multivariate dispersion test was significant ($P < 0.05$), post hoc Tukey pairwise comparisons were performed to determine which groups exhibited unequal multivariate dispersions. In addition to PERMANOVAs, nonmetric multidimensional scaling (NMDS) ordinations were used to visualize diet overlap between lake basin and season among Walleye and Yellow Perch in each prey category. The ordinations used the numerical count and biomass of the prey items consumed in

each prey category. Centroids were plotted for lake basin and season using the “ordiellipse” function in the vegan package (Oksanen et al. 2025). The ellipses represent the 95% confidence interval for lake basin and season. Mercury concentrations were added as a vector within the NMDS plots, using the function “envfit” from the vegan package in R (Oksanen et al. 2025), to observe how mercury values interacted with the diets.

In addition to quantifying diet data, Walleye and Yellow Perch diets were categorized by basin (Upper and Lower Red Lake) and season (spring, summer, and fall). For each prey category, the Index of Relative Importance (IRI) was calculated following Pinkas et al. (1971) and Martin et al. (1996) using the equation:

$$IRI = \%F \times (\%N + \%M)$$

where %F represents the frequency of occurrence, %N represents numerical abundance, and %M represents the mass contribution of each prey group. The IRI provides a composite measure of prey importance by integrating frequency, abundance, and biomass, thereby reducing biases that can arise when diets include rare but large prey items or small yet abundant taxa. IRI values were subsequently standardized to percentages to allow comparisons of diets between basins and across seasons for both Walleye and Yellow Perch. Confidence intervals (95%) for IRI values were generated using bootstrapping procedures to assess variability in prey importance.

Mercury concentrations were analyzed separately for Walleye and Yellow Perch. Akaike Information Criterion (AIC) scores were used to identify the best-supported model, with candidate predictors including length, biomass, sex, season, and basin. Linear models were then fitted to evaluate the significance of mercury concentrations in response to the selected predictors for each species. To test for differences in mercury concentrations of prey items between Upper and Lower Red Lake, data was first assessed for normality using the Shapiro–Wilk test. Prey items with non-normal distributions ($P < 0.05$) were analyzed using Wilcoxon rank-sum tests, while those meeting the assumption of normality ($P > 0.05$) were compared using two-sample t-tests. All statistical analyses were performed in R (R version 4.5.0; R Core Team 2025).

Results

A total of 525 Walleye ranging from 186 to 624 mm and 322 Yellow Perch varying from 95 mm to 332 mm were collected for mercury analysis. For diet analysis purposes, 504 Walleye and 325

Yellow Perch stomachs were collected. There were a total of 1,703 diet items successfully identified from Walleye diets. Additionally, in Yellow Perch diets, 4,774 prey items were successfully identified. A total of 1,770 prey fish species were collected ranging from 34 mm to 110 mm in length and a sum of 2,285 invertebrates were collected for mercury analysis. The best fit model for explaining variation in mercury concentration for Walleye (Table 2) and Yellow Perch (Table 3) was $Hg \sim TL.mm * Sex * Season * Basin$, with an AIC score of -1238.9 for Walleye and -1604.4 for Yellow Perch. This model accounts for interactions between fish length, sex, season, and basin. The second best fit model for Walleye and Yellow Perch was $Hg \sim TL.mm * Season * Basin$ with AIC scores of -1233.5 and -1580.5. The best fit model was used to generate figures that illustrate seasonal and spatial differences in mercury concentrations in Walleye (Figure 2) and Yellow Perch (Figure 3) from Upper and Lower Red Lake as a function of fish length (mm). Mercury levels increased with Walleye and Yellow Perch Length (mm) in all seasons and both basins, reflecting typical bioaccumulation patterns. As expected, mercury concentrations were substantially higher in Walleye compared to Yellow Perch, with Walleye more frequently exceeding consumption advisory thresholds across all size classes and seasons.

Walleye from Upper Red Lake consistently exhibited higher mercury concentrations than those from Lower Red Lake, with the most pronounced differences occurring in spring and fall. During these seasons, a few Upper Red Lake Walleye exceeded the 0.47 mg/kg threshold, placing them in the “1 meal per month or avoid” consumption advisory category. Although mercury levels were generally lower in summer, Upper Red Lake Walleye still tended to have higher concentrations than similarly sized individuals from Lower Red Lake. Most Walleye across both basins fell within the “1 serving per week” category or better (Figure 2). Overall, mercury concentrations in Walleye ranged from 0.03 mg/kg in smaller fish (211 mm; 64 g) to 0.64 mg/kg in larger individuals (421 mm; 612 g) (Figure 2). The largest Walleye sampled, a mature female from Lower Red Lake measuring 624 mm and weighing 2,244 g had a mercury concentration of 0.31 mg/kg. Yellow Perch from Upper Red Lake also exhibited higher mercury concentrations than those from Lower Red Lake, particularly in summer and fall. These seasonal differences were most evident in the fall, when Upper Red Lake Perch approached or exceeded the 0.15 mg/kg threshold corresponding to the “1–2 servings per week” advisory. In contrast, spring mercury concentrations were comparable between basins, though fewer Yellow Perch were collected during that season. The

majority of Yellow Perch were classified within the “2–3 servings per week” advisory category, indicating relatively low mercury-related consumption risk (Figure 3). Across all samples, Yellow Perch mercury concentrations ranged from 0.009 to 0.16 mg/kg, corresponding to individuals from 184 mm (69 g) to 258 mm (156 g) (Figure 3). The largest individual, a 332 mm, 476 g mature female from Lower Red Lake had a mercury concentration of 0.09 mg/kg. While sex is an important predictor in mercury models, it did not appear to strongly influence concentrations between basins in this study. Consumption advisories were used based on the state of Minnesota’s findings and recommendations.

Walleye permutational multivariate ANOVA test based on prey counts revealed significant basin-level differences during spring ($p = 0.008$, $R^2 = 0.03$) and summer ($p = 0.001$, $R^2 = 0.22$), but not in fall ($p = 0.591$, $R^2 = 0.01$; Table 4). Tests of permutation multivariate dispersion indicated that dispersions did not differ significantly between basins in spring ($p = 0.377$), but were significantly different in summer ($p = 0.001$). Post hoc Tukey pairwise comparisons confirmed this result, showing a significant difference in dispersion between Upper and Lower Red Lake in summer ($p < 0.001$). Therefore, while basin-level differences in spring reflect true differences in prey counts, the significant effect observed in summer may be influenced in part by unequal dispersions between basins. Similarly, PERMANOVA indicated that Walleye diets by biomass differed between basins only during the summer ($R^2 = 0.20$, $p < 0.001$), while no significant basin effects were detected in spring ($p = 0.065$, $R^2 = 0.02$) or fall ($p = 0.138$, $R^2 = 0.03$) (Table 5). Tests of permutation multivariate dispersion showed dispersion differed significantly between basins in summer ($p = 0.005$). A post hoc Tukey test, however, did not identify significant pairwise differences between Upper and Lower Red Lake basins ($p = 0.718$), suggesting that the summer PERMANOVA result primarily reflects differences in dietary composition rather than heterogeneity of dispersion. Count-based Non-metric Multidimensional Scaling (NMDS) revealed notable differences in Walleye diet composition between Upper Red Lake and Lower Red Lake across seasons (Figure 4; A). While some overlap in diet was observed during certain seasons, distinct lake-level patterns were apparent. In spring, Upper Red Lake diets were more broadly distributed, indicating a more variable diet, whereas Lower Red Lake diets formed a tighter cluster, closely associated with prey such as Shiners. During summer, diets between Upper Red Lake and Lower Red Lake were clearly separated in ordination space. Walleye from Upper Red Lake

