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Going deep: evaluating deep and shallow water drivers of mercury in Lake Champlain fish

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PROJECT SUMMARY

We completed a survey of mercury concentrations in five game fish species from Lake Champlain in 2022-2023, which found mercury levels in most species have declined since the previous survey in 2016-2017. Based on isotopic techniques, we found precipitation to be a less important source of mercury to the fish in the deep, pelagic waters of the lake than in Laurentian Great Lakes, inferring that watershed sources of mercury are important to bioaccumulation throughout the lake. Drivers of mercury concentrations in the water column, seston and zooplankton varied between deep and shallow basins, and with temperature and river influence. Concentrations of mercury in zooplankton in the hypolimnion increased throughout the summer, despite declining methylmercury concentrations in the water column over this time period. We established an ongoing working group with members from Quebec, New York, and Vermont to discuss fish consumption guidelines and communication strategies in the Lake Champlain region. Finally, an outreach plan was developed to communicate up-to-date information on mercury in fish and fish consumption advisories to Lake Champlain anglers.

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1. PROJECT INTRODUCTION

Concentrations of mercury (Hg) in sport fish in Lake Champlain frequently exceed the EPA criterion calculated to protect human health. Fish Hg surveys have been conducted on Lake Champlain since the early 1980's, with the most recent in 2016/17 (Swinton and Nierzwicki-bauer, 2018), and have assembled a valuable record of Hg concentrations in fish species commonly caught by recreational fishers. Data from these surveys inform state environmental and health agencies tasked with establishing fish consumption advisories, which provide guidance on the numbers of fish meals that can be safely consumed. Fish surveys also record changes in Hg concentrations over time and across the lake basins, which serve as indicators of the effectiveness of Hg source control policies and of other environmental changes in the lake affecting Hg bioaccumulation in fish. In the past decade, regional atmospheric inputs of Hg have decreased based on data from the Mercury Deposition Network site in Underhill, VT (NADP, 2016). This would predict that Hg concentrations in fish would also decline throughout the lake. However, in the most recent study of Hg in Lake Champlain, this was not the case since there were differences in Hg concentrations in recreationally caught fish species between basins of the lake, as well as differences between basins in the temporal trends for fish Hg (Swinton and Nierzwicki-Bauer, 2019). Not surprisingly, higher concentrations of Hg were found in top trophic level species that inhabit the cold, deep waters of the lake (eg. walleye, lake trout) (Swinton and Nierzwicki-bauer, 2018). However, yellow perch and smallmouth bass, which are found throughout the lake, had higher Hg concentrations in deep basins of the Main Lake Extension (**see Fig. 1** - North Main, Main and South Main Lake) than the eastern basins (shallow Missisquoi Bay and Northeast Arm, and deeper Malletts Bay). Moreover, Hg concentrations in these fish had increased compared to previous years, whereas in other basins, they remained the same or decreased over time (Swinton and Nierzwicki-Bauer, 2019). Since atmospheric inputs of Hg have declined, this suggests climatic and ecological factors, such as changes in temperature, oxygen depletion, tributary discharge, and food web assemblage are indirectly caused increases in fish Hg in the Main Lake Extension. In alignment with Lake Champlain Basin Program (LCBP) priorities both for research on contaminants in the lake, and replication of previous assessments of mercury concentrations in fish tissues, we repeated the fish Hg survey using comparable sample sizes, fish species and protocols as the previous assessment. Fish tissue samples were collected opportunistically for this survey through collaboration with Vermont Fish and Wildlife, the LCBP Boat Launch Steward program, and Lake Champlain International. Data from the surveys was shared with Vermont Department of Health. Fish mercury levels were analyzed for differences across basins, and for temporal trends compared to past surveys.

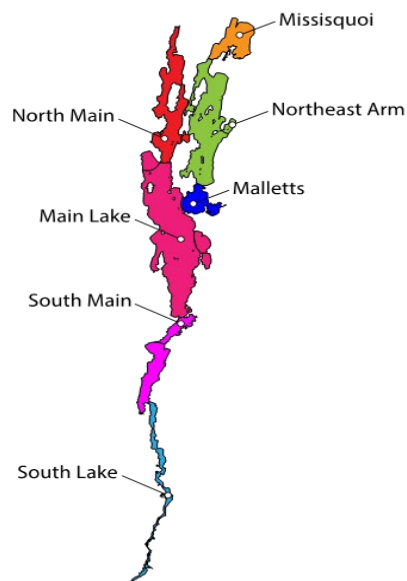


Fig. 1 Lake Champlain Basins

In aquatic environments, Hg, when converted to its toxic and bioavailable form, methylmercury (MeHg), bioaccumulates from the water column to phytoplankton at the base of the food web, and biomagnifies up the food chain reaching high concentrations in top trophic species (Driscoll et al., 2007). The primary source of MeHg can vary across regions of the lake, as do the factors controlling bioaccumulation through the food web. In this work, we evaluated mercury stable isotopes as well as carbon and nitrogen stable isotopes in a subset of fish tissue

from the survey, to assess the major sources of mercury and pathways of mercury uptake in top trophic level game fish. We also conducted a study of the levels of mercury in the water column and in zooplankton over three time points from six basins of the lake (Malletts, Missisquoi Bay, St. Alban's Bay (in the Northeast Arm), and three segments of the Main Lake (South, Main and North). We combined our data with ancillary parameters and water quality variables monitored by the Lake Champlain Long-term Monitoring Program, to determine the response of the deep and shallow basins to environmental stressors and long-term weather change (Isles et al., 2017). This work responded to LCBP's Goal 1 Opportunity for Action management plan, to perform ongoing monitoring and targeted research to support clean waters and identify the levels of toxic contaminants in sport fish.

In addition to our research activities, we collaborated with regional stakeholders and local anglers and communicated the risks and benefits of consuming fish from Lake Champlain to potentially at-risk communities. These outreach activities included forming a working group of government stakeholders and researchers from Vermont, New York and Quebec to discuss the differences in fish consumption advice between states/provinces, share new surveys and data, and compare strategies for communicating safe eating guidelines to anglers and the public. We have also communicated with community leaders including the Chiefs of two Abenaki tribes and the Burlington Cultural Broker's group, about fish consumption practices and awareness of safe eating guidelines among different communities. These outreach activities will also fulfill Goal 4 by informing the public of up-to-date, local fish consumption guidelines.

2. TASKS COMPLETED

Task 1: Form a Project Advisory Committee (PAC). Our PAC was led by Jamie Shanley, a colleague and regional Hg expert, who is part of the LCBP Technical Advisory Committee. Our PAC also includes Anna Gallagher (Vermont Department of Environmental Conservation (VTDEC)), Sarah Hobson (VT DEC), Margaret Murphy (Vermont Fish and Wildlife Department (VTFWD)), Pam Wadman (Vermont Department of Health (VTDOH)), Meg Modley (LCBP), Sarah Janssen (US Geological Survey (USGS)), Kate Crawford (Middlebury College). We met with the PAC three times over the course of the project and discussed the progress and received feedback.

Task 2: Develop a QAPP. The QAPP was approved and provides details of a Quality Assurance/Quality Control plan for sample collection, chemical analyses, and data storage, as well as data availability upon completion of the study.

Task 3: Regional stakeholder meeting. We have hosted three online meetings (Dec 2, 2022; March 25, 2025 and May 5, 2025) attended by regional government stakeholders from VT, NY and QC (QC did not attend the first meeting). At these meetings we discussed i) the needs of the state/provincial agencies for setting fish consumption advisories, and which survey data support the agencies' needs; ii) current differences in fish advisories for the lake between different states and provinces, and how they may be unified; iii) needs and means for making fish advisories more accessible (eg. translate to other languages, angler surveys to understand demographics and current practices). These meetings were attended by DEC, VTDOH and VTFWD from VT and NY, and the Ministries of Environment and Health from QC.

Task 4: Outreach Plan. We explored different avenues of communication for determining the needs of anglers to make informed decisions about fish consumption. We engaged with anglers fishing by boat through collaboration with the LCBP Boat Launch Stewards program. During

initial training for the Boat Launch Stewards program, the Project PI gave a presentation to the Boat Launch Stewards about Hg in fish and how to collect samples. The Stewards communicated with anglers about Hg in the lake and provided information via rack card. To reach a broad community of anglers on the lake, information on the survey was posted on local fishing forums (eg. IceShanty.com, 802-fishing Facebook page, www.lakechamplainunited.com/forums, Trout Unlimited), and rack cards with links and QR codes were distributed at fishing derbies, marinas and bait shops.

One change in our Outreach Plan has been that the FishApp, which was intended to be completed through matching funds from VT DEC, was never brought to fruition due to delays in release of VT's safe eating guidelines and the software for the app becoming obsolete. We instead produced printouts of the new safe eating guidelines, which were posted in grocery stores around the state.

Over the course of the grant, we communicated bi-directionally with Chief Don Stevens of the Nulhegan Band of the Coosuk Abenaki and Chief Joanne Crawford of the Missisquoi Band of Abenaki, and learned about fishing practices and shared information on Hg in fish. In addition, and in close communication with the LCBP staff, we engaged with organizational leaders of multiple groups through the Burlington Cultural Broker's group to identify communities who are consuming fish from the lake. We also learned about these groups through a complementary research effort supported by Lake Champlain Sea Grant. Research was led by Dr. Bindu Panikkar and Dr. Ari Chiapella to support an undergraduate honors thesis by Eli Stein (Stein 2023) and a master's thesis by Saurav Lamichhane (Lamichhane 2024). These researchers held focus groups with Arabic, Nepali and Somali community members to better understand their fish-eating habits and knowledge of safe fish consumption guidelines. They identified a gap in knowledge of these new American communities about safe fish consumption guidelines and strong evidence that these groups commonly fished for subsistence.

These results informed an outreach plan developed through this project. This plan describes the setting and situation as related to fish consumption by anglers in Lake Champlain, and suggests ways to build upon engagement pathways established by this project. Further, the outreach plan includes recommendations based upon community needs identified through this study and the complementary studies (e.g., need for translation of fish consumption advisories into multiple languages, delivery of information in a manner that reaches these communities where they gather/frequent). The outreach plan also describes data gaps (e.g., species for which we need Hg data).

Tasks 5 and 6: Fish collection and analysis. These tasks are for the same activities and data, but were split over two years (Task 5: 2022, Task 6: 2023). Samples of sport fish tissue were collected opportunistically across all seven segments of the lake (South Lake, South Main Lake, Main Lake, North Main Lake, Mallets Bay, Northeast Arm, Missisquoi Bay) using comparable protocols to previous studies (fishing derbies, local anglers, collaboration with other research and monitoring efforts)(Swinton and Nierzwicki-bauer, 2018). A total of 372 samples were collected and analyzed; the number of fish per species/basin are shown in Table 5.1 below.

Table 5.1 - Number of fish from each species and basin included in the study.

	Missisquoi	Northeast Arm	North Main	Malletts	Main	South Main	South
Lake Trout			6		22	12	
Smallmouth Bass	17	43	22	10	15	8	10
Walleye	2	2	14	13	13	6	2
White Perch	10	10	10	6	3	13	10
Yellow Perch	10	21	12	10	8	22	10

We note that sample numbers are low for walleye for some basins – this is in part because ice fishing was limited due to ice conditions both winters during the survey. Adult walleye overwinter in the tributaries then move to the deeper lake basins where they feed throughout the summer. For this reason, the spatial distribution of Hg concentrations across basins is not as meaningful for this species as it is for the smaller forage fish, which feed from a more localized area. We also note that smallmouth bass and walleye were sampled mostly from fishing derbies which focus on catching large fish. This resulted in samples predominantly from large fish, and also captured a large size distribution of fish. Because size has a strong influence on Hg concentration, the large size distribution introduces some uncertainty on the size-normalized estimate of Hg concentration..

Differences in fish Hg concentration by species, and trends in Hg concentration over time, compared with previous surveys on the lake were analyzed by Mixed Linear Models. Concentrations of Hg in Lake Champlain fish were also compared to fish from the Laurentian Great Lakes as a regional comparison. Results were reported to LCBP for inclusion in the 2024 State of the Lake Report, and to stakeholders at VT DOH, VT DEC, as well as through workshops with government stakeholders from QC and NY.

Task 7: Fish isotope analysis. A subset of fish tissues from the survey were submitted for Hg stable isotope ($\delta^{202}\text{Hg}$, $\Delta^{199}\text{Hg}$, $\Delta^{200}\text{Hg}$) and “conventional” stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) analyses. This study focused on the top predator fish, walleye ($n = 23$) and lake trout ($n = 15$), which provide information on the sources of Hg to the lake food webs, allowing for comparison with data from the Laurentian Great Lakes. Composites of yellow perch tissue ($n=10$ total) from each basin of the lake were also submitted for analysis. Isotope signatures of these forage fish provide insight into the processes governing Hg cycling lower in the food chain in different basins of the lake.

Task 8: Water column sampling and analysis. Water and zooplankton samples were collected from six basins (South Main Lake, Main Lake and North Main Lake, Malletts Bay, St. Albans Bay, Missisquoi Bay) and three tributaries (Lamoille River, Mill River and Missisquoi River) at 3 timepoints in the summer of 2023. Difficulties with boat access and the major storm event of July 2023 caused some variability in sampling time points between sites, particularly in July. All water samples were filtered and analyzed for MeHg and Hg in the dissolved and particulate phases. Ancillary data were also collected across the depth profile at each site by data sonde (chlorophyll, temperature, conductivity, turbidity, dissolved oxygen, CDOM). Plankton samples (>150 μm mesh size) were analyzed for MeHg, Hg, and $\delta^{13}\text{C}$ / $\delta^{15}\text{N}$; a subsample was also analyzed for taxonomy.

Task 9: Evaluate water column drivers of Hg bioaccumulation. Concentrations of MeHg and Hg in the water column and in zooplankton were compared across basins, and compared to different drivers, including water quality parameters (chlorophyll-a, CDOM, temperature and TSS). Plankton Hg concentrations were evaluated relative to coarse taxonomy, $\delta^{15}\text{N}$, and concentrations in the particulate to explain trends.

Task 10: Analyze changes in MeHg relative to environmental stressors. Concentrations of MeHg in the Lake Champlain food web and their response to environmental stressors (water temperature, storm events) were compared to predicted trends in stressors for the lake. These trends include longer periods of stratification, higher surface water temperatures, and more frequent and severe storm events. The potential impacts of invasive species on fish Hg concentrations were also considered.

3. METHODOLOGY

Tasks 5 and 6: Fish collection and analysis

Fish collection: Tissue samples were collected as muscle plugs from live fish at fishing derbies, and from smallmouth bass and walleye caught in gill nets during the VTFWD fish surveys, using a protocol approved by Institutional Animal Care and Control at Dartmouth. White and yellow perch and lake trout were collected from the VTFWD monitoring surveys. Fish were euthanized on the boat, stored in coolers during transport, and frozen at the lab at Dartmouth. Fish tissue samples were also collected from anglers by the LCBP Boat Launch Stewards. Fish were either donated whole, as sections of filets, or Boat Launch Stewards took muscle plugs from (dead) fish that anglers intend to keep. Samples were kept in coolers for the remainder of the day, then stored in the freezer at LCBP prior to returning to Dartmouth. Fish that were sampled whole were fileted at Dartmouth. Information forms were filled out with the length and weight of each fish, and transferred along with fish samples to Dartmouth College, where information was entered into a spreadsheet.

Fish tissue was transferred to labelled, pre-weighed 20 mL glass vials and a wet tissue weight was recorded. Samples were freeze dried, re-weighed, and homogenized in a Teflon ball mill. Dried tissue was stored at room temperature until analysis (Tasks 5, 6, and 7).