consumed more Yellow Perch, while Lower Red Lake diets were more associated with Freshwater Drum and Trichoptera. In the fall, diet composition between Upper Red Lake and Lower Red Lake was increasingly similar, with overlapping ellipses centered near Unknown Fish and Shiners, indicating a convergence in diet at this time of year. A vector for mercury concentration (Hg.ppm) pointed toward prey such as Zygoptera, Darters, and Sunfish suggesting that Walleye who fed on these diet items had elevated mercury levels within their tissue. Non-metric Multidimensional Scaling based on prey mass revealed differences in the biomass composition of Walleye diets between Upper Red Lake and Lower Red Lake across seasons (Figure 4; B). While patterns were generally consistent with those observed in the count-based Non-metric Multidimensional Scaling, the mass-based ordination highlighted key differences in dominant prey by biomass. In spring, both lakes showed overlap, but Upper Red Lake still exhibited a broader spread, suggesting a more variable diet. Lower Red Lake spring diets were again more tightly grouped and associated with Shiners. In summer, Upper Red Lake and Lower Red Lake diets remained distinct. Upper Red Lake samples were associated with Yellow Perch, while Lower Red Lake diets clustered near Freshwater Drum and Trichoptera, indicating different prey items were contributing increased biomass in each lake during the summer. Fall diets in both lakes overlapped considerably, particularly around unknown Fish, suggesting convergence in biomass-dominant prey during that season. The vector representing mercury concentration (Hg.ppm) pointed toward the lower left quadrant of the plot, closely aligned with Shiners. This suggests Walleye who fed on shiners also had increased mercury concentrations in their tissue.

Yellow Perch permutational multivariate ANOVA indicated diets differed between Upper and Lower Red Lake depending on season and whether diets were assessed by prey counts (Table 4) or biomass (Table 5). In spring, basin effects approached but did not reach significance for counts ($p = 0.085$, $R^2 = 0.34$) or biomass ($p = 0.077$, $R^2 = 0.36$). In summer, significant basin-level differences were detected for both counts ($p = 0.001$, $R^2 = 0.07$) and biomass ($p = 0.001$, $R^2 = 0.06$), with no evidence of heterogeneous dispersion (counts $p = 0.97$; biomass $p = 0.326$). Fall diets showed the strongest basin differences (counts $p = 0.001$, $R^2 = 0.26$; biomass $p = 0.001$, $R^2 = 0.41$), again with dispersions remaining homogeneous (counts $p = 0.662$; biomass $p = 0.45$). Collectively, these results suggest that basin-level differences in Yellow Perch diets were strongest in fall, moderate in summer, and weakest in spring, and that observed differences reflected true

compositional changes rather than differences in group variability. In the count-based NMDS ordinations (Figure 5; A), spring diets showed the greatest similarity between basins, with both clustering around Chironomidae and Darters. Upper Red Lake summer diets clustered with Notonectidae and unidentified fish, whereas Lower Red Lake summer diets were more closely associated with Trichoptera and Bivalves. Fall diets showed no overlap between basins, with Lower Red Lake strongly associated with Gastropods, while Upper Red Lake diets were linked to Amphipods. Yellow Perch biomass ordinations (Figure 5; B) revealed some overlap and separation between basins when compared with seasonal variation. In spring, some overlap occurred between basins around Chironomidae, Trichoptera, Bivalves and unknown invertebrates, but Lower Red Lake diets were also associated with Zygoptera, Darters, Amphipods and Notonectidae. In summer some overlap was present between the basins. Lower Red Lake summer diets were dominated by invertebrate biomass, including Trichoptera, Bivalves, Chironomidae, and unknown invertebrates, while Upper Red Lake diets were closer to Darters, Notonectidae, and Amphipods. Fall diets again showed no overlap, with Lower Red Lake strongly associated with Gastropods and Upper Red Lake clustering with Amphipods, Darters and Notonectidae. The Hg.ppm vector in count and biomass ordinations aligned strongly with Gastropods indicating Yellow Perch who fed on Gastropods also had increased mercury concentrations within their tissue.

Index of relative importance (IRI) analysis showed that Walleye in both Upper and Lower Red Lake relied heavily on fish, but the composition of their diets varied by basin and season (Figure 6). In Upper Red Lake, diets were consistently dominated by fish species, with shiners and Trout Perch common in spring, Yellow Perch almost exclusively consumed in summer, and a broader mix of shiners, Yellow Perch, and other fish taken in fall. This pattern reflects a strong dependence on fish prey throughout the year. Lower Red Lake Walleye, however, displayed a more diverse feeding strategy that included substantial use of invertebrates. Spring diets featured shiners alongside a variety of aquatic invertebrates, while summer diets shifted to a strong reliance on Trichoptera and Freshwater Drum. By fall, fish once again dominated, particularly shiners and unidentified fish, with Yellow Perch and Freshwater Drum also present. Together, these findings demonstrate that Walleye in Upper Red Lake fed almost exclusively on fish, whereas those in Lower Red Lake incorporated a wider range of prey, particularly invertebrates, into their diets. Overall, Walleye fed primarily on species of shiners, Yellow Perch and Freshwater drum, adding species of invertebrates like Trichoptera when the opportunity was present in the Red Lakes.

IRI results indicated that Yellow Perch primarily consumed invertebrates in both basins, but individuals in Upper Red Lake incorporated a wider variety of fish into their diets (Figure 7). In Upper Red Lake, spring diets were dominated by Chironomidae with only minor amounts of fish and other invertebrates. Summer diets became more diverse, including Yellow Perch, unknown fish, Freshwater Drum, darters, shiners, and several invertebrate groups. By fall, diets shifted back almost exclusively to invertebrates, particularly Amphipods, with small contributions from other prey. In Lower Red Lake, Yellow Perch diets remained more strongly focused on invertebrates across seasons. In spring, only Zygoptera and Chironomidae were consumed, while summer diets broadened to include Trichoptera as the dominant prey along with a range of invertebrates and occasional fish such as Trout Perch and Burbot. By fall, diets narrowed again, with Gastropods becoming the primary prey type and only small amounts of other invertebrates present. Overall, these findings suggest that Yellow Perch in both basins rely heavily on invertebrates, but those in Upper Red Lake incorporate fish more frequently into their diets compared to those in Lower Red Lake. In conclusion, Yellow Perch between both basins fed mostly on invertebrates like Amphipods, Chironomidae, Zygoptera and Trichoptera but fed on other prey like fish when the opportunity was presented.

Mercury concentrations in various prey types from Upper Red Lake exhibited consistently higher mercury levels than those from Lower Red Lake (Figure 8). This pattern was particularly evident in species such as shiners, Walleye, Yellow perch, and Trout Perch which showed both elevated concentrations and greater variability in Upper Red Lake. In contrast, prey types such as Amphipods, Chironomidae, Gastropods, and Hirudinea displayed low mercury concentrations across both basins. Overall, all fish and invertebrate prey items collected possessed a higher mean and standard deviation of mercury concentrations in Upper Red Lake compared to Lower Red Lake (Table 8). Wilcox tests and T-tests showed Anisoptera, shiners, Trout Perch, Walleye and Yellow Perch have significant differences in mercury concentrations ($p < 0.05$), with Upper Red Lake species having higher concentrations of mercury (Table 8). Anisoptera specifically had higher concentrations of mercury compared to other invertebrate species, including Zygoptera, who are in the same family. Freshwater Drum was the only prey type that could not be successfully found or collected in the seine hauls. Previous research done by a Red Lake Nation College student

showed mercury concentrations in Freshwater Drum adults were considerably lower compared to Walleye and other fish in the Red Lakes.

Discussion

Mercury analysis of prey species reinforces that organisms in Upper Red Lake exhibit higher mercury concentrations than those in Lower Red Lake. Nearly all fish and invertebrate taxa collected from Upper Red Lake showed elevated mean mercury levels, particularly shiners, Trout Perch, Walleye, and Yellow Perch, as well as invertebrates such as Anisoptera, which reached concentrations of 0.016–0.046 mg/kg Hg. Anisoptera are predatory insects that feed on fish and can remain in aquatic habitats for two to three years during their larval stages, allowing substantial time for mercury bioaccumulation (Wu et al. 2021). Comparable Anisoptera samples collected in Minnesota by the United States Geological Survey exhibited similar concentrations (0.008–0.027 mg/kg), with lower levels observed farther south in the state (USGS 2020). In Nova Scotia, Anisoptera mercury levels ranged from 0.01–0.12 mg/kg (Buckland et al. 2014). Among prey taxa, shiners had the highest mercury concentrations in the Red Lakes (0.009–0.119 mg/kg). For context, Spottail Shiners from regions of Canada and New York exhibited mercury concentrations between 0.01–0.30 mg/kg depending on environmental conditions (Suns et al. 1995; Choy et al. 2009). Elevated prey mercury concentrations in Upper Red Lake provide a clear mechanism for the higher mercury burdens observed in the Walleye and Yellow Perch populations, and suggest increased mercury concentrations in Upper Red Lake is present within all species in the Red Lakes.

As expected for higher trophic-level predators, Walleye consistently exhibited greater mercury concentrations than Yellow Perch across all size classes, seasons, and basins. Mercury levels increased with fish length, reflecting standard bioaccumulation trends, and were consistently higher in Upper Red Lake than in Lower Red Lake, corroborating Orgon et al. (2023). Walleye from Upper Red Lake exceeded the 0.47 mg/kg consumption advisory threshold, particularly in spring and fall, indicating elevated human exposure risk in this basin. Across all samples, Walleye mercury concentrations ranged from 0.03–0.64 mg/kg, consistent with Orgon et al. (2023), who reported values between 0–0.6 mg/kg for fish of similar lengths (200–600 mm) respectively. Yellow Perch also showed elevated mercury in Upper Red Lake, especially during summer and fall, with some individuals approaching or exceeding the 0.15 mg/kg advisory threshold. In this

study, Yellow Perch mercury concentrations ranged from 0.009–0.162 mg/kg (95–332 mm total length). Similar concentrations (0.036–0.281 mg/kg) have been reported for Yellow Perch in Canadian and northeastern Minnesota lakes (Harris et al. 1998; Kidd et al. 1995). Collectively, these results demonstrate that elevated mercury in Upper Red Lake is a persistent and basin-wide phenomenon, not a temporary or anomalous event.

Environmental factors likely drive these differences between basins. Upper Red Lake’s extensive wetland areas promote methylmercury production, increasing the pool of bioavailable mercury (Orgon et al. 2023). Additionally, this basin provides less favorable habitat conditions for plankton, fish and invertebrates (P. Brown, personal communication, December 9, 2024). With an average depth of only 5 meters, Upper Red Lake is a shallow, bowl-shaped basin composed primarily of sandy substrate, offering minimal habitat structure (P. Brown, personal communication, December 9, 2024). Reduced prey availability and habitat quality force organisms to allocate more energy to foraging and less to growth, reducing somatic growth dilution and enhancing mercury accumulation (Karimi et al. 2016). Thus, the combination of greater methylmercury production and lower growth efficiency could explain the consistently higher mercury levels across species in Upper Red Lake. In contrast, Lower Red Lake bordered primarily by upland forest, retains mercury in soils and limits its conversion to bioavailable forms (Orgon et al. 2023). Its greater depth (9 meters) and more complex habitat structure support abundant zooplankton, invertebrate, and fish communities, increasing prey availability (P. Brown, personal communication, December 9, 2024). When food resources are plentiful, organisms can devote more energy to growth and reproduction rather than foraging, increasing somatic growth dilution and reducing tissue mercury concentrations (Karimi et al. 2016). Supporting this, Walleye from Lower Red Lake exhibit higher growth rates in specific year classes, suggesting increased growth efficiency (Orgon et al. 2023).

Dietary analyses (NMDS, PERMANOVA, and IRI) revealed significant basin and season specific differences in prey composition for both Walleye and Yellow Perch. Walleye in Upper Red Lake relied primarily on fish prey, whereas those in Lower Red Lake consumed a greater proportion of invertebrates in addition to fish. These results are consistent with previous findings showing Walleye diets dominated by Yellow Perch and shiner species in Minnesota and Lake Erie (Kamden et al. 2023; Knight et al. 1982). Yellow Perch diets were dominated by invertebrates in both basins, though piscivory increased in Upper Red Lake. Earlier studies of Red Lake Yellow Perch reported

similar reliance on Chironomidae and zooplankton (Pycha et al. 1955), and research in Lake Michigan and Lake Erie also found that perch diets shift based on habitat and prey availability (Happet et al. 2015; Knight et al. 1982). Overall, both species demonstrate flexible, opportunistic feeding patterns influenced by local prey resources. NMDS and PERMANOVA results further suggest diets differed between the basins in both Walleye and Yellow Perch. Thus, diet composition reflects both ecological differences and trophic pathways influencing mercury accumulation in the two basins.

Findings from this study and Orgon et al. (2023) also suggest that fish movement between the basins may be more limited than previously thought, contributing to distinct mercury profiles. Earlier work by Smith et al. (1952) estimated that approximately 20% of Walleye migrate between basins. Future tagging and mark-recapture studies could clarify current movement patterns and their role in mercury dynamics. Additionally, the shallow depth of the basins prevents thermal stratification, leading to elevated temperatures, especially in Upper Red Lake (P. Brown, personal communication, December 9, 2024). Elevated temperatures in smaller or shallower lakes have been associated with increased mercury accumulation in fish (Harris et al. 1998). Therefore, exploring temperature-related metabolic processes and other bioenergetic factors could help explain how physiological and environmental variables interact to influence mercury uptake and retention. Overall, this research provides a foundational understanding of mercury trophic transfer in the Red Lake system, with direct implications for fisheries management, fish consumption advisories, and the protection of Red Lake Nation's health and cultural practices. Integrating these findings with long-term monitoring and stable isotope analyses will help clarify mercury pathways and inform targeted management strategies to reduce human and ecological exposure.

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TABLES

Table 1. Common names, family names, scientific names, Ojibwe names, and abbreviations used to represent 21 prey categories observed in diets of Walleye and Yellow Perch in Upper and Lower Red Lake.

Prey category	Abbreviation
Amphipoda	AMPH
Anisoptera (Oboodashkwaanishiinh)	ANIS
Bivalve (Es)	BIVA
Burbot <i>Lota lota</i> (Mizay)	BUB
Chironomidae	CHIRO
Decapoda (Ashaageshiinh)	DECA
Darter <i>Etheostoma</i> spp.	DAR
Hirudinea (Ozagaskwaajime)	HIRU
Freshwater Drum <i>Aplodinotus grunniens</i> (Maanashigan)	FRD
Gastropoda (Obiimiskodisii)	GASTRO
Northern Pike <i>Esox Lucius</i> (Ginoozhe)	NOP
Notonectidae	NOTO
Shiner <i>Notropis</i> spp.	SHI
Sunfish <i>Lepomis</i> spp. (Agwadaashi)	SUN
Trichoptera	TRICO
Trout Perch <i>Percopsis omiscomaycus</i>	TRP
Unidentified Fish	UNKF
Unidentified Invertebrate	UNKI
Walleye <i>Sander vitreus</i> (Ogaa)	WAE
Yellow Perch <i>Perca flavescens</i> (Asaawens)	YEP
Zygoptera	ZYGO

Table 2. AIC models used to predict mercury (Hg) concentrations in Walleye from Upper and Lower Red Lake.