Hg analysis: Samples for total mercury in fish tissue were analyzed by Direct Mercury Analyzer (DMA) using U.S. EPA Method 7473 (U.S. EPA 2007). Approx ~ 30 mg of dried fish tissue was analyzed for each sample. Duplicate, blank and standard reference material samples were run every 10 samples. Analytical run reports were exported into excel on the instrument computer and stored and backed up with project data. Samples were analyzed as dry weight and concentrations converted to wet weight for reporting.

Data analysis: Data were first visualized using the ggplot2 package in R. Data from previous game fish surveys were obtained from LCBP. Fish length and log₁₀Hg concentrations were standardized for analyses. For years where data was scarce, multiple years were grouped. Trend analyses of trout and walleye Hg concentrations were analyzed by linear model, using the *lm* package in R. Models assigned scaled, log-transformed Hg concentrations as the dependent variable, year as a categorical independent variable, length as the co-variate, and an interaction term between length and year. Concentrations of Hg were back-transformed for visualization and interpretation. Mean Hg concentration for each year (or group of years) was assessed using

the *emmeans* package, and differences between means were evaluated by Sidak's test using the *multcomp* package.

For yellow perch, white perch, and smallmouth bass, Hg concentrations across years (or groups of years) were analyzed by mixed linear models using the *lme4* package in R. Mixed models account for dependencies within the data, in this case variability across locations (basins), giving a more accurate statistical assessment. Here scaled, log-transformed Hg concentrations were modeled with year and length as fixed effects, and basin as a random effect. Models were produced with random intercept and random slope effects and compared to one another by ANOVA (using the *ranova* test in *lme4*). Where no significant difference between models was observed, the simpler model (random intercept) was chosen. Mean Hg concentration for each year (or group of years) was assessed using the *emmeans* package, and differences between means were evaluated by Sidak's test using the *multcomp* package. As above, concentrations of Hg were back transformed for visualization and interpretation.

To better understand the trends in fish Hg across basins, a subset of data for smallmouth bass, yellow and white perch from 2011 to 2023 was selected, as earlier surveys had poor spatial distribution. Samples were analyzed by mixed linear models as above, with length, year and basin as fixed effects, and length/(year and basin) as random effects.

Concentrations of Hg in lake trout and walleye from Lake Champlain were compared with large surveys of these species from the Great Lakes (Lepak et al., 2018; Zananski et al., 2011). Fish in this study were caught opportunistically, and samples taken from fillet tissue. In a large survey of fish from the Great Lakes through the GLFMP program between 2004 and 2013, fish were collected by gill net, selecting for specific size distributions – 600 to 700 mm length for trout and 400 to 500 mm for walleye, which gave similar Hg concentrations between species (Zananski et al., 2011). More recent data are available, but currently Hg concentrations can only be accessed as dry weight Hg concentrations (Lepak et al., 2025). Fish from the Great Lakes studies were analyzed for whole body Hg concentrations. To compare with the Lake Champlain data, we estimated fillet Hg concentrations from whole Great Lakes fish Hg concentrations using the equation from (Peterson et al., 2005):

$$\text{Log}_{10}\text{Hg (whole body)} = -0.2710 + 0.9005 * \text{Log}_{10}\text{Hg (biopsy/fillet)}$$

Fillet Hg concentrations from Lake Champlain were then normalized to 650mm (trout) and 450mm (walleye) by prediction from the covariate models. Length-normalized concentrations were estimated for each individual fish by adding back the residuals from the model to the predicted value (Eagles-Smith and Ackerman, 2014).

Task 7: A subset of 50 dried, homogenous samples were sent to USGS for stable Hg isotope analysis and University of California (UC) Davis for $\delta^{13}\text{C}/\delta^{15}\text{N}$ analysis. Samples of walleye and lake trout were analyzed as individual fish, whereas yellow perch samples were composites of equal amounts of five fish each from the same basin. Samples for stable Hg isotope analysis were reported as $\delta^{202}\text{Hg}$, $\Delta^{199}\text{Hg}$, $\Delta^{200}\text{Hg}$ by standard protocols of notation (Blum and Bergquist, 2007). Samples for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis (~1 mg) were weighed to 0.01 mg in tin capsules, and shipped to the UC Davis Stable Isotope Facility.

Due to the variable influence of fish lipid concentration on $\delta^{13}\text{C}$ ratios across species, bulk $\delta^{13}\text{C}$ were corrected for lipid content ($\delta^{13}\text{C}_{\text{lipid_free}}$) using a mathematical model based on molar C:N ratio (Hoffman et al., 2015).

Data analysis: Mercury stable isotope ratios have been used to distinguish different sources of mercury and identify the processes driving Hg cycling in aquatic systems (Bergquist and Blum, 2007; Lepak et al., 2015b, 2018). Stable isotope ratios of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) provide information on fish foraging and habitat ($\delta^{13}\text{C}$), and on trophic positions ($\delta^{15}\text{N}$) (Peterson and Fry, 1987). Combining these isotopic measures in fish tissue provides interpretation of the Hg sources and food/energy pathways of Hg bioaccumulation (Janssen et al., 2021; Lepak et al., 2025, 2022).

Mercury isotope systematics are complex and are delineated through three different processes of fractionation: mass-dependent fractionation (MDF) is denoted by $\delta^{202}\text{Hg}$, and provides information on Hg sources (eg. terrestrial versus atmospheric) and reaction pathways (Demers et al., 2013; Lepak et al., 2015b). Hg is also susceptible to mass-independent fractionation (MIF), which affects odd mass isotopes, and is denoted as $\Delta^{199}\text{Hg}$. Odd-MIF is caused by photochemical processes due to sunlight exposure, and results in large positive shifts in $\Delta^{199}\text{Hg}$ in clear surface waters. Differences in the ratio of $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ have been shown to distinguish between photochemical reduction of inorganic Hg (~1.00) versus photodemethylation of MeHg (~1.36) (Bergquist and Blum, 2007; Das et al., 2009; Kwon et al., 2015). Photochemical reactions causing odd-MIF also impact MDF ratios, causing shifts in $\delta^{202}\text{Hg}$. A third type of fractionation is even-MIF, which though not fully understood is thought to be due to processes in the high tropopause, which are conserved during Hg deposition. Even-MIF is denoted as $\Delta^{200}\text{Hg}$, and is interpreted as Hg derived from precipitation (Kwon et al., 2020; Lepak et al., 2015b, 2018). Biomagnification of Hg through food webs conserves $\Delta^{199}\text{Hg}$ and $\Delta^{200}\text{Hg}$ ratios, and to a large extent $\delta^{202}\text{Hg}$ ratios; these Hg isotope values therefore represent MeHg taken up at the base of the food web. Isotope notations follow the convention described by Blum and Bergquist (Blum and Bergquist, 2007).

As with concentration data, isotope ratios across species and locations were visualized using *ggplot2* in R. Regressions between isotope ratios were analyzed by York regression, which finds the best-fit line when both the dependent and independent variables have measurement uncertainties (York et al., 2004). York regressions were analyzed using the package *isoplot* in R.

Task 8: Water samples were collected from three deep basins (South Main Lake, Main Lake and North Main Lake, adjacent to Lake Champlain Long-term Monitoring Program stations 9, 19, and 36) and three near shore basins (Malletts Bay, St. Albans Bay, Missisquoi Bay, adjacent to Lake Champlain Long-term Monitoring Program stations 25, 40, 51, and adjacent to VT EPSCoR/LCBP monitoring platforms). The Main Lake sites and Malletts Bay were sampled from the SUNY-Plattsburgh LCRI vessels, and Missisquoi and St. Albans Bays from the UVM Henley Shiner. At deep water stations, water samples were collected at 5 depths by trace metal clean go-flo bottles, which feature a close-open-close operation triggered by a messenger to avoid sample contamination and exchange of water from different depths. Each sample was split into duplicates on deck. Samples were collected in duplicate at 2 depths (near (~0.05 m) the sediment and near (~0.5 m) the surface) from St. Albans and Missisquoi Bay using acid washed tubing and a peristaltic pump. In addition, water from the tributaries feeding into the nearshore sites were collected in duplicate at each time point, near the USGS gauges / Lake Champlain Long-term Monitoring stations at Lamoille River, Mill River and Missisquoi River. Ancillary water quality parameters were measured by sonde across the depth profile at each site (chlorophyll, temperature, conductivity, turbidity, dissolved oxygen, CDOM).

Zooplankton tows (150 μm mesh size) were also conducted at each site and time point. Vertical tows were collected across the hypolimnion and epilimnion in the deep basins, and across the entire water column in the shallow basins. Tows were repeated until sufficient sample was collected; sample from tows were combined to obtain three discrete samples per site/compartments.

A subsample of zooplankton were treated with carbonated water and preserved in ethanol for taxonomy. The remainder were frozen, then freeze-dried. Net zooplankton density was calculated, samples were counted by major taxonomic group (eg. cladocera, copepoda, rotifera). Plankton were analyzed for Hg, MeHg and $\delta^{13}\text{C}/\delta^{15}\text{N}$ to determine trophic level and food sources at the base of the foodweb across basins. All data is summarized in Appendix 8.1, and in the deliverables section below.

Coarse taxonomy of the plankton samples were determined for the hypolimnion and epilimnion of each basin across the sampling time points. The relative amount of phytoplankton to zooplankton was also determined by microscopy. Briefly, a 1 mL aliquot of zooplankton slurry was transferred to a Sedgewick-Rafter counting cell. For zooplankton, mean cell area was estimated from the formula for an oval for each cell. For phytoplankton, the average area of 10 phytoplankton per sample was estimated; all cells within one grid were counted and multiplied by the average area. The % zooplankton relative to the total seston by area was reported.

4. QUALITY ASSURANCE TASKS COMPLETED

Scientific fish licenses were obtained from Vermont and New York for 2022-23. Training of Boat Launch Stewards for fish collection was conducted by the Outreach Manager and the Project Manager, through both an on-line introductory session and an in-person hands-on training.

Clean sample and specimen handling were reviewed with staff and volunteers helping with fish collection. Protocols for live fish collection or handling by project staff were approved by Dartmouth's Institution for Animal Care and Use Committee (IACUC). All staff working in Dartmouth Laboratories for this project took required Bioraft Laboratory Safety Training through Dartmouth's Environmental Health and Safety office.

Task 5-6: Quality control measures for mercury analysis in game fish tissue were submitted with the finalized data. All quality control metrics were met.

Task 7: Quality control measures for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope analysis performed at UC Davis, and for Hg isotope analysis performed at the USGS Mercury Isotope Laboratory were reported with the data.

Task 8: Quality control measures for mercury speciation in water, particles, and plankton samples were submitted along with the data.

5. DELIVERABLES SUBMITTED

Task 1: Our Project Advisory Committee met annually, and includes government stakeholders and researchers from the Lake Champlain region, as well as national experts in mercury science. Our last meeting will be prior to acceptance of the Final Report.

Task 2: The project QAPP was approved on June 15, 2022.

Task 3: The document “Summary of Meetings with State and Provincial Agencies related to Safe Fish Consumption Guidelines” was delivered August 5, 2025.

Over the project period, we held three meetings with State and Provincial staff who have responsibility to set safe fish consumption guidelines for Lake Champlain waters in New York, Quebec, and Vermont. The first meeting was held in December 2022 and the following two were held in March and May 2025, respectively.

The meetings were attended by representatives from the three governments. This included staff representing the New York State Departments of Environmental Conservation and Health, the Vermont Department of Health, and the Quebec Ministry of the Environment. In addition to project team members from the University of Vermont (UVM) and Dartmouth, other participants represented the Lake Champlain Basin Program and UVM. Between 10 and 13 people attended each meeting.

The first meeting provided an opportunity to share our research goals, information about historic mercury testing in Lake Champlain, and processes to collect data in the present study. The second meeting allowed us to share study results as well as to learn about and share with attendees results of complementary studies. All three meetings allowed each government agency to share current safe fish consumption guidelines and processes for data collection and decision-making about their guidelines, which do not align with one another. The meetings were intended to open a dialogue among these scientists and decision makers to promote possible future collaborations that may allow for better alignment of safe fish consumption guidelines in the future.

At the 2022 meeting, Pam Wadman with the Vermont Department of Health presented about their ongoing efforts to update Vermont’s fish consumption guidelines that had not been updated since 2014. She presented that while Vermont’s guidelines would be more similar to New Yorks, they would not align exactly as Vermont based guidelines on average weights of children (23 kg) and adults (70 kg). For instance, New York has a 0-meal guideline for some fish for sensitive populations such as pregnant people and small children, while Vermont anticipated not having any guidelines that would recommend these sensitive populations eat no fish meals per month. Wayne Richter with the NYS Department of Environmental Conservation shared information about New York’s ongoing mercury testing (which began in 1970), though with limited data from Lake Champlain. Meg Modley with the Lake Champlain Basin Program offered to assist Wayne in getting fish samples to him for analysis to supplement those he had. Henry Spliethoff with the New York State Department of Health reported that New York had updated guidelines in 2021 (www.health.ny.gov/fish/background.htm), which were anticipated to be updated again in 2024. These were based on parts per million of contaminants of concern. Sensitive populations were women under age 50 and children under age 15. The meeting participants were open to meeting again in the future and thought there may be possibility to align ages of sensitive populations better (Vermont uses age 6; New York age 15 and EPA age 11). There was also discussion of PFAS data being collected and the impact that may have on safe fish consumption guidelines.

At the 2025 meetings, participants learned about the research outcomes of a Lake Champlain Sea Grant-funded social science effort led by Dr. Ari Chiapella and Dr. Bindu Panikkar with students Eli Stein and Saurav Lamichhane. These researchers carried out surveys of Lake Champlain anglers, and held focus groups with Arabic, Nepali, and Somali communities. They

found evidence of subsistence fishing by these communities and lack of awareness of safe fish consumption guidelines. They assigned lowest risk to the Arabic community due to limited fishing time/success, a moderate risk to Nepali community members who fished more often and had more dependence on fish as a food resource, and a high risk for Somali community members who ate fish 3-4 times per week and ate everything except fish intestines. WhatsApp chat groups among community members, integration of safe fish consumption guidelines into workshops led by Lake Champlain Sea Grant, Lake Champlain Basin Program and Vermont Fish and Wildlife, and outreach through youth in schools were recommended means of improving these communities' knowledge of safe fish consumption guidelines. Following this presentation, Vivien Taylor shared findings of the most recent mercury in fish sampling carried out through the current study. Participants learned that collection methods biased larger fish sizes due to collaborations with fishing derby participants to collect muscle samples. Results suggested that mercury sources in fishes caught in Lake Champlain had a great influence of watershed sources of mercury than atmospheric sources deposited to the lake in precipitation. Vivien shared that, in general over the past 15 years, that mercury levels in Lake Champlain fish seemed to increase and decrease at varied times and mercury levels are not tracking as much as previously with atmospheric inputs as in the past.

State and Provincial agency staff also shared updates. Pam Wadman shared Vermont's recently released fish meal mercury calculator and Eat Fish, Choose Wisely card (both available at: <https://www.healthvermont.gov/environment/recreational-water/mercury-fish>). Audrey Vangenechten and Mark Swinton from New York State shared how New York's guidelines are set using a risk management approach (see: www.health.ny.gov/fish/background.htm). New York's guidelines take into account not only mercury but also PCBs, dioxins, chlordane and other chemicals, which is why their sensitive age group is older than Vermont's. Regional advisories have been developed in the past few years, with a statewide advisory for mercury added in 2024 building upon a regional Adirondacks (and also Catskills) advisory from 2022-23. There are do not eat recommendations for northern pike and walleye for sensitive populations. There are also new restrictions on bluegill and rock bass coming in Cumberland Bay due to PFOS. These will also set a do not eat recommendation for both fish species. There is an active Hudson River fish advisory project with regional brochures and color-coded maps available in many languages that were tested with the public in 2023 (see: www.health.ny.gov/fish/maps.htm). Denis Laliberté shared an overview of how fish consumption guidelines were determined in Quebec. Samples have been collected since 1978 at 300 sites (40-70 sites/year). They are analyzed for mercury, PCBs, and other organic chemicals. However, Quebec fishing guidelines were primarily based upon mercury samples. They also define a general population and a sensitive population and recommend people eat smaller fish such as yellow perch. They divide fish into three size classes for consumption guidelines. They will be sampling in Missisquoi Bay this year. They have the Quebec Fishing Guide available to share consumption guidelines (<https://www.quebec.ca/en/health/nutrition/healthy-eating-habits/fish-consumption-and-health/recommendations>).

Vermont has an interest and need for an evaluation of the recently (Dec 2024) released Safe Eating Guidelines for Fish You Catch in Vermont (<https://www.healthvermont.gov/sites/default/files/document/env-rw-eat-fish-choose-wisely-text.pdf>) to assess if communication of these guidelines has caused a behavioral change in fish consumers. evaluate mercury in fish communications and to assess if those are affecting behavioral changes. Pam has done some initial work with a call survey, so has baseline

information, but would like to partner with others to assess this with new guidelines. New York has done some assessment of their safe eating guidelines – by connecting with individuals at events and following up with them 4-6 months later with another survey. Feedback included the public hearing a negative message, which affected fishing license sales. One solution has been to direct people to other, safer locations to fish. New Hampshire has done some work related to seafood consumption with state residents.

New York would like to collaborate on relaying fish consumption advice through an app.

New York also reported having a draft technical document about PFOS developed among Great lakes states that will be completed in the next 6-12 months. Audrey indicated she could share that once it is available. Audrey also had some information from a statewide survey that included responses from Lake Champlain. She shared those results in a follow up email after the meeting: “This was a statewide survey that was distributed via the NYS DEC licensed angler email list in 2023. We received ~7,800 in about two weeks. These are the answers for the subset (n=241) who marked Lake Champlain as one of three waters they fish most often (you will see Lake Champlain as the top choice as a result). Link to Lake Champlain focused survey responses: https://www.surveymonkey.com/results/SM-7k1a3sac8YtFICZC28OyZw_3D_3D/.”

Task 4: The summary was prepared by Kris Stepenuk and delivered August 5, 2025. Details are below.

Overview

This outreach plan was developed to communicate up-to-date information on mercury in fish and fish consumption advisories to Lake Champlain anglers.

Setting

Lake Champlain lies between New York and Vermont and forms a portion of the border between these states and the province of Quebec, Canada. The lake is 120 miles (193 km) long and 12 miles (19 km) wide at its widest. Its watershed area is 8,234 miles² (21,326 km²). Lake Champlain has nine major tributaries and itself drains north into the Richelieu River in Quebec. Primary land uses in the watershed include forest (67%), farmland (14%), and urban (6%).

The lake is important to the local and regional recreation and tourism economy, including as a fishing destination. Anglers on Lake Champlain include private individuals who access the lake from private homes, boat landings, and marinas. Anglers also fish in Lake Champlain during chartered trips and through fishing tournaments.

Lake Champlain is impacted by mercury carried through the air and deposited to the lake where it transforms into the highly dangerous methylmercury. It biomagnifies as it moves through the aquatic food chain. As such, individuals who eat Lake Champlain fish should be aware of consumption guidelines related to eating specific species of fish at the same time as having awareness of the health benefits of eating fish.

Communications between study researchers and Burlington’s Cultural Brokers (i.e., community leaders representing a variety of refugee communities and indigenous communities in the Lake Champlain Basin who meet regularly to address community challenges especially as related to health and safety), anecdotal evidence through complementary fishing education workshops,

and findings of complementary studies (e.g., Stein 2023; Lamichhane 2024) suggest that there is a gap in knowledge about mercury levels in fish commonly caught and eaten by subsistence anglers. As these anglers are often opportunistic, eating whatever they catch (Stein 2023), there is need for additional data to be collected about species not traditionally tracked through regular mercury monitoring efforts. This includes brown bullhead catfish (also called bullpout) and carp (Stein 2023). Other Lake Champlain fish consumed by cultural community anglers for which mercury data are not available include northern pike, white sucker, bluegill, and eel (Lamichhane 2024). In addition, meetings with Chiefs of the Nulhegan and Missisquoi Abenaki tribes during this project revealed that, in addition to fish we sampled, tribal community members eat smelt, northern pike, salmon, pumpkinseeds, and eel. Tribal members may prefer larger fish over smaller fish, which enhances the potential for higher methylmercury concentrations. These communities often fish from shore and ice fish. They often freeze (to eat later) and sometimes sell their catch. They also compost parts of the fish not eaten. Separately, and a possible area for future research is that tribal members also eat wild foods, such as wild rice, mammals (muskrat, beaver, otter), and waterfowl, which may be a source of exposure to mercury and other contaminants.

Key Target Audiences:

Lake Champlain Anglers who eat fish for subsistence:

Recent studies suggest that between 15% (Karimi 2022) and 21% of Lake Champlain anglers (Malchoff et al. 2024) fish for subsistence or as a supplemental food source and/or opportunistically (Stein 2023). This includes both indigenous people and non-indigenous cultural community groups such as those from such locations as Nepal, Sudan, Egypt, and Somalia (Stein 2023, Lamichhane 2024). This is important as those likely most affected by methylmercury from fish caught from Lake Champlain are those communities who rely upon or who more commonly eat fish from the lake. We know from conversations with chiefs of the Nulhegan and Missisquoi Abenaki tribes during the project period that tribal community members often eat the fish they catch and fish throughout the year.

White people were found to have significantly more knowledge of safe fish consumption guidelines than Asian or Middle Eastern/African people (Stein 2023). As such, greater effort in future outreach might be made to reach non-white anglers over white anglers to help build awareness among these populations about safe fish consumption guidelines.

Recreational Lake Champlain Anglers who occasionally eat fish from the lake:

Nonetheless, other recreational anglers on Lake Champlain may also be at risk due to consumption of fish from the lake and should also be aware of current consumption guidelines.

Recommended Outreach Plan

To reach these audiences with critical information about mercury in fish and fish consumption guidelines, a variety of outreach activities are recommended. These should be informed by study results and, as appropriate, New York (New York State 2025), Quebec (Government of Quebec 2024), and recently updated State of Vermont fish consumption guidelines (State of Vermont 2025). The fish consumption guidelines across the three locations do not align with one another based on differences in how each government calculates risk.

Presentations and Tabling

A primary tool for dissemination of research findings and fish consumption guidelines is recommended to be through events and educational programs. These may be carried out in-person or remotely. It is recommended that a translator be employed as necessary to ensure those targeted to learn the information are able to fully understand the information shared.

Possibilities to provide education include:

- Presentations made at community meetings or gatherings (e.g., Cultural Brokers/Health Disparities Cultural Competency Council, religious gatherings, neighborhood meetings, Town meetings).
- Presentations about safe fish consumption during fishing education workshops (e.g., those hosted by Lake Champlain Sea Grant, Lake Champlain Basin Program, VT Fish and Wildlife Department).
- Webinars hosted in partnership with local groups (e.g., Cultural Brokers, Lake Champlain United).

One-on-One Communications

- Sharing of knowledge through tabling at fishing derbies (e.g., Father's Day fishing derby, [other fishing derbies](#)) or other types of events (e.g., farmer's markets, summer evening community events).
- Canvassing local fishing spots to share a summary of study results and fishing guidelines, both of which should be made available in multiple languages. The 15 most popular fishing spots of respondents to a fish consumption survey in the Burlington, VT, area were recently identified (Stein 2023). These could be targeted. They include (in order of popularity from most to least popular), Mallets Bay (Lake Champlain), Winooski River mouth, Colchester Point Access Area, Winooski River (generally), Lake Champlain (generally), Salmon Hole (Winooski River), Lamoille River, Island Line Causeway (Lake Champlain), Burlington Bay (Lake Champlain), Inland Sea/Northeast Arm (Lake Champlain), Otter Creek (Vergennes), Shelburne Pond, Waterbury Reservoir, Huntington River, and at the Intervale (Winooski River). In addition, chiefs of the Nulhegan and Missisquoi Abenaki tribes reported tribal members commonly fish in St Albans Bay (Lake Champlain), Lake Memphremagog, Lake Carmi, Missisquoi Bay/sandbar (Lake Champlain), Kingsland Bay (Lake Champlain).
- During the project, we communicated directly with Abenaki chiefs who, in turn, shared messaging with their constituents/members. For future outreach about study findings and fish consumption guidelines, we recommend working with the tribal chiefs again, as they have existing communications networks and could share the message and key information through those.
- To reach anglers and those eating fish from Lake Champlain more broadly, we recommend asking individuals who are reached through outreach efforts to pass along their knowledge to at least one other person.

Rack Cards / Fact Sheets

To support education programs and tabling events, study results could be summarized in a fact sheet or rack card. These could also be distributed to local bait and tackle shops and marinas. They might also be carried by Lake Champlain Basin Program Boat Launch Stewards in the event a boater or angler has interest in communicating about mercury in fish and safe fish consumption.

This rack card should be supplemented by governmental agency fact sheets describing current fish consumption guidelines. The summary and guidelines should be made available in multiple languages.

Lists

Building upon our work to share information about this research study and to recruit anglers to assist in collecting fish that were used in mercury analyses, we recommend sharing project results and fish consumption guidelines (particularly the updated Vermont guidelines) via local fishing forums including IceShanty.com, 802-fishing page on Facebook, and www.lakechamplainunited.com/forums.

Media

To share study results and fish consumption guidelines, a press release could be developed and forwarded to media outlets. If carried out by Lake Champlain Sea Grant, UVM Extension maintains an extensive list of media contacts, including printed and online news, television, and radio. A press release could be crafted in partnership with Lisa Halvorsen, who can then share it with various media sources.

With funding to support translation, we could develop a short video in multiple languages through the [Vermont Language Justice Project](#).

Finally, to reach a broader audience, we can propose this as a topic for UVM Extension's daily TV show shown on WCAX, called *Across the Fence*. This show has a viewership of 20,000 households across Vermont, and in northern New York, northern New Hampshire and southern Quebec.

Tasks 5 and 6: Fish collection and analysis.

Data was submitted for these tasks in April 2024, and included in the 2024 State of the Lake Report.

Introduction: In this study, temporal trends in Hg concentration for yellow perch, white perch, smallmouth bass, lake trout and walleye (based on data from our 2022-2023 survey as well as previous LCBP surveys) were evaluated across the lake. Spatial trends in yellow perch, white perch and smallmouth bass were also assessed. Lake trout and walleye are long-lived, slow growing piscivorous species that are established indicator species for mercury bioaccumulation in whole lake studies (Bartz et al., 2023; Lepak et al., 2018). Trout are apex predator fish in large lakes, whereas walleye are typically a lower trophic level; both species have been used to evaluate mercury levels at the top of the food chain in lakes (Bartz et al., 2023; Lepak et al., 2025, 2019, 2018; Zananski et al., 2011). Lake trout are only found in the deep basins of the North Main, Main and South Main Lake segments of Champlain, whereas adult walleye migrate to the tributaries in winter to spawn, returning to the deep basins in spring where they feed throughout the summer and fall. Large adult walleye are rarely found in the shallow regions of

the lake after leaving the tributaries in early spring, and feed most of the year from the deep basins, also making them good sentinels of the deep basins. Yellow and white perch and smallmouth bass can inhabit all basins of the lake year-round. While these species do migrate, their movements are generally more localized than walleye. Perch and bass travel to nearshore regions in spring and early summer to spawn and move throughout the year depending on water temperature and food availability.

As well as assessing temporal trends, concentrations of Hg in walleye and trout were compared to data from the Great Lakes, to give insight into the drivers of Hg bioaccumulation in large lakes. Length-normalized Hg concentrations for both species were compared to data from the Great Lakes, though considerable uncertainty in these comparisons are derived from the length corrections over a large range of fish sizes in this study, and from estimating whole fish Hg concentrations from filet Hg (in this study), which was needed to harmonize the different datasets.

Results: Concentrations of Hg were plotted across species (Figure 5_6.1). As expected, the predator species – trout, walleye and smallmouth bass, had higher concentrations of Hg than yellow and white perch. Across the predator species, bass and walleye had a larger range of Hg concentrations than lake trout. Despite lake trout being apex predators and Hg concentrations being driven by biomagnification, other factors, particularly growth rates, can influence Hg concentrations across species (Johnston et al., 2022; Lavigne et al., 2011). The lower growth rates of walleye than lake trout have been attributed to their higher Hg concentrations in other northern lakes (Lavigne et al., 2011). In this dataset, smallmouth bass and walleye were sampled from fishing derbies which biased the samples toward large individuals, and Hg is strongly correlated to size.

Trends in fish Hg concentration are shown in Figures 5_6.2a-e. Significant differences in mean Hg were evident between years sampled, with length as a covariate for both trout ($F = 16.27$ d.f. = 11/193, $p < 0.0001$) and walleye ($F = 17.87$ d.f. = 11/207, $p < 0.0001$). For lake trout, an interaction effect between log Hg and fish length was evident, particularly for the 2000 and 2011 survey data (Figure 5_6.1S), which may confound the interpretation of these years. The model-estimated mean Hg concentration for length-normalized lake trout ranged from 335 to 662 ng g^{-1} (ww) (Figure 5_6.2a). There was a significant decline in mean trout Hg from the early surveys (1987-1993) to 2011, then values increased in 2016-2017, and decline in 2022-2023 to a value intermediate to the 2011 and 2016-2017 surveys. For walleye there was also an interaction effect for 2004 (Figure 5_6.2S). The temporal trend for walleye was less clear than for trout, though Hg (972 ng g^{-1}) in the early survey (1996-1998) was significantly higher than the more recent surveys (575 ng g^{-1} in 2022-2023 (Figure 5_6.2b)).

Mixed linear effect models for perch and smallmouth bass are shown in Table 5_6.1 to 5_6.3, and in Figures 3S to 5S. For yellow perch, a random slope model explained more of the variation in Hg, whereas for white perch and smallmouth bass, there was no difference between models and the simpler random intercept model was chosen. Length-normalized estimated mean concentrations of yellow perch among surveys ranged from 107 to 193 ng g^{-1} . The temporal trend for yellow perch was similar to that of lake trout, with a significant decline in mean perch Hg from the early surveys (1988-1998) to 2011, then values increased in 2016-2017, and decline in 2022-2023 to a value intermediate to the 2011 and 2016-2017 surveys (Figure 5_6.3). Estimated mean concentrations of Hg in white perch were higher than in yellow perch 144 to 235 ng g^{-1} , attributed in part to the larger size of these fish (mean length 239 mm

(white perch) versus 203 mm (yellow perch)). There was much less variability in white perch between 1988-1998 and 2016-2017 than in yellow perch. The most recent survey (2022-2023) estimates a decline in white perch Hg concentrations for the first time across the record. Estimated mean smallmouth bass concentrations across surveys ranged from 427 to 585 ng g⁻¹. Though confidence levels were high due to small sample sizes in the earliest surveys, smallmouth bass had a similar trend to yellow perch and lake trout, with the 2016-2017 fish having higher Hg than those from 2011 and 2022-2023.