Walleye				
Function	AIC	AIC	R ²	
Hg~TL.mm*Sex*Season*Basin	-1238.9	0.0	0.59	
Hg~TL.mm*Season*Basin	-1233.5	-5.3	0.56	
Hg~TL.mm*Sex*Basin	-1221.9	-16.9	0.55	
Hg~TL.mm+Sex+Basin	-1220.0	-18.9	0.54	
Hg~TL.mm+Sex+Season+Basin	-1218.9	-20.0	0.54	
Hg~TL.mm+Sex*Basin	-1218.7	-20.1	0.54	
Hg~TL.mm+Basin	-1211.1	-27.8	0.53	
Hg~TL.mm*Basin	-1210.9	-28.0	0.53	
Hg~TL.mm+Season+Basin	-1210.0	-28.8	0.53	
Hg~TL.mm*Sex	-1142.9	-95.9	0.46	
Hg~TL.mm+Sex	-1140.8	-98.0	0.46	
Hg~TL.mm*Season*Sex	-1135.6	-103.3	0.47	
Hg~TL.mm+Season	-1127.1	-111.7	0.45	
Hg~TL.mm	-1126.4	-112.4	0.44	
Hg~TL.mm*Season	-1126.1	-112.8	0.45	
Hg~TL.mm+Weight.g	-1124.7	-114.2	0.44	
Hg~Weight.g	-1092.7	-146.1	0.41	
Hg~Basin	-846.8	-392.1	0.05	
Hg~Season	-836.6	-402.3	0.03	
Hg~Sex	-821.9	-417.0	0.00	

Table 3. AIC models used to predict mercury (Hg) concentrations in Yellow Perch from Upper and Lower Red Lake.

Yellow Perch			
Function	AIC	AIC	R ²
Hg~TL.mm*Sex*Season*Basin	-1604.4	0.0	0.40
Hg~TL.mm*Season*Basin	-1580.5	-23.9	0.32
Hg~TL.mm+Basin	-1576.4	-28.0	0.27
Hg~TL.mm+Sex+Basin	-1575.8	-28.6	0.27
Hg~TL.mm*Basin	-1575.0	-29.4	0.27
Hg~TL.mm*Sex*Basin	-1574.4	-30.0	0.29
Hg~TL.mm+Season+Basin	-1574.2	-30.2	0.27
Hg~TL.mm+Sex*Basin	-1574.1	-30.3	0.27
Hg~TL.mm+Sex+Season+Basin	-1573.5	-30.9	0.27
Hg~TL.mm*Season*Sex	-1559.5	-44.9	0.27
Hg~TL.mm*Season	-1550.0	-54.3	0.22
Hg~TL.mm	-1543.6	-60.8	0.18
Hg~TL.mm+Weight.g	-1542.3	-62.1	0.18
Hg~TL.mm+Sex	-1541.8	-62.6	0.18
Hg~TL.mm*Sex	-1541.4	-63.0	0.19
Hg~TL.mm+Season	-1541.0	-63.4	0.19
Hg~Weight.g	-1540.2	-64.2	0.17
Hg~Basin	-1486.6	-117.8	0.02
Hg~Sex	-1484.9	-119.5	0.02
Hg~Season	-1477.1	-127.3	0.00

Table 4. Results of permutational multivariate ANOVA tests (A) and permutation tests of multivariate dispersion (B) in Walleye and Yellow Perch diets by numeric count. Significant results ($P < 0.05$) are indicated by an asterisk (*). Walleye and Yellow Perch results ($p > 0.05$) of permutation tests of multivariate dispersion (B) indicate that significant permutational multivariate ANOVA (A) results were driven by differences in overall diets rather than differences in multivariate dispersions.

(A) Response variable	Predictor variable (URL vs LRL)	df	<i>F</i>-statistic	<i>P</i>-value
Walleye diets	Spring	1	3.180	0.008*
	Summer	1	21.94	0.001*
	Fall	1	0.597	0.591
Yellow Perch diets	Spring	1	3.557	0.085
	Summer	1	5.538	0.001*
	Fall	1	22.28	0.001*
(B) Response variable	Predictor variable (URL vs LRL)	df	<i>F</i>-statistic	<i>P</i>-value
Walleye diets	Spring	1	0.743	0.337
	Summer	1	14.98	0.001*
Yellow Perch Diets	Summer	1	9e-04	0.970
	Fall	1	0.218	0.662

Table 5. Results of permutational multivariate ANOVA tests (A) and permutation tests of multivariate dispersion (B) in Walleye and Yellow Perch diets by biomass. Significant results ($P < 0.05$) are indicated by an asterisk (*). Walleye and Yellow Perch results ($p > 0.05$) of permutation tests of multivariate dispersion (B) indicate that significant permutational multivariate ANOVA (A) results were driven by differences in overall diets rather than differences in multivariate dispersions.

(A) Response variable	Predictor variable (URL vs LRL)	df	<i>F</i>-statistic	<i>P</i>-value
Walleye diets	Spring	1	2.043	0.065
	Summer	1	18.99	0.001*
	Fall	1	1.733	0.138
Yellow Perch diets	Spring	1	3.985	0.077
	Summer	1	3.462	0.001*
	Fall	1	30.76	0.001*
(B) Response variable	Predictor variable (URL vs LRL)	df	<i>F</i>-statistic	<i>P</i>-value
Walleye Diets	Summer	1	11.53	0.005*
Yellow Perch Diets	Summer	1	0.953	0.326
	Fall	1	0.665	0.450

Table 6. Seasonal breakdown of Walleye diet index of relative importance (IRI) percentages by prey category in Upper Red Lake (URL) and Lower Red Lake (LRL), with associated confidence intervals.

Species	Spring URL	Summer URL	Fall URL	Spring LRL	Summer LRL	Fall LRL
AMPH				2.44 (-2.92, 4.59)		
ANIS				0.94 (-1.77, 1.78)		
BIVA					0.004 (-0.03, 0.01)	
BUB					0.01 (-0.09, 0.02)	
CHIRO				0.23 (-1.06, 0.47)		
DAR				0.02 (-0.16, 0.03)		
DECA						
FRD		1.14 (-1.91, 2.21)			26.2 (10.0, 39.1)	1.18 (-12.8, 2.36)
GASTRO						
HIRU						
NOP		0.04 (-0.42, 0.08)				
NOTO				0.004 (-0.03, 0.01)		
SHI	81.5 (66.7, 134)	0.89 (-2.84, 1.78)	45.8 (18.3, 71.1)	92.4 (87.7, 104)	1.01 (-2.14, 1.97)	23.5 (-31.4, 46.2)
SUN		0.03 (-0.32, 0.06)	0.35 (-1.59, 0.70)			
TRICO					67.7 (55.4, 84.1)	
TRP	11.5 (-32.1, 22.8)	0.27 (-1.39, 0.54)		0.25 (-2.26, 0.50)		
UNKF	6.29 (-23.3, 11.9)	0.72 (-3.48, 1.44)	38.8 (11.7, 62.7)	0.73 (-1.10, 1.33)	2.11 (-3.91, 3.93)	72.9 (48.1, 131)
UNKI				0.004 (-0.04, 0.01)		
WAE	0.23 (-3.31, 0.46)					
YEP	0.52 (-4.10, 1.04)	96.9 (95.1, 103)	15.1 (-7.36, 27.5)	1.54 (-3.95, 3.05)	2.99 (-4.91, 5.88)	2.38 (-22.5, 4.77)
ZYGO	0.04 (-0.87, 0.08)			1.47 (-1.22, 2.61)		

Table 7. Seasonal breakdown of Yellow Perch diet index of relative importance (IRI) percentages by prey category in Upper Red Lake (URL) and Lower Red Lake (LRL) with associated confidence intervals.