Trends across year and location (basin) were assessed for yellow and white perch, and smallmouth bass, for the surveys between 2011 and 2023 (Figures 5_6.3a-c). Across basins, yellow perch from Mallett's Bay were elevated particularly in the 2011 and 2022-2023 surveys; concentrations were significantly higher in Malletts than in Northeast Arm and Main Lake in 2011, than Main Lake in the 2016-2017 survey, and than Northeast Arm, North Main Lake, Main Lake and South Main Lake in the 2022-2023 survey (Table 5_6.4S). These higher concentrations are consistent with more efficient bioaccumulation in Malletts Bay (Chen et al., 2012; Miller et al., 2012), which aligns with the lower productivity in this basin. The trend of higher Hg in the deep basins in 2016-2017 was previously reported (Swinton and Nierzwicki-Bauer, 2019). Across the deep basins, the fish in the North Main basin had higher Hg concentrations in 2016-2017 than in 2011, but were not significantly different than 2022-2023. In the Main Lake, the 2016-2017 fish Hg was higher than those from the survey before or after. There were no significant differences in Hg in the South Main Lake fish across these three time points (Table 5_6.4S). In the previous work, fish from the deep basins were combined to show a strong increase in Hg concentrations between 2011 and 2016/17 (Swinton and Nierzwicki-Bauer, 2019).

For white perch, there were fewer differences across basins and between surveys. While the Hg concentrations were lower in 2022-2023 than previous years in the whole lake, differences across surveys in individual years were less evident, and white perch from 2022-2023 were only significantly lower than the 2016-2017 survey in Northeast Arm (Table 5_6.5S). Smallmouth bass Hg concentrations were only significantly higher in 2016-2017 than in 2011 in South Main Lake, with no difference in 2023. This relationship was reported previously by Swinton and Nierzwicki-Bauer (2019), but with the three deep Main Lake basins combined. As with yellow perch, concentrations were found to increase from 2011 to 2016-2017 in the deep lake only in that study. The higher concentrations of Hg in Malletts Bay observed in yellow perch were not present in white perch or smallmouth bass.

Concentrations of Hg in lake trout and walleye from Lake Champlain were compared with concentrations in the Laurentian Great Lakes (Figure 6_5.4). Lake trout and to a lesser extent, walleye, are open-water, pelagic predators that have been used as sentinels of Hg bioaccumulation across whole lakes (Lepak et al., 2022). Length-adjusted concentrations of Hg in walleye and trout from Lake Champlain were compared to concentrations from the Great Lakes adjusted to estimated filet-concentrations. Concentrations estimated in Lakes Superior and Huron were higher than those in Lakes Michigan, Erie and Ontario, which had similar concentrations to Champlain. The higher bioaccumulation in Lakes Superior and Huron is attributed to more efficient bioaccumulation in clear, oligotrophic waters (Ogorek et al., 2021) and to higher atmospheric versus watershed or industrial inputs, as direct atmospherically deposited Hg is thought to be more bioavailable (Harris et al., 2007). This comparison with the Great Lakes, which have a low watershed to lake ratio, ranging from 3.4:1 (Ontario) to 1.6:1

(Superior), is intended to explore the watershed influence in Champlain, which has a large and steep drainage area giving it a ratio of 19:1, discussed further in Task 7.

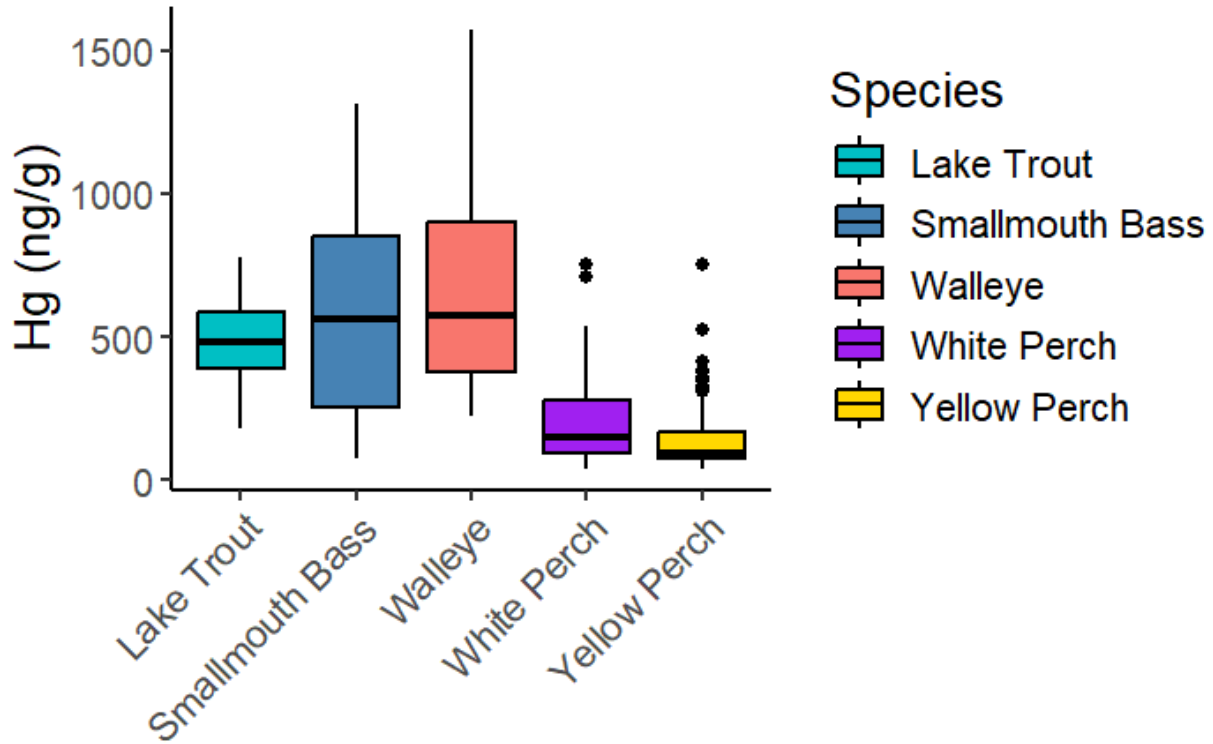


Figure 5_6.1) Concentration of Hg (ng/g WW-wet weight) in fish from the 2022-2023 survey. In this plot across species, concentrations are untransformed.

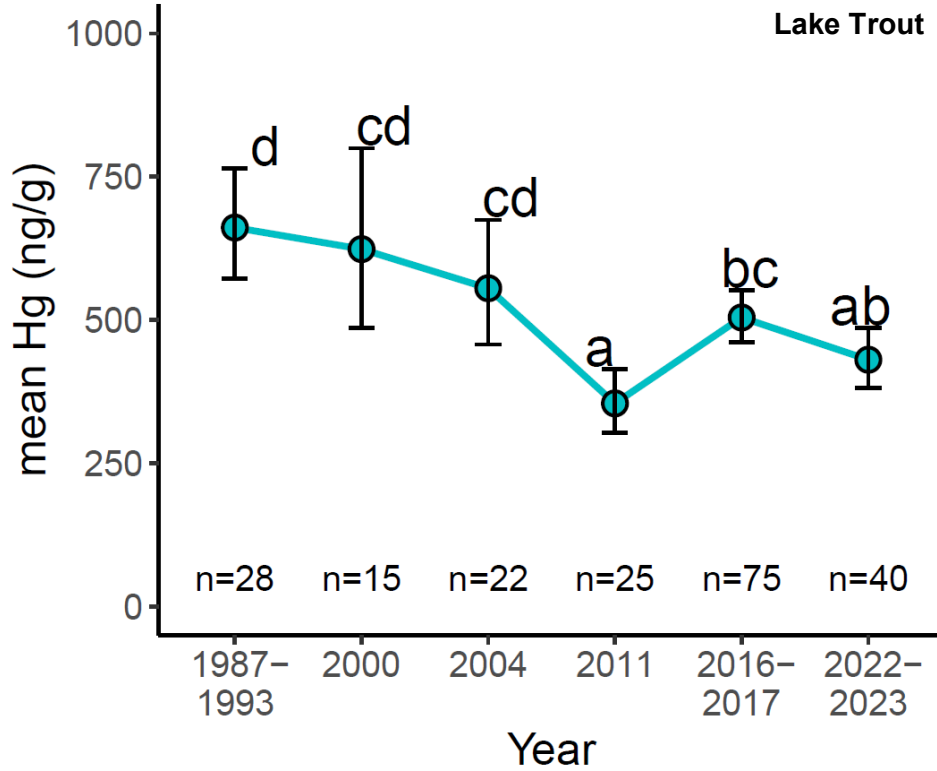


Figure 5_6.2a) Estimated length-corrected mean concentrations (+/- 95% confidence limits) of Hg in lake trout over time. Surveys from 1987 and 1993 were combined due to small sample numbers (n). Survey years were treated as categorical variables and differences in Hg across surveys were significant ($F = 16.27$ d.f. = 11/193, $p < 0.0001$). Letters (a-d) signify differences in mean Hg by Tukey's post hoc test: if two surveys share the same letter, mean Hg is not significantly different.

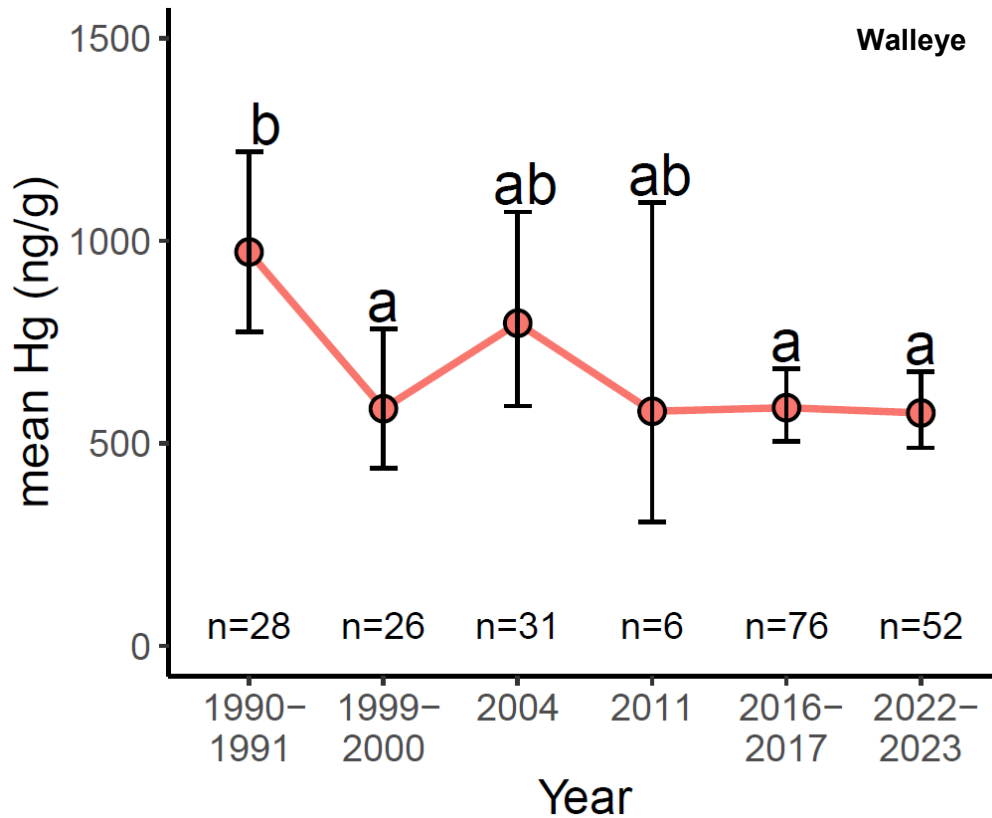


Figure 5_6.2b) Estimated length-corrected mean concentrations (\pm 95% confidence limits) of Hg in walleye over time. Surveys from some years combined due to small sample numbers (n). Survey years were treated as categorical variables and differences in Hg across surveys were significant ($F = 17.87$ d.f. = 11/207, $p < 0.0001$). Letters (a-b) signify differences in mean Hg by Tukey's post hoc test: if two surveys share the same letter, mean Hg is not significantly different.

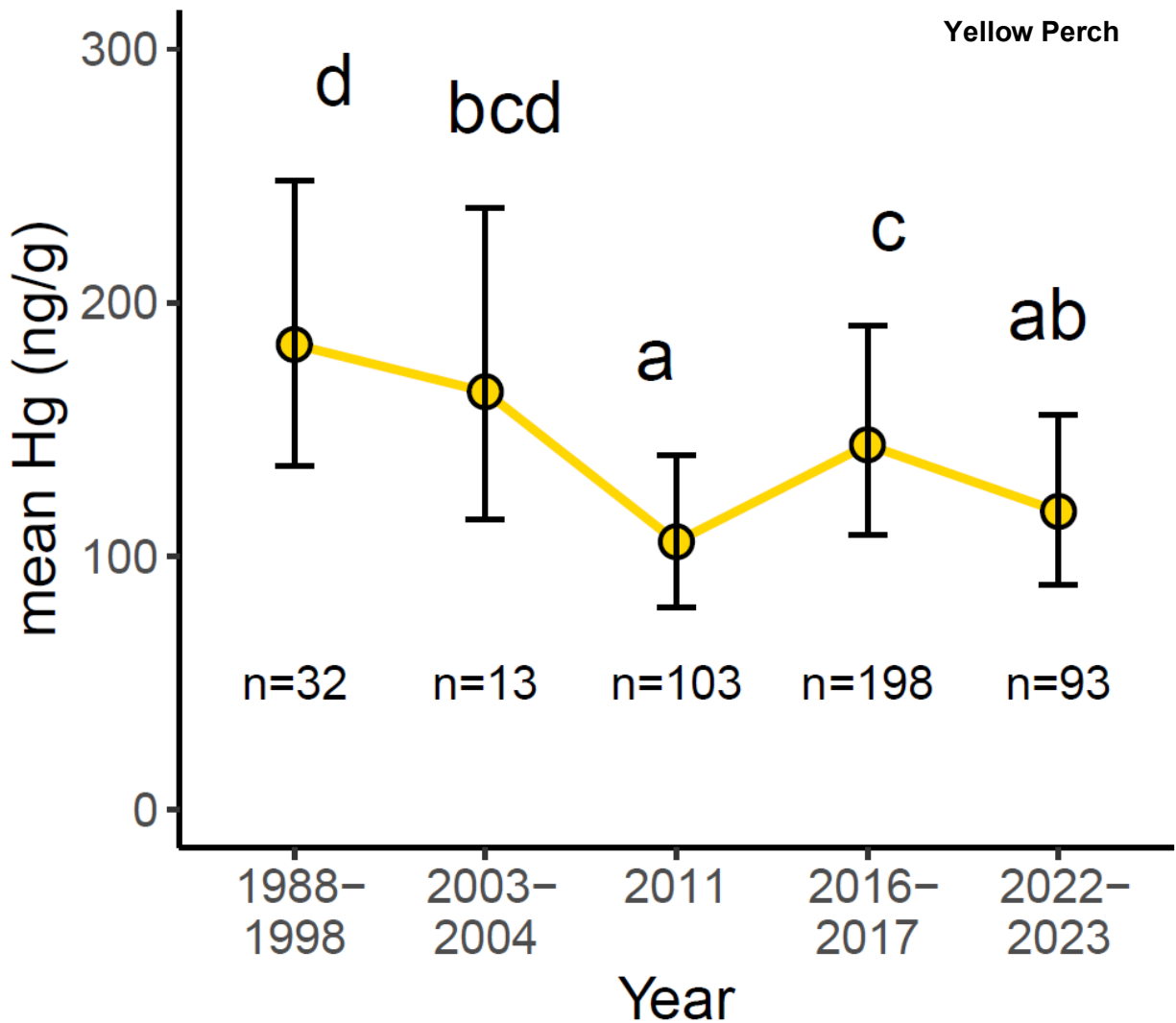


Figure 5_6.2c) Estimated length-corrected mean concentrations (\pm 95% confidence limits) of Hg in yellow perch over time, modelled by mixed effects model with year and length as fixed effects and basin as a random effect. Surveys from 1988 to 1998 were combined due to small sample numbers (n). Letters (a-d) signify differences in mean Hg by Tukey's post hoc test: if two surveys share the same letter, mean Hg is not significantly different.

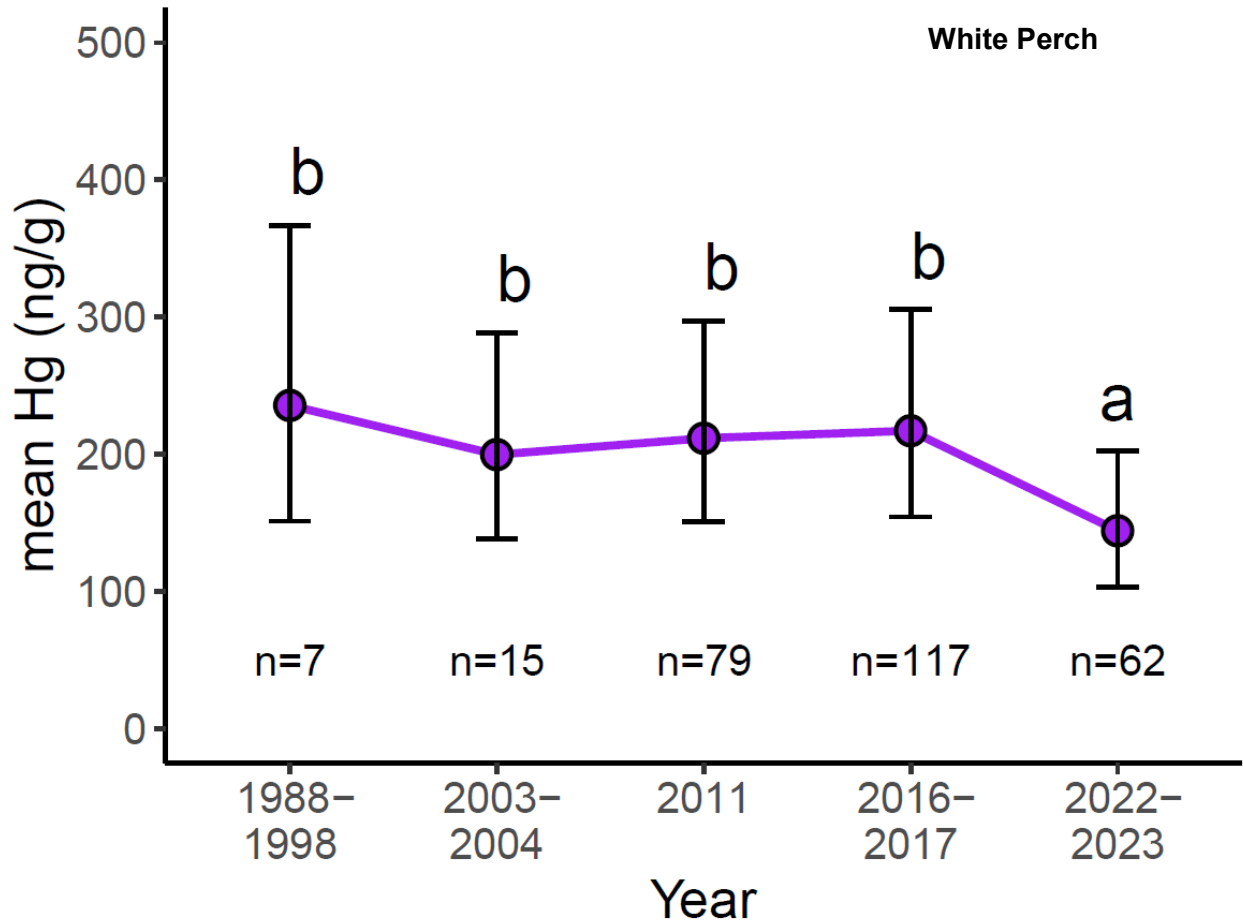


Figure 5_6.2d) Estimated length-corrected mean concentrations (\pm 95% confidence limits) of Hg in white perch over time, modelled by mixed effects model with year and length as fixed effects and basin as a random effect. Surveys from 1988 to 1998 were combined due to small sample numbers (n). Letters (a-b) signify differences in mean Hg by Tukey's post hoc test: if two surveys share the same letter, mean Hg is not significantly different.

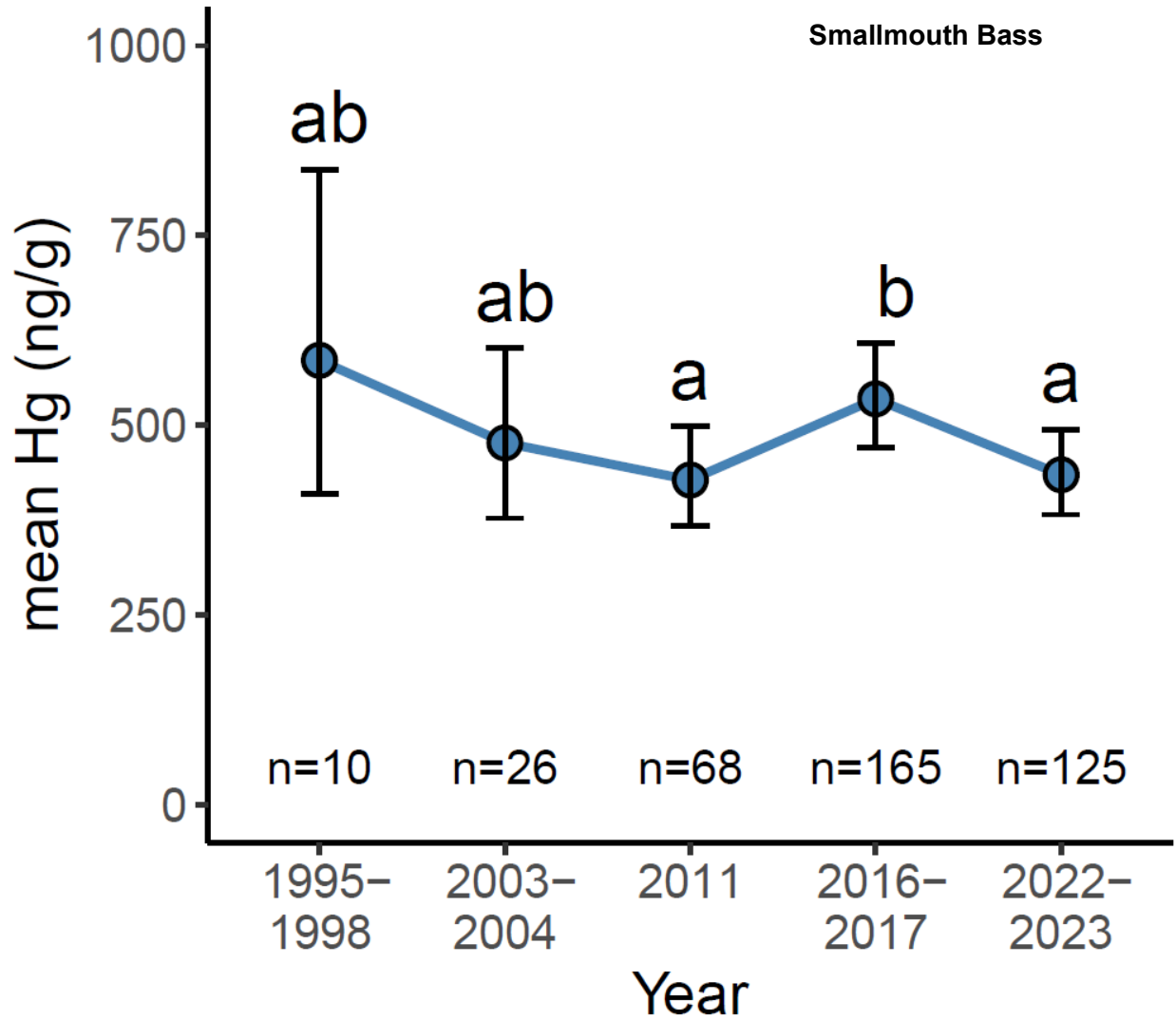


Figure 5_6.2e) Estimated, length-transformed mean concentrations (\pm 95% confidence limits) of Hg in smallmouth bass over time, modelled by mixed effects model with year and length as fixed effects and basin as a random effect. Surveys from 1995 to 1998 were combined due to small sample numbers (n). Letters (a-b) signify differences in mean Hg by Tukey's post hoc test: if two surveys share the same letter, mean Hg is not significantly different.

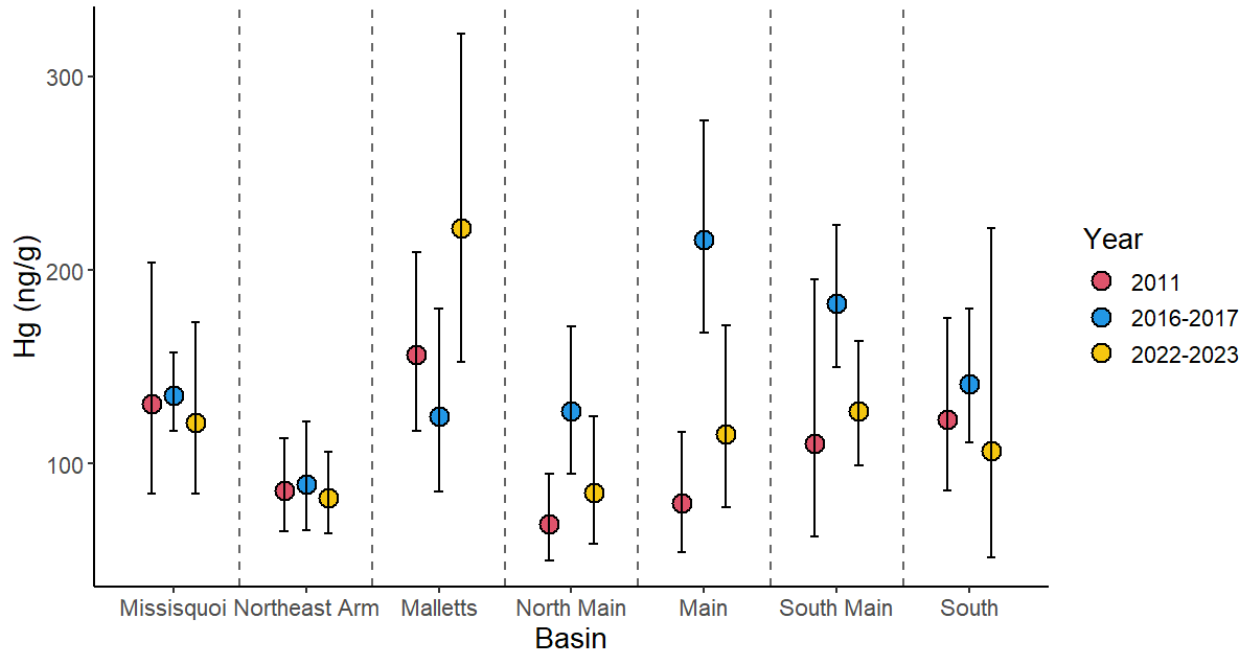


Figure 5_6.3a) Estimated length-corrected mean concentrations (+/- 95% confidence limits) of Hg in yellow perch by basin in the 2011 to 2023 surveys, modelled by mixed effects model with year, length and basin as fixed effects and basin and length/year:basin as a random intercept effect.

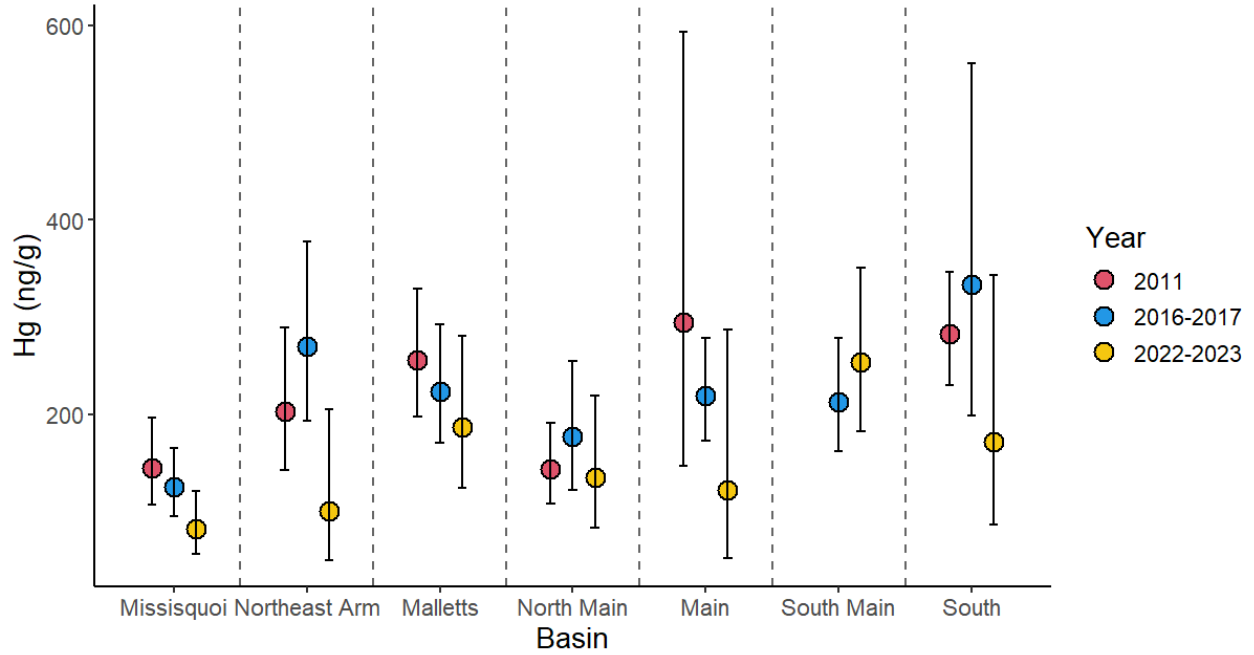


Figure 5_6.3b) Estimated length-corrected mean concentrations (+/- 95% confidence limits) of Hg in white perch by basin in the 2011 to 2023 surveys, modelled by mixed effects model with year, length and basin as fixed effects and basin and length/year:basin as a random intercept effect.

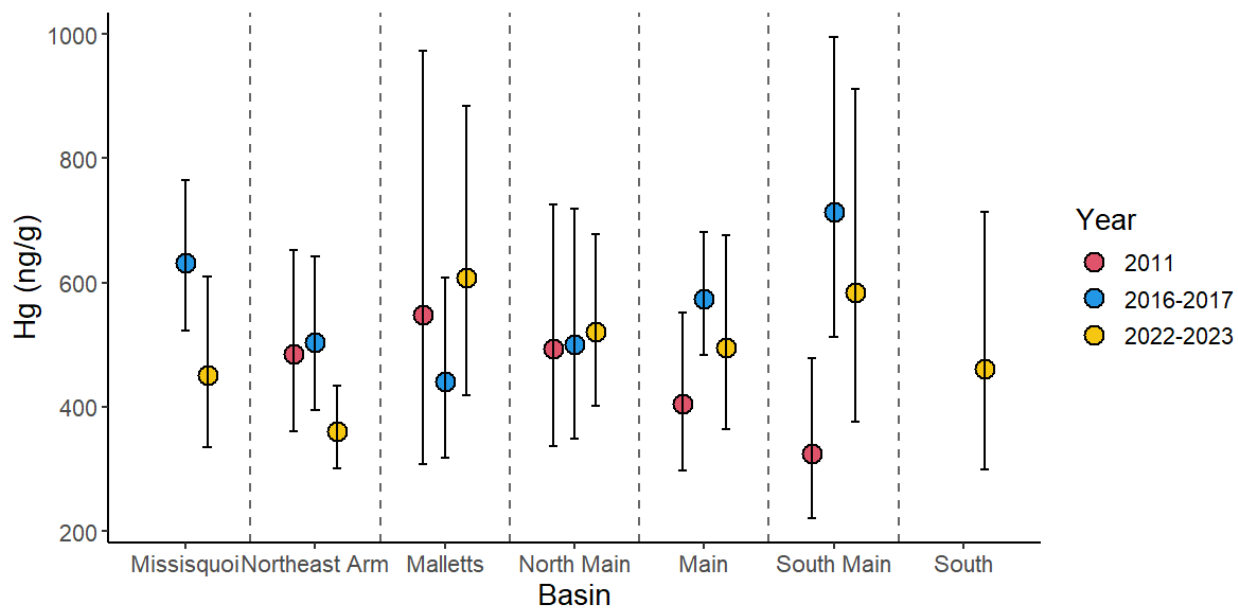


Figure 5_6.3c) Estimated length-corrected mean concentrations (+/- 95% confidence limits) of Hg in smallmouth bass by basin in the 2011 to 2023 surveys, modelled by mixed effects model with year, length and basin as fixed effects and basin and length/year:basin as a random intercept effect.