Species	Spring URL	Summer URL	Fall URL	Spring LRL	Summer LRL	Fall LRL
AMPH	0.07 (-0.37, 0.15)	16.6 (-10.5, 33.3)	97.4 (95.5, 105)		3.68 (-8.85, 7.25)	
ANIS						
BIVA					1.11 (-1.28, 2.09)	0.40 (-1.70, 0.80)
BUB					0.46 (-3.24, 0.91)	
CHIRO	99.1 (98.2, 111)		0.98 (-2.44, 1.86)	33.3(-33.3, 66.7)	1.37 (-2.05, 2.67)	0.32 (-2.02, 0.64)
DAR		3.73 (-11.2, 7.47)				
DECA					0.33 (-2.50, 0.65)	
FRD		4.01 (-23.5, 9.02)				
GASTRO						
HIRU					0.11 (-0.53, 0.22)	96.6 (93.4, 112)
NOP					2.46 (-8.29, 4.93)	
NOTO		4.95 (-2.75, 9.58)			0.20 (-1.26, 0.40)	
SHI		2.22 (-13.4, 4.44)	0.84 (-3.37, 1.68)			
SUN			0.39 (-3.11, 0.78)			
TRICO	0.15 (-0.73, 0.29)	1.49 (-3.07, 2.97)			62.7 (41.0, 102)	2.67 (-11.6, 5.34)
TRP		1.19 (-8.46, 2.40)	0.42 (-2.24, 0.85)		0.88 (-6.64, 1.75)	
UNKF	0.69 (--13.4, 1.38)	14.7 (-23.0, 28.3)			1.99 (-3.12, 3.90)	
UNKI		0.12 (-1.75, 0.25)				
WAE						
YEP		50.5 (20.6, 92.0)			0.75 (-2.41, 1.49)	
ZYGO				66.7 (33.3, 133)	23.9 (-8.59, 45.4)	

Table 8. Sample size (n), standard deviation (sd), mean, test type, W or T statistic and *P*-value if present for each prey item that was collected for mercury concentrations (Hg.ppm) between Upper (URL) and Lower Red Lake (LRL). Prey abbreviations are defined in Table 1. Significant results ($P < 0.05$) are indicated by an asterisk (*).

Prey Item	URL (n)	LRL (n)	URL (sd)	LRL (sd)	Mean URL	Mean LRL	Test Type	<i>W</i> -statistic	<i>T</i> -statistic	<i>P</i> -value
AMPH	5	12	0.0077	0.0035	0.0122	0.0097	t-test	N/A	-0.7153	$p > 0.05$
ANIS	6	3	0.0126	0.0008	0.0311	0.0173	t-test	0	N/A	$p < 0.05^*$
BIVA	1	0	N/A	N/A	0.0044	N/A	N/A	N/A	N/A	N/A
BUB	1	0	N/A	N/A	0.0072	N/A	N/A	N/A	N/A	N/A
CHIRO	3	2	0.0009	0.0008	0.0090	0.0021	t-test	N/A	0	$p > 0.05$
GASTRO	10	5	0.0052	0.0017	0.0073	0.0055	Wilcoxon	21	N/A	$p > 0.05$
HIRU	2	2	0.0150	0.0055	0.0181	0.0148	Wilcoxon	2	N/A	$p > 0.05$
NOTO	0	1	N/A	N/A	N/A	0.0307	N/A	N/A	N/A	N/A
SHI	88	81	0.0166	0.0066	0.0316	0.0168	Wilcoxon	881	N/A	$p < 0.05^*$
SUN	0	2	N/A	0.0081	N/A	0.0343	N/A	N/A	N/A	N/A
TRICO	0	4	N/A	0.0028	N/A	0.0072	N/A	N/A	N/A	N/A
TRP	12	15	0.0086	0.0031	0.0206	0.0145	t-test	N/A	-2.3508	$p < 0.05^*$
WAE	70	8	0.0194	0.0026	0.0427	0.0124	Wilcoxon	31	N/A	$p < 0.05^*$
YEP	40	8	0.0194	0.0057	0.0468	0.0103	Wilcoxon	14	N/A	$p < 0.05^*$
ZYGO	2	6	0.0045	0.0060	0.0133	0.0129	Wilcoxon	5	N/A	$p > 0.05$

FIGURES

Figure 1: Map of study area, located in northcentral Minnesota and within the ancestral land of Red Lake Nation and ceded territories. Red border represents Red Lake Nation boundaries. Red circles represent Walleye and Yellow Perch gill net sample locations and black circles represent prey sample locations.

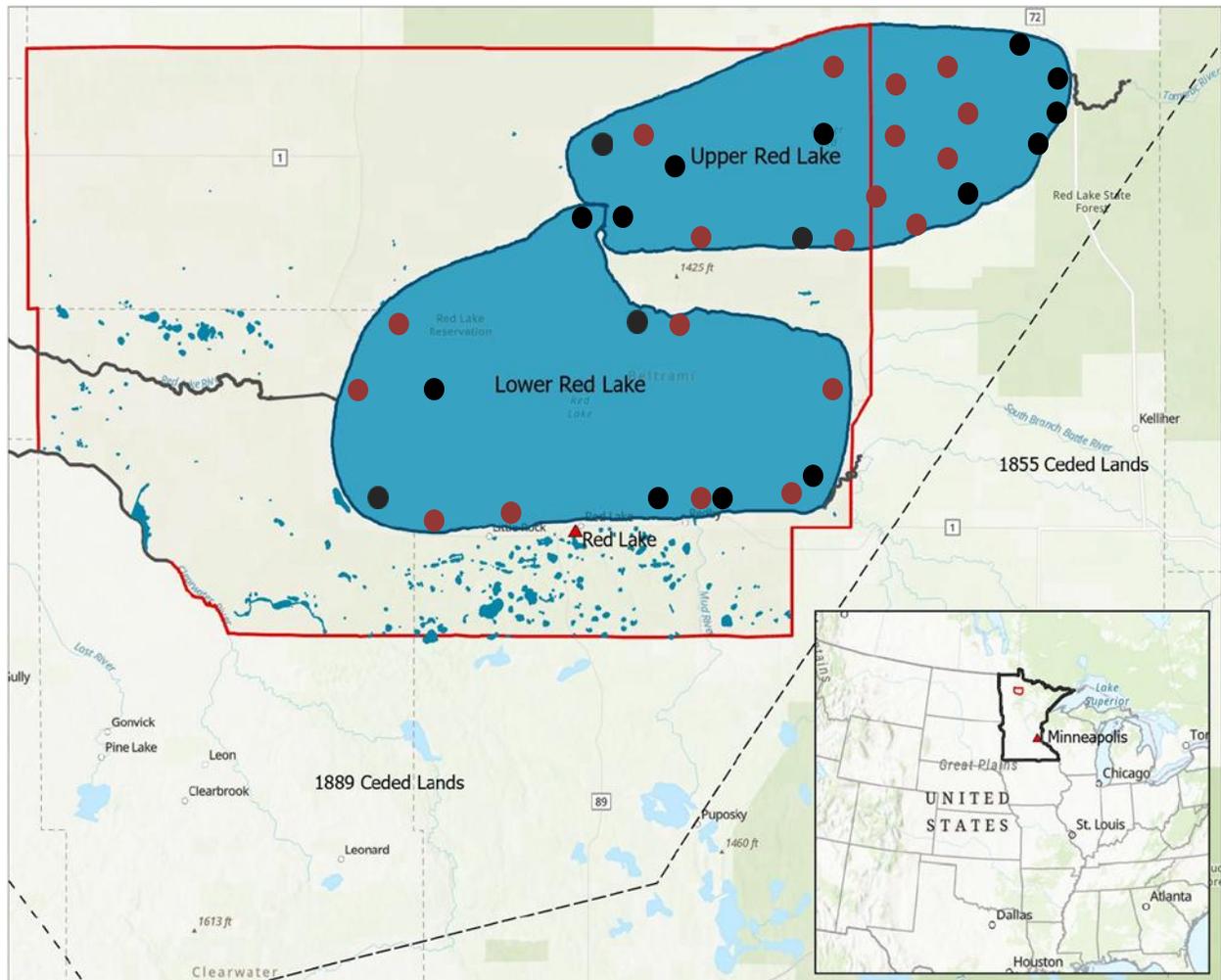


Figure 2. Mercury concentrations (Hg (mg/kg)) by length (mm) as a response to sex and season in Walleye from Upper Red Lake (URL; pink) and Lower Red Lake (LRL; black) collected by Red Lake DNR, Red Lake Commercial Fisheries and MN DNR during 2024 and 2025. The square shape represents male Walleye and the circle shape represents female. Green line represents 2-3 servings/week, blue line represents 1-2 servings/week, below the pink line represents 1 serving/week and concentrations within the shaded red area recommend 1 meal/month or avoid completely.

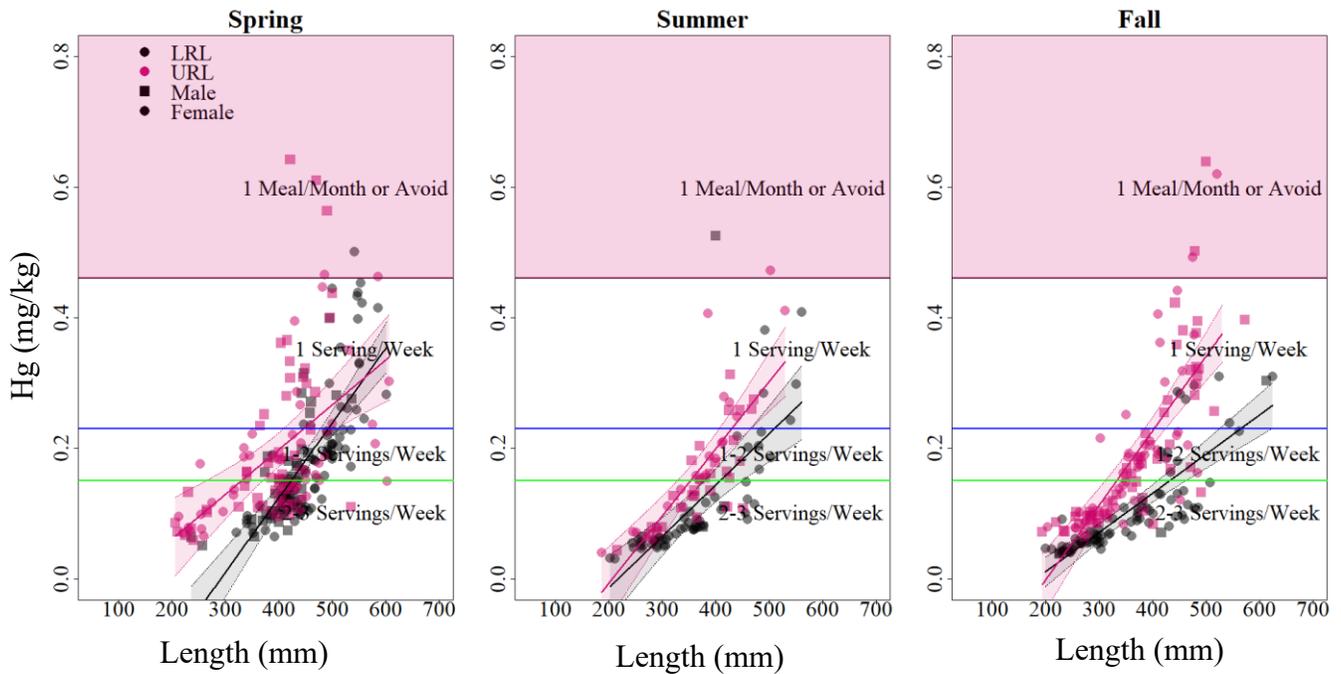


Figure 3. Mercury concentrations (Hg (mg/kg)) by length (mm) as a response to sex and season in Yellow Perch from Upper Red Lake (URL; pink) and Lower Red Lake (LRL; black) collected by Red Lake DNR, Red Lake Commercial Fisheries and MN DNR during 2024 and 2025. The square shape represents male Yellow Perch and the circle shape represents female. Below the green line represents 2-3 servings/week and above suggests 1-2 servings/week. Yellow Perch mercury concentrations are significantly lower compared to Walleye mercury concentrations.

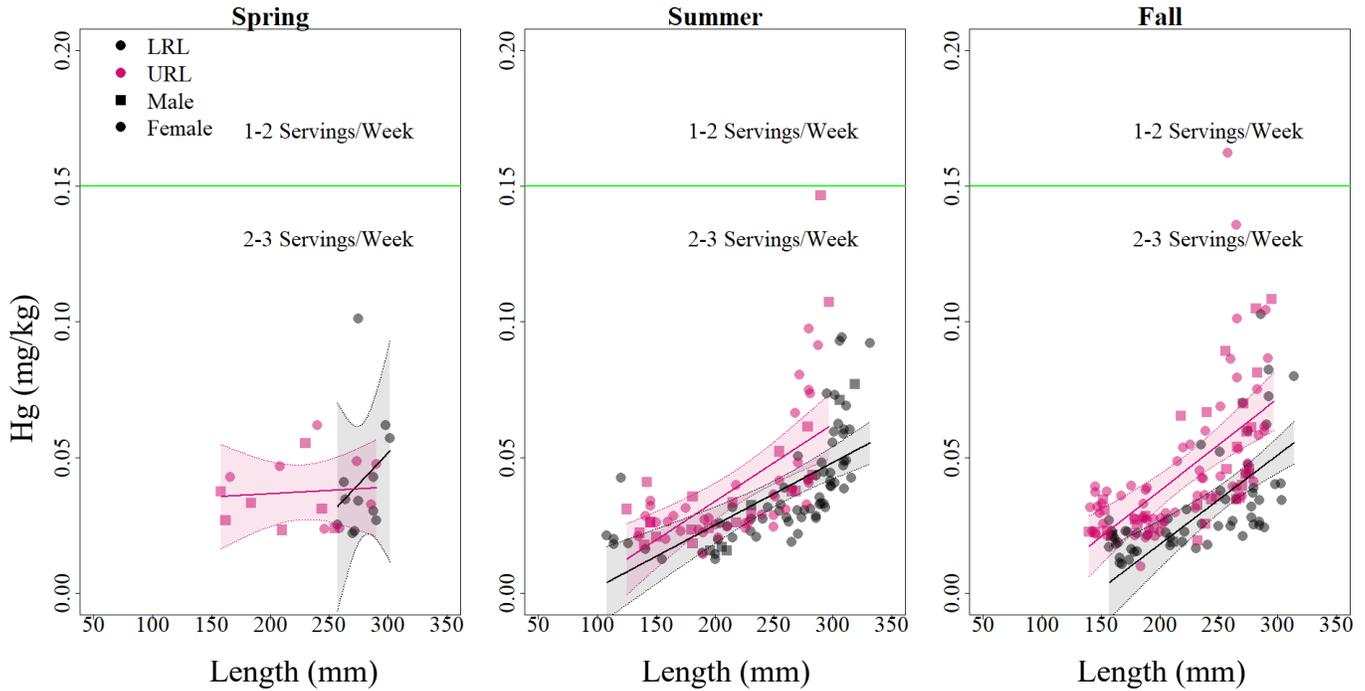


Figure 4. Nonmetric multidimensional scaling (NMDS) ordinations of Walleye diets in spring (green), summer (orange), and fall (blue) between Upper (URL) and Lower (LRL) Red Lake. Figures represent count (A) and biomass (B) of Walleye diet composition. Ellipses represent the 95% CIs centered on centroids of each species' overall diet in multivariate space, and prey categories are presented in gray text. Prey abbreviations are defined in Table 1.