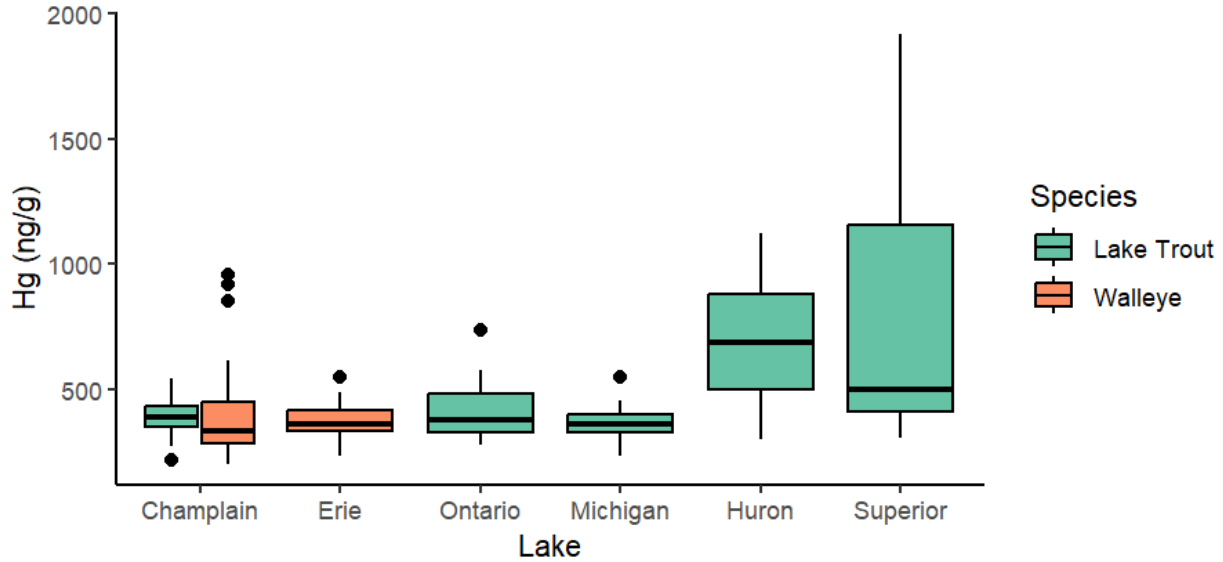


Figure 5_6.4) Hg concentrations for Lake Trout and Walleye from Lake Champlain (2022-2023) and the Great Lakes (2004 to 2013). Earlier Great Lakes fish were used in this plot because wet weight Hg concentrations were not available for 2020-2021 surveys. Lake Champlain fish Hg concentrations were length-normalized to 650 mm for Lake Trout and 450 mm for Walleye. To compare with the Lake Champlain data, fillet Hg concentrations were estimated from whole Great Lakes fish Hg concentrations using the equation from (Peterson et al., 2005).

Task 7: Hg, C, and N isotopes:

Ratios of Hg, C and N in pelagic predator fish (lake trout, walleye) and forage fish (yellow perch) from Lake Champlain were determined to better understand the sources of Hg to a large deep lake with substantial watershed inputs. We also compare Hg isotope signatures in Lake Champlain fish to those from the Great Lakes.

Molar ratios of C:N were first evaluated (Figure 7.1) to determine the influence of lipids on $\delta^{13}\text{C}$; lipids are composed primarily of C and H, with low concentrations of N, whereas proteins and carbohydrates have a higher N content. As expected, lake trout, which are fatty, cold-water fish, have much higher C:N ratios than walleye or trout. Because lipid content and metabolism has a strong influence on $\delta^{13}\text{C}$ ratios, lake trout bulk $\delta^{13}\text{C}$ ratios were mathematically corrected for lipid effects using the C:N ratio (Hoffman et al., 2015). Lipid-free $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ are shown in Figure 7.2. Lake trout have a tight distribution of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ compared with higher variability in the other species, reflecting the more consistent diet and habitat of the deepwater species. Lake trout are apex predators in the lake, whereas walleye have lower $\delta^{15}\text{N}$ values signifying a lower trophic level, and yellow perch, which are forage fish have the lowest $\delta^{15}\text{N}$. Ratios of $\delta^{13}\text{C}_{\text{lipid free}}$ broadly reflect basal carbon sources and feeding zones, and are typically enriched in nearshore and depleted in pelagic carbon sources (Lepak et al., 2025; Peterson and Fry, 1987). Across the three species, $\delta^{15}\text{N}$ was strongly associated with log₁₀-transformed Hg ($r^2 = 0.51$, $p < 0.0001$), consistent with the biomagnification of Hg with increasing trophic level. Within species, the relationship between $\delta^{15}\text{N}$ and Hg was only evident for walleye ($r^2 = 0.53$, $p < 0.0001$). The smaller number of samples, and the small distribution of $\delta^{15}\text{N}$ values limited evaluation of this relationship in lake trout. Previous studies of temperate lakes have found $\delta^{15}\text{N}$ to be a strong predictor of Hg in walleye, perch and trout (Johnston et al., 2022; Kidd et al., 1995). There was no influence of $\delta^{13}\text{C}$ on Hg concentrations across all species; $\delta^{13}\text{C}_{\text{lipid free}}$ was positively associated with log₁₀-transformed Hg ($r^2 = 0.44$, $p < 0.0260$) in lake trout. Conversely, In a study of Hg in fish from temperate lakes, $\delta^{13}\text{C}$ was found to have less of an influence on Hg in pelagic species than benthivores (Johnston et al., 2022).

Hg isotope ratios in fish from Lake Champlain are shown in Figures 7.3 to 7.5. The large range in $\Delta^{199}\text{Hg}$, and the linear relationship between $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ (Figure 7.3), is typical of lake fish Hg, and indicates both isotope ratios are largely driven by photochemical effects (Lepak et al., 2022, 2018). Photochemical fractionation occurs prior to MeHg bioaccumulation at the base of the food web, and isotope ratios are conserved through bioaccumulation and trophic transfer (Armstrong et al., 2023; Kwon et al., 2012). The $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ ratios are therefore representative of residual MeHg in the water column. The ratio of $\Delta^{199}\text{Hg}$ to $\Delta^{201}\text{Hg}$ (Figure 7.4) provides further information on the photochemical fractionation pathway: the slope of $\Delta^{199}\text{Hg} / \Delta^{201}\text{Hg}$ by York regression was 1.334 ± 0.010 , which is consistent with photodemethylation, by comparison to laboratory experiments that differentiated odd-MIF from MeHg photodemethylation ($\Delta^{199}\text{Hg} / \Delta^{201}\text{Hg} = 1.36$) and from Hg photoreduction ($\Delta^{199}\text{Hg} / \Delta^{201}\text{Hg} = 1.00$) (Bergquist and Blum, 2007; Zheng and Hintelmann, 2009). Photodemethylation, and associated odd-MIF, is driven by the penetration of UV light in the water column, and is related to water clarity (Lepak et al., 2018). Lake trout, which feed from clear, offshore pelagic reservoirs, have a small distribution of elevated $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ ratios, whereas walleye, which predominantly feed in deep, offshore basins, but migrate to shallower areas with higher DOC and turbidity to spawn, had more variability in $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$, though overlapping with lake trout. As expected, yellow perch, which feed from littoral food webs, had a wide range of

$\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ ratios, with significantly lower mean values than for walleye and trout ($\delta^{202}\text{Hg}$: $F = 24.55$; $p < 0.0001$; $\Delta^{199}\text{Hg}$: $F = 39.03$; $p < 0.0001$; Figure 7.3). There were no significant differences in $\Delta^{200}\text{Hg}$ ratios between species.

Differences in Hg and N isotope ratios in yellow perch were evident between basins. There was a correlation between $\Delta^{199}\text{Hg}$ and both $\delta^{15}\text{N}$ ratio ($r^2 = 0.52$, $p < 0.0035$) and Hg concentration ($r^2 = 0.64$, $p < 0.0006$) (Figure 7.5), driven by high values of all three variables in Malletts Bay (though $\delta^{15}\text{N}$ and Hg were not correlated). It is noted that these values are from composites of five yellow perch per datapoint. Fish from Malletts Bay were not significantly larger than fish from the other basins, as fish size would confound interpretation of the higher Hg concentrations and trophic level ($\delta^{15}\text{N}$). The apparent relationship between these variables is likely driven by the oligotrophic status of Malletts Bay, which promotes higher water clarity (and therefore higher $\Delta^{199}\text{Hg}$) and more efficient Hg bioaccumulation (Ogorek et al., 2021) than the more productive basins. Unlike trout and walleye, yellow perch were collected from the littoral zones of the deep Main Lake basins, which may explain the much lower $\Delta^{199}\text{Hg}$ values for that lake segment. The cause of the high $\delta^{15}\text{N}$ in Malletts Bay perch is unclear; variability in fish $\delta^{15}\text{N}$ across lakes and over time (years) in the Great Lakes is attributed to biogeochemical differences and productivity, but also changes in fish diet (Lepak et al., 2025; Sierszen et al., 2006). $\delta^{15}\text{N}$ in perch from Malletts Bay may reflect more pelagic versus littoral feeding (nearshore has lower $\delta^{15}\text{N}$, Lepak et al., 2025) in fish from the other basins. This pattern in $\delta^{15}\text{N}$ in perch is being examined in more detail in a current project.

Comparison with Great Lakes: To further evaluate the sources of Hg to Lake Champlain, Hg isotope ratios were compared to those from fish surveys in the Great Lakes (Lepak et al., 2025, 2018). Across lakes, fish had a wide range of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ (Figure 7.6), with the most elevated values in the oligotrophic Upper Great Lakes (Superior, Michigan and Huron), reflecting higher photodemethylation in clearer waters (Lepak et al., 2018). Lake Champlain trout and walleye had $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ values intermediate to the East and West Basins of Lake Erie (fish populations have been found not to mix from these regions of the lake (Lepak et al., 2018)), and lower than the other four Great Lakes. Mean $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ ratios for each lake were also plotted against Secchi depth (Figure 7.7), a measure of water clarity, which was shown to be strongly related to odd-MIF in the Great Lakes (Lepak et al., 2018). Secchi depth in the Main Lake basin of Champlain was also intermediate to the East and West Basins of Lake Erie, though drivers of water clarity are different between these lakes/basins. Light penetration in lakes is limited by colored DOC and particulates (Effler et al., 2010; O'Donnell et al., 2013), but in low DOC waters such as Champlain and the Great Lakes, the deep chlorophyll maxima also plays a role in the depth of UV penetration, and therefore the degree of MIF (Lepak et al., 2018). The Main Lake basin of Champlain has higher concentrations of DOC than the Great Lakes (Zhou et al., 2016), as well as higher levels of colored DOM (O'Donnell et al., 2013). The mesotrophic deep main basins of Lake Champlain have a deep chl-a max (Chiapella et al., 2023; Smeltzer et al., 2012), whereas the shallow West Basin of Lake Erie is highly eutrophic with a near-surface chl-a max, and water clarity is limited by sediment resuspension (Donnell et al., 2010). The deeper and less eutrophic East Basin of Erie has a deep chl-a max, and slightly higher MIF than Champlain. The relationship between MIF and Secchi depth across these basins support the ascertain that MIF, and MeHg loss through photodemethylation, are broadly driven by water clarity but less specific to the limnologic conditions which drive it (Dimento and Mason, 2017; Motta et al., 2019).

While photodegradation is the exclusive cause of MIF, it is also a major driver of MDF ($\delta^{202}\text{Hg}$) in clear lakes, though the extent of $\delta^{202}\text{Hg}$ fractionation varies with dissolved organic matter concentration and character, and phytoplankton density (Armstrong et al., 2023). While $\delta^{202}\text{Hg}$ fractionation is consistent under a specific set of water quality conditions, $\Delta^{199}\text{Hg}/\delta^{202}\text{Hg}$ can vary substantially across conditions with no discernible pattern elucidated to date (Armstrong et al., 2023). Mathematical corrections of $\delta^{202}\text{Hg}$ for photodemethylation have been proposed and their application may be justifiable within lake, but not across lakes with varying limnologic character (Armstrong et al., 2023; Lepak et al., 2025). In sediments, where *in situ* photodegradation of Hg is minimal, $\delta^{202}\text{Hg}$ is a strong indicator of source, with watershed derived Hg having strongly negative values relative to precipitation (Lepak et al., 2015b). In fish, $\delta^{202}\text{Hg}$ corrected for photodemethylation has been shown to reflect changes in atmospheric Hg source and declines in Hg within lakes (Lepak et al., 2025). Across Champlain and the Great Lakes, $\delta^{202}\text{Hg}$ in Champlain is more negative than the West Basin of Erie, despite only slightly lower values of $\Delta^{199}\text{Hg}$. This may reflect more watershed derived Hg to Lake Champlain, though the fractionation caused by photodemethylation makes this difficult to confirm.

Values of $\Delta^{200}\text{Hg}$ in fish were plotted across lakes (Figure 7.8). Here, values of $\Delta^{200}\text{Hg}$ from Lake Champlain fish were significantly lower than those from the Great Lakes (Fstat = 21.297, $p < 0.0001$). The processes driving even-MIF ($\Delta^{200}\text{Hg}$) are not fully understood, but this isotopic fractionation occurs in the upper atmosphere, and values in fish and sediment have been attributed to Hg derived from precipitation (Chen et al., 2012, 2016; Lepak et al., 2018, 2015). This infers that a smaller portion of Hg delivery to Champlain waters is from precipitation, than in the Great Lakes.

Conclusions: A goal of this work is to better understand the sources and delivery of Hg to Lake Champlain fish, particularly due to the lake's large and steep watershed. Using a mass balance approach and measures of Hg inputs (tributary loading, precipitation) and export (sediment deposition, river outlet), a previous study found the watershed to be the major source of Hg to the lake (Gao et al., 2006; Shanley and Chalmers, 2012). However, offshore basins where large, pelagic fish feed can be decoupled from nearshore zones that receive Hg from the watershed, and epilimnetic coastal waters have been found to receive Hg predominantly from precipitation (Štok et al., 2015). Furthermore, Hg directly deposited to the epilimnion has been shown to be more bioavailable than Hg from the watershed (Harris et al., 2007). Here, we used a multiple isotope approach to better understand the pathways and processes controlling Hg concentrations in Lake Champlain fish.