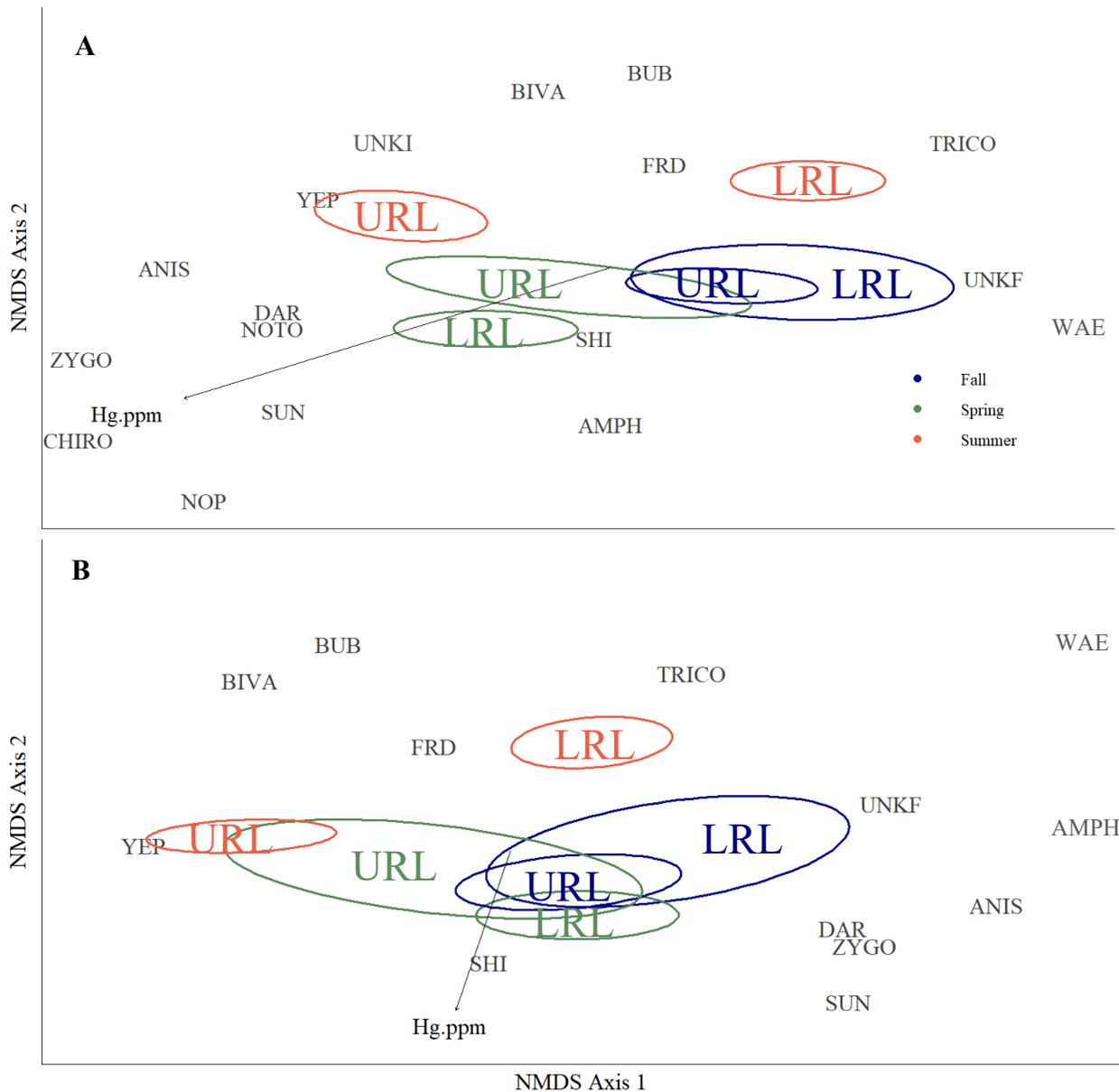


Figure 5. Nonmetric multidimensional scaling (NMDS) ordinations of Yellow Perch diets in spring (green), summer (orange), and fall (blue) between Upper (URL) and Lower (LRL) Red Lake. Figures represent count (A) and biomass (B) of Yellow Perch diet composition. Ellipses represent the 95% CIs centered on centroids of each species' overall diet in multivariate space, and prey categories are presented in gray text. Prey abbreviations are defined in Table 1.

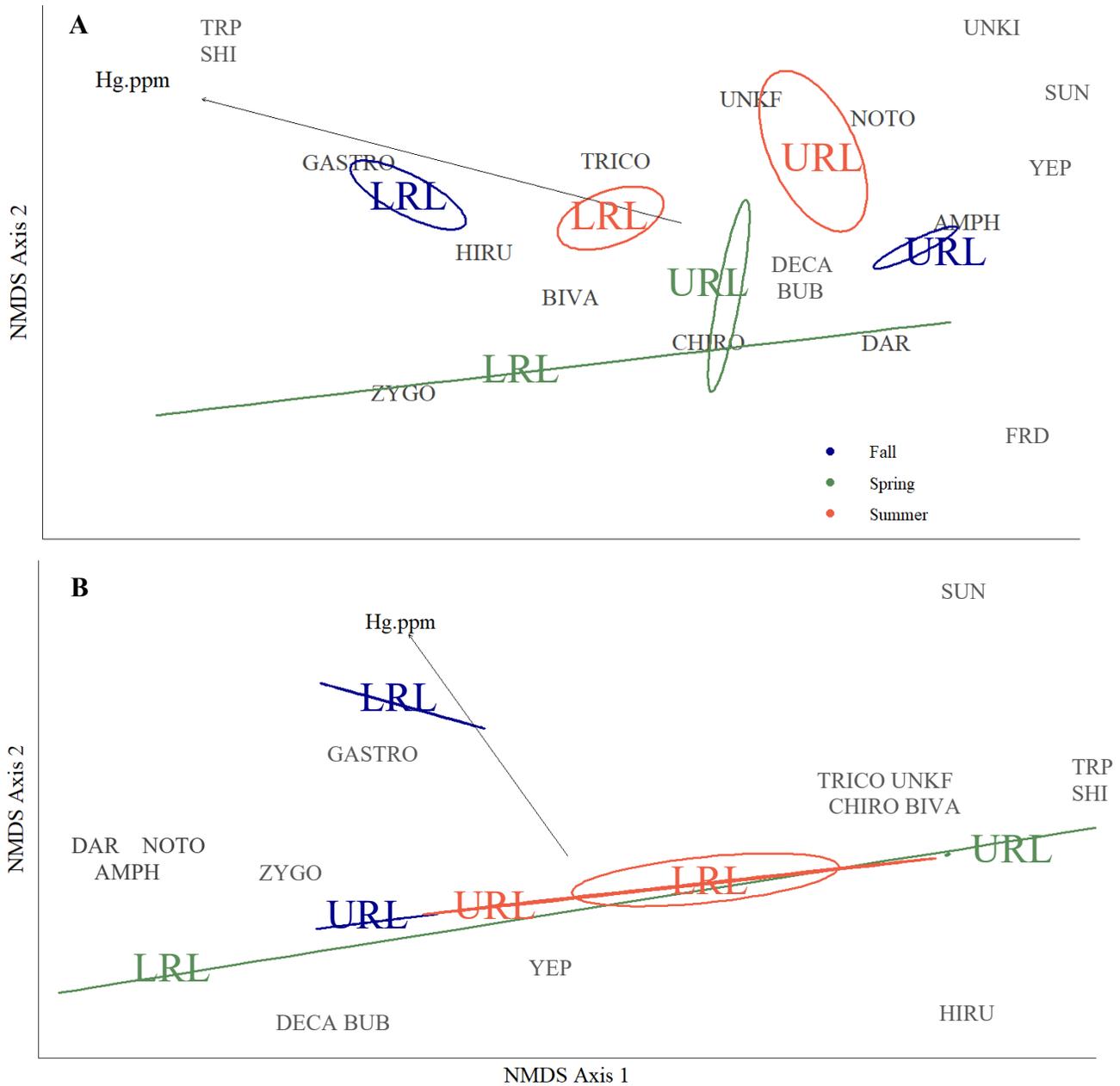


Figure 6. Percent index of relative importance (IRI) for prey categories in diets of Walleye in spring, summer and fall between Upper (URL) and Lower (LRL) Red Lake. Presented prey categories include everything that was present within the diets. Prey abbreviations are defined in Table 1.