This work provides a baseline of Hg and C/N isotope ratios, which have been a powerful tool for understanding temporal trends in the Great Lakes (Lepak et al., 2025). From this data, the strongest evidence of watershed-derived Hg in the epilimnion of Lake Champlain are the lower $\Delta^{200}\text{Hg}$ values in fish, relative to the Great Lakes. The delivery of Hg to Champlain from the tributaries is supported by its large watershed:lake ratio and suggests a portion of watershed derived Hg does reach the offshore basin and is bioaccumulated. The controlling role of photodemethylation on the $\Delta^{199}\text{Hg}$ and $\delta^{202}\text{Hg}$ ratios in fish reflects the influence of in-lake processes controlling Hg cycling in the epilimnion. Differences in MIF in forage fish across the basins of Lake Champlain also reflect the variable limnologic characteristics of different segments of the lake. Temporal trends in Hg concentrations and isotopic signatures in the Great Lakes which depart from atmospheric trends have been associated with changes in water quality and prey availability driven by invasive species (Lepak et al., 2025, 2015a). The temporal

changes in Hg concentrations in Champlain are not clearly aligned with an invasion, and have been suggested to be driven by an increase in major storm events (Swinton and Nierzwicki-Bauer, 2019). The impacts of storms and increased watershed runoff are likely to have indirect effects on Hg levels in the lake, through changes in water quality conditions that alter photodemethylation and bioavailability.

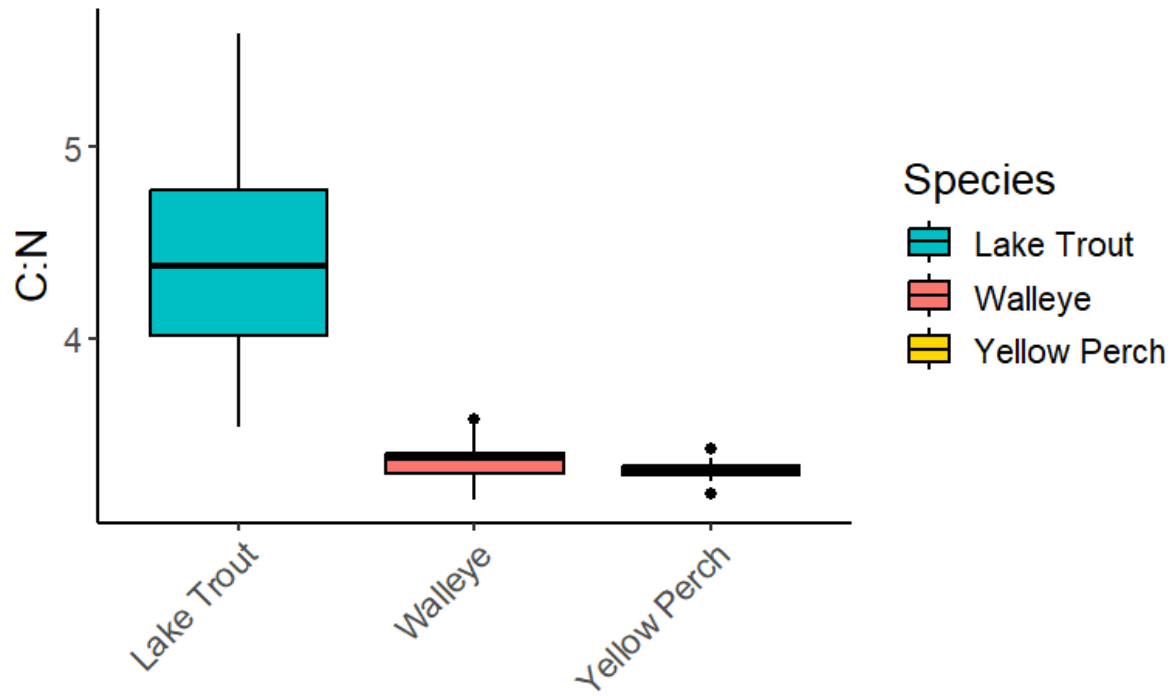
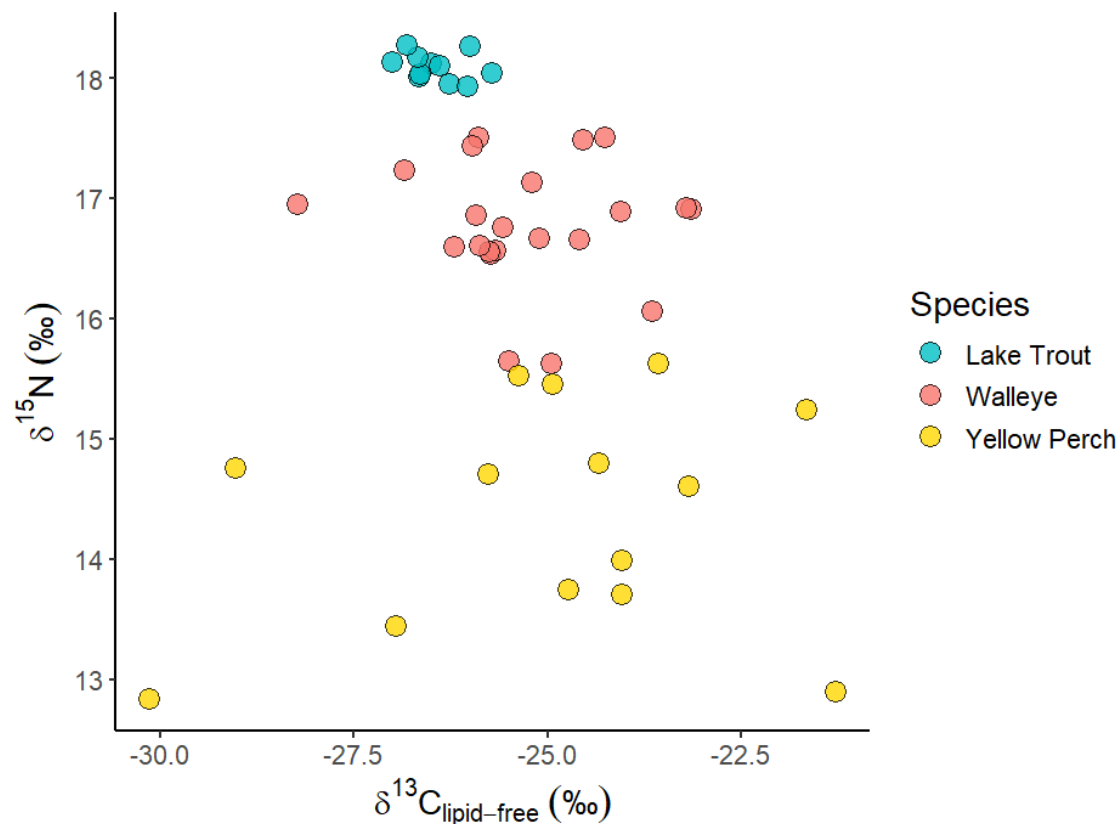


Figure 7.1) Molar C:N ratio, a proxy for lipid content in fish, for a subset of fish selected for isotopic analysis.



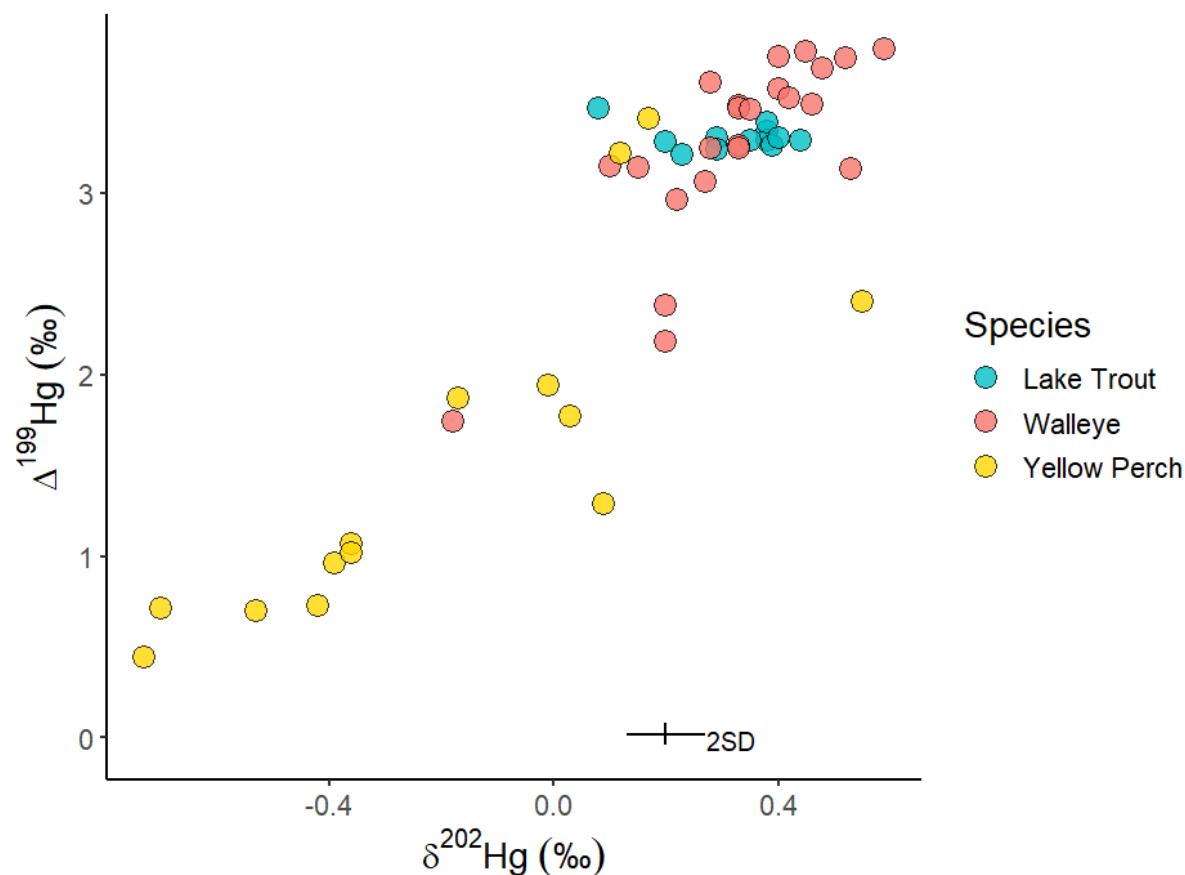


Figure 7.3) $\delta^{202}\text{Hg}$ versus $\Delta^{199}\text{Hg}$ for subset of fish selected for isotopic analysis. Lake trout and walleye samples were analyzed as individuals, and yellow perch samples were analyzed as composites of 5 individuals. The 2SD marker denotes precision metrics as 2 times the standard deviation based on repeated measures. $\Delta^{199}\text{Hg}$ values reflect photochemical effects from exposure to sunlight, with highest values found in fish feeding from the clear offshore waters where sunlight penetrates deep into water column. $\delta^{202}\text{Hg}$ fractionation occurs from a multitude of reactions, with photochemical effects having a large, but unpredictable effect. Both $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ ratios in lakes are predominantly driven by MeHg exposure to sunlight in the water column prior to bioaccumulation.

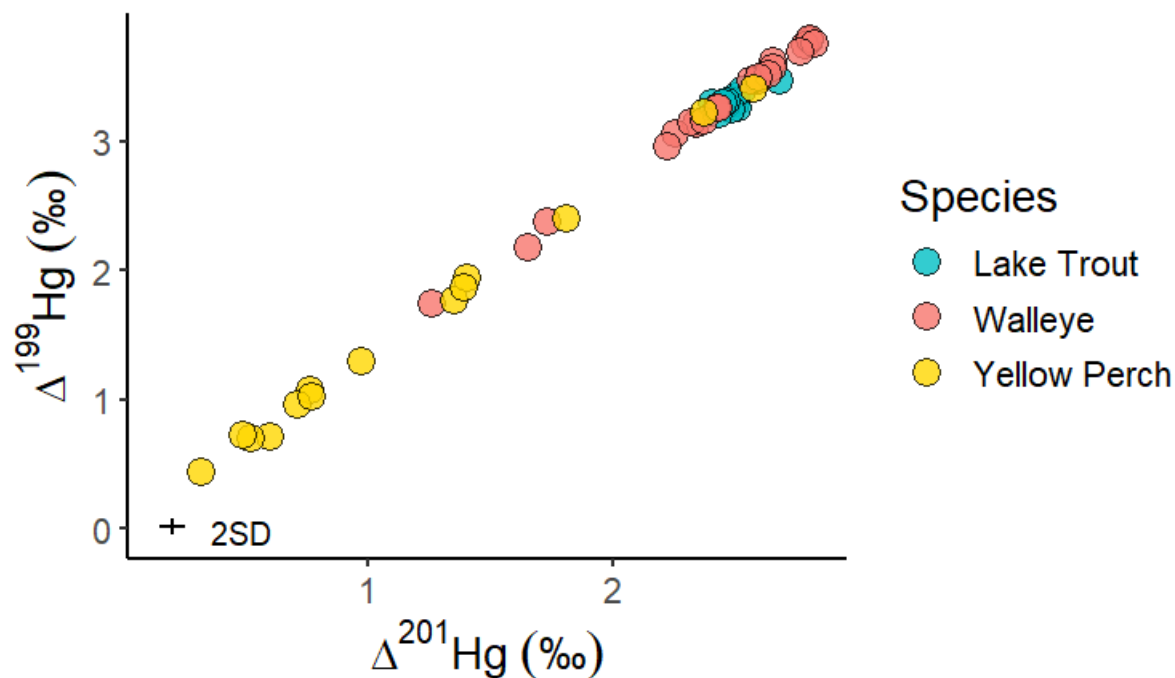


Figure 7.4) $\Delta^{201}\text{Hg}$ versus $\Delta^{199}\text{Hg}$ for subset of fish selected for isotopic analysis. Lake trout and walleye samples were analyzed as individuals, and yellow perch samples were analyzed as composites of 5 individuals. The 2SD marker denotes precision metrics as 2 times the standard deviation based on repeated measures. The ratio of $\Delta^{199}\text{Hg}$ to $\Delta^{201}\text{Hg}$ distinguishes two types of photochemical reactions; the slope of $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$ of 1.334 ± 0.010 indicates that photodegradation of MeHg (not photoreduction of Hg^{2+}) in the water column is the major reaction driving $\Delta^{199}\text{Hg}$ fractionation. This is typical of fish Hg, which is predominantly MeHg.

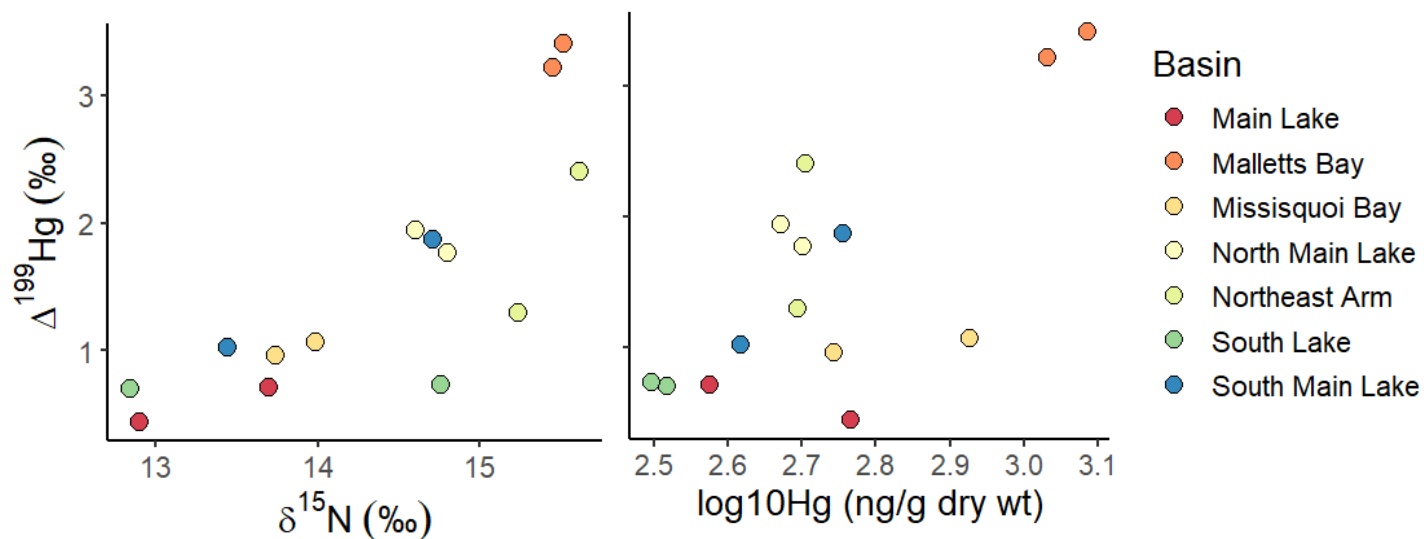


Figure 7.5) $\delta^{15}\text{N}$ versus $\Delta^{199}\text{Hg}$ ratios and $\log_{10}\text{Hg}$ concentrations in yellow perch across basins in Lake Champlain. Each data point is a composite of 5 individual yellow perch. The higher $\Delta^{199}\text{Hg}$ relative to $\delta^{15}\text{N}$ and $\log_{10}\text{Hg}$ are driven by the values in Malletts Bay, the most oligotrophic basin. Higher $\Delta^{199}\text{Hg}$ is driven by more light penetration in this oligotrophic (clear) basin; and higher $\log_{10}\text{Hg}$ are also related to higher Hg bioaccumulation in more oligotrophic waters. The higher $\delta^{15}\text{N}$ in Malletts Bay and apparent relationship with $\Delta^{199}\text{Hg}$ is unclear, and may be due to more pelagic than littoral feeding.