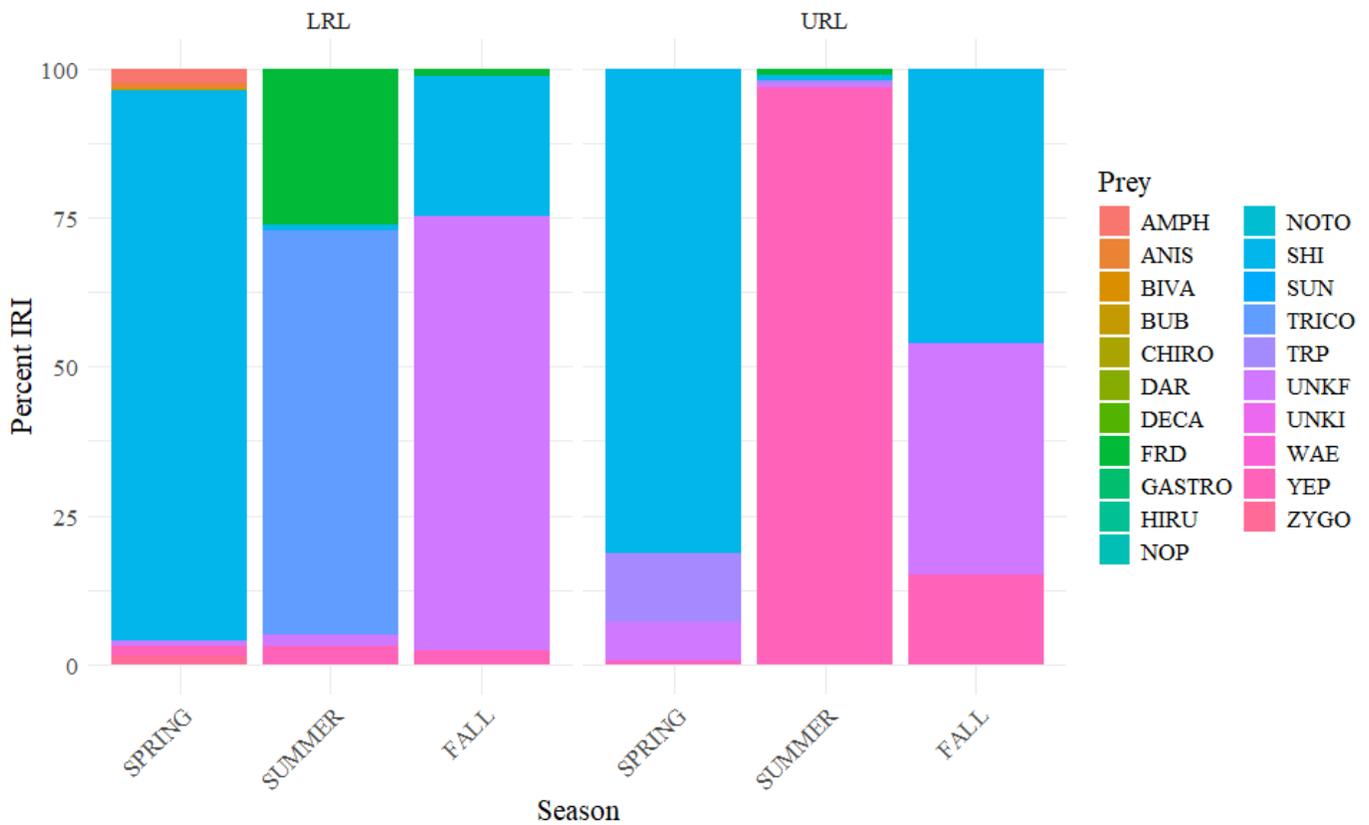


Figure 7. Percent index of relative importance (IRI) for prey categories in diets of Yellow Perch in spring, summer and fall between Upper (URL) and Lower (LRL) Red Lake. Presented prey categories include everything that was present within the diets. Prey abbreviations are defined in Table 1.

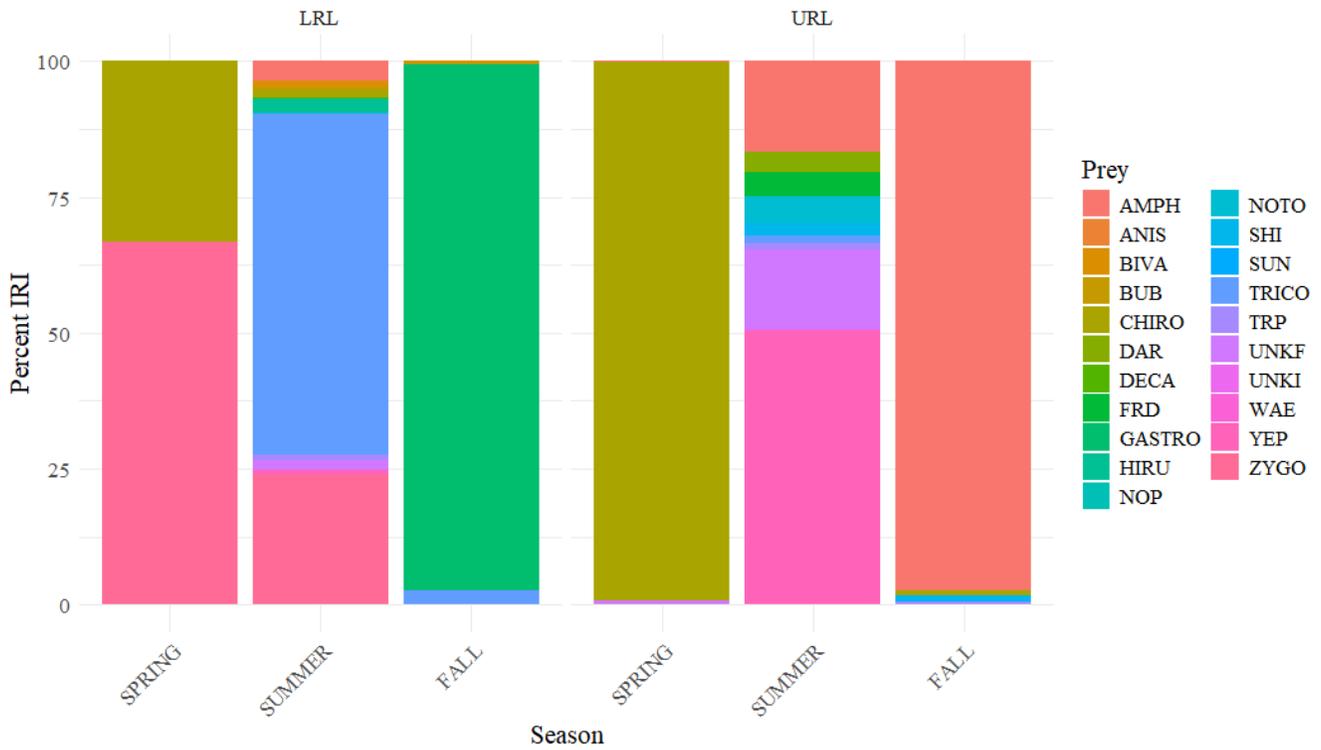


Figure 8. Mercury concentrations (Hg (mg/kg)) across various prey types in Upper (URL; black) and Lower (LRL; pink) Red Lake. Prey abbreviations are defined in Table 1.