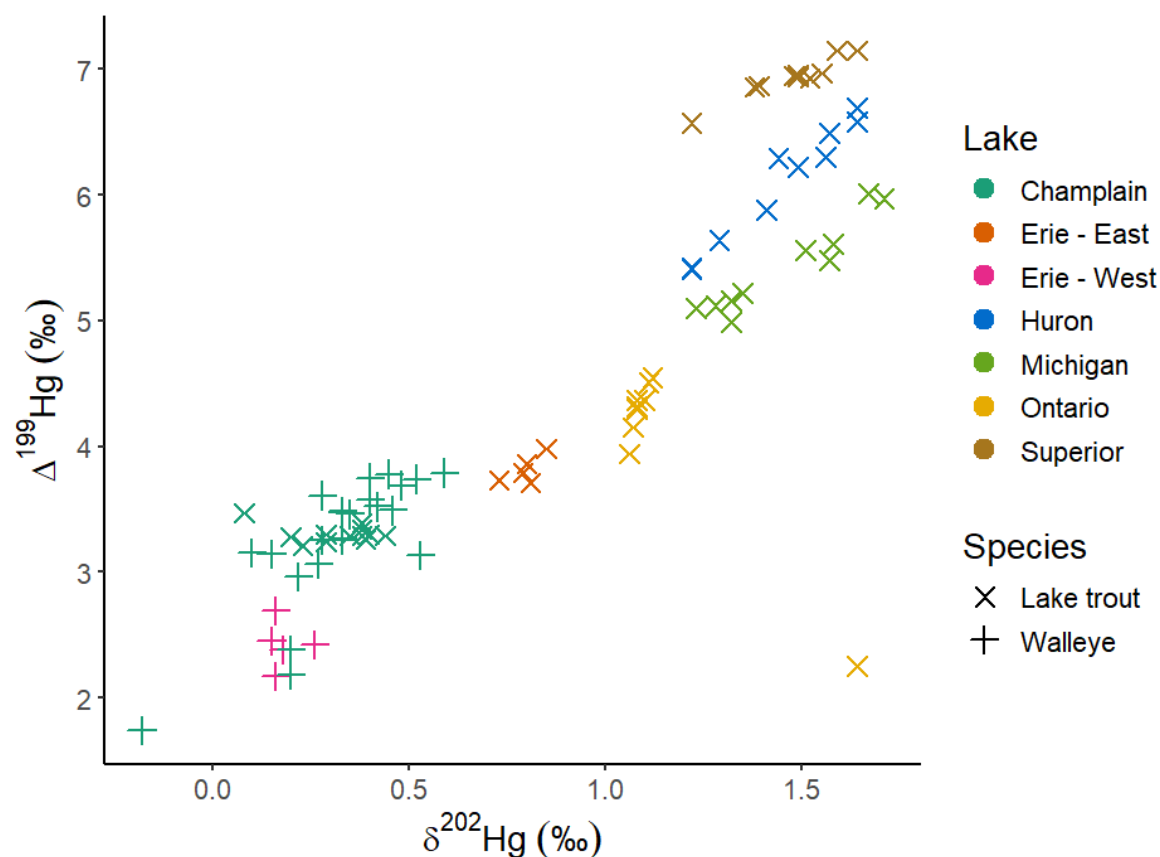


Figure 7.6) $\delta^{202}\text{Hg}$ versus $\Delta^{199}\text{Hg}$ ratio for lake trout and walleye from Lake Champlain (2022-2023) and the Great Lakes (2020-2021; data from Lepak et al., 2025). Fish from Lake Erie are divided into East and West as populations from these regions were found not to mix (Lepak et al., 2018). Across Champlain and the Laurentian Great Lakes, the values of both $\Delta^{199}\text{Hg}$ and $\delta^{202}\text{Hg}$ are driven by sunlight penetration, and reflect water clarity. The upper Great Lakes, which are most oligotrophic have the highest $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$.

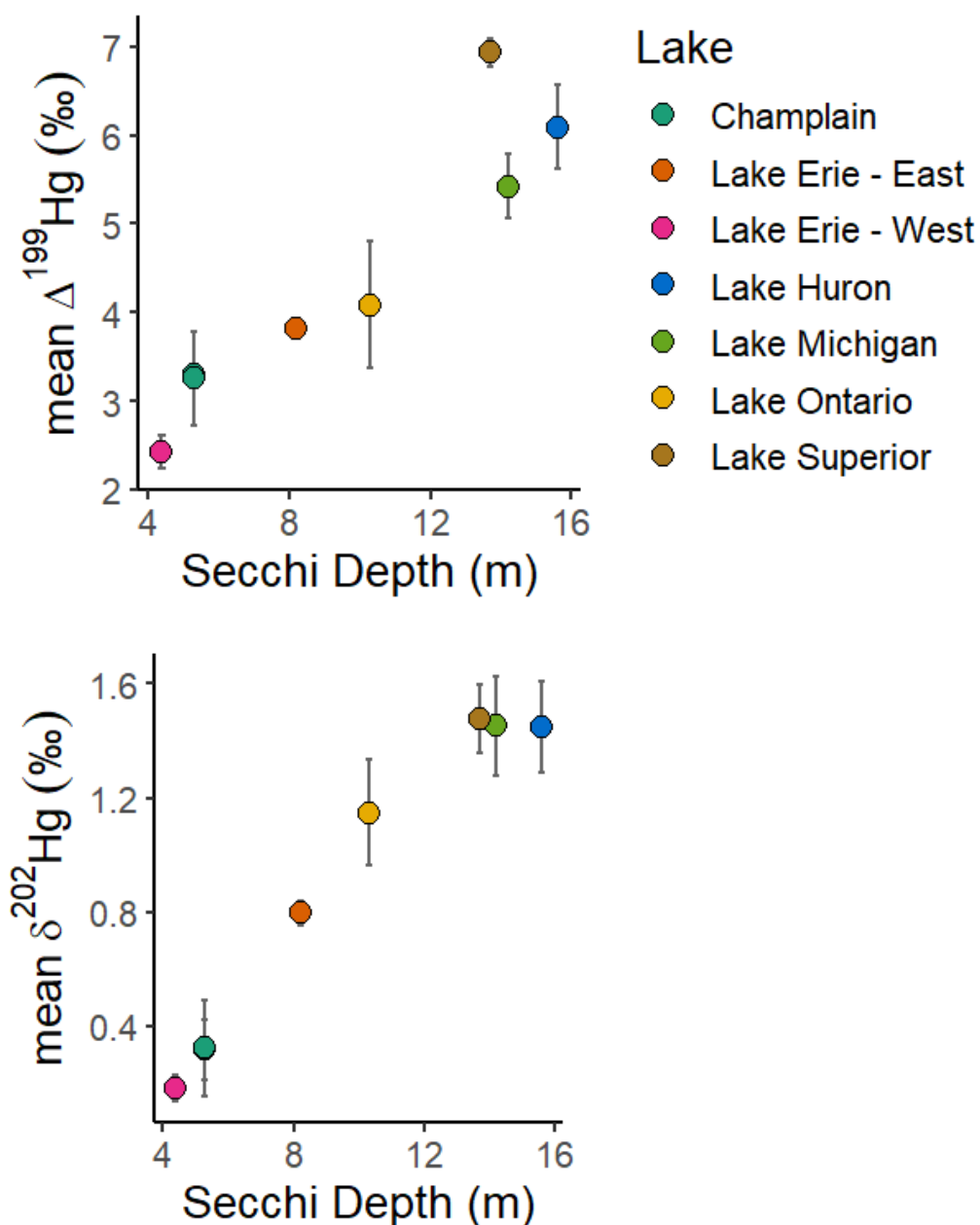


Figure 7.7) $\Delta^{199}\text{Hg}$ (top) and $\delta^{202}\text{Hg}$ (bottom) versus Secchi depth for lake trout and walleye from Lake Champlain (2022-2023) and the Great Lakes (2020-2021; data from Lepak et al., 2025). Fish from Lake Erie are divided into East and West as walleye populations from these regions were found to be distinct (Lepak et al., 2018). Secchi depth for Lake Champlain was taken from the VTDEC Longterm Monitoring database, using the mean value for the Main Lake Basin (5.3 m) in 2022-2023. Across Champlain and the Great Lakes, the values of both $\Delta^{199}\text{Hg}$ and $\delta^{202}\text{Hg}$ are strongly related to Secchi depth, a measure of water clarity.

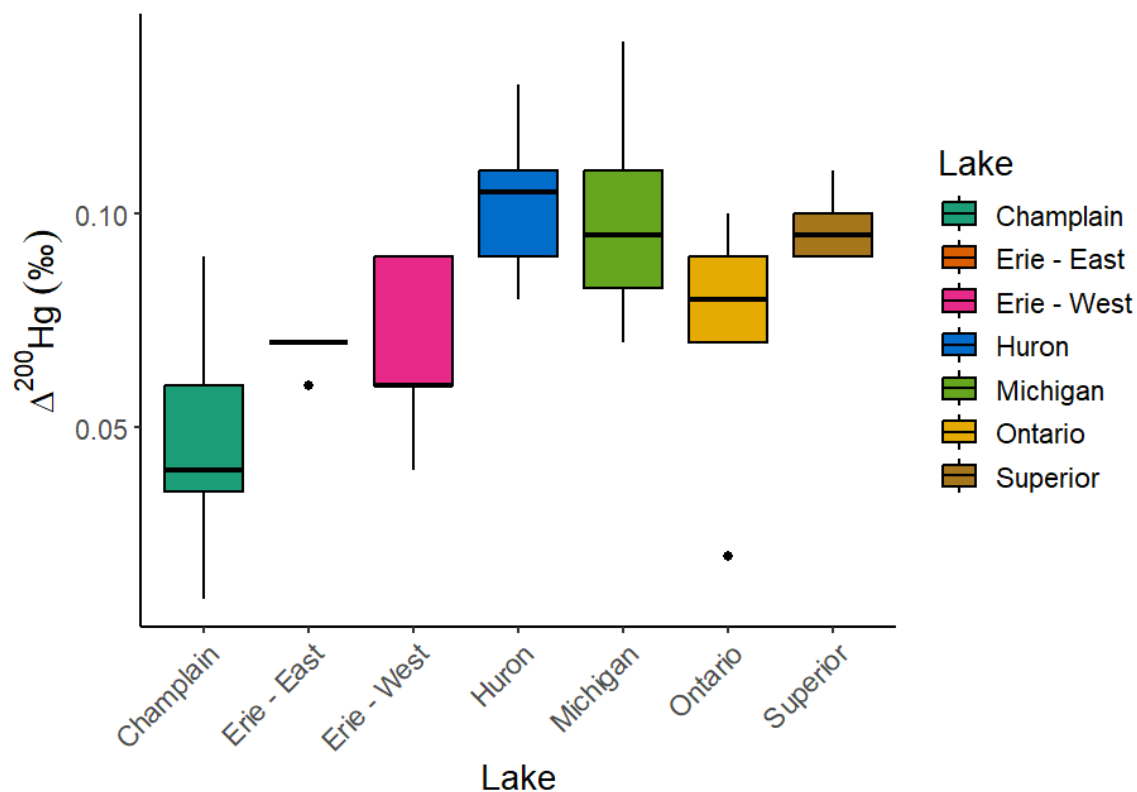


Figure 7.8) $\Delta^{200}\text{Hg}$ ratios for lake trout and walleye from Lake Champlain (2022-2023) and the Great Lakes (2020-2021; data from Lepak et al., 2025). Fish from Lake Erie are divided into East and West as walleye populations from these regions were found to be distinct (Lepak et al., 2018). $\Delta^{200}\text{Hg}$ ratios are driven by precipitation-derived Hg; Lake Champlain has a lower proportion of Hg from wet deposition relative to the Great Lakes.

Task 8: Water column sampling and analysis.

All Hg data and ancillary measures were submitted to LCBP on 05/20/2024.

Depth profiles of MeHg and THg (both filtered (ng/L) and particulate (ng/g)) are shown in Figures 8.1 to 8.4. There were no discernable trends in MeHg or Hg with depth. Enriched MeHg in the hypolimnion of smaller lakes has been observed when bottom waters of lakes become anoxic, attributed to methylation of Hg by anaerobic bacteria in the water column and at the sediment water interface (Armstrong et al., 2025a; Eckley and Hintelmann, 2006; Todorova et al., 2009). However, MeHg enrichment was not evident in the deep waters of the Laurentian Great Lakes (Mason and Sullivan, 1997), where Hg concentrations are low and water circulation limits oxygen depletion. In Lake Champlain hypoxia only rarely occurs in the deep Main Lake basins, and usually in the Otter Creek section (South Main Lake) in late summer, where river influence is strong and water circulation is limited. Malletts Bay can become hypoxic under calm conditions in late summer. The shallow, nearshore bays (Missisquoi and St. Albans) are polymictic, and can become anoxic during dense algal blooms. The year of sampling (2023) had unusually frequent summer storms, including a 1000-year storm on July 10-11, 2023, and lake water levels were uncharacteristically high throughout the year (Figure 8.5). Periods of hypoxia were not captured at any site in this study, though Malletts Bay and Missisquoi Bay became briefly hypoxic in September (Lake Champlain Long-term Monitoring Program data) after sampling was complete.

Concentrations of MeHg in the epilimnion of the lake and in tributaries (both filtered and particulate) are shown in Tables 8.1 to 8.6. Concentrations of MeHg in the water column of the lake were low and in a similar range to our earlier study (Jackson et al., 2009; Miller et al., 2012). In the Main Lake basins and Malletts Bay, filtered and volumetric particulate MeHg concentrations (ng/L) were similar across basins and time points, with the exception of an increase in South Main Lake at the 08/02/23 time point. In the eutrophic shallow bays, dissolved MeHg increased between June and late August in St. Albans Bay; whereas in Missisquoi Bay, concentrations peaked on (07/19/23) following the storm. Concentrations of particulate MeHg (ng/g) in the water column declined across all basins of the lake from June to late August.

As is typical, concentrations of MeHg (ng/L) were higher in the rivers than the lake sites. Discharge in the rivers and sampling time points are shown in Figure 8.6. Mill River is a much smaller stream than the other two tributaries, with lower discharge. Concentrations of MeHg were previously found to increase with high flow conditions in Lake Champlain tributaries (Shanley and Chalmers, 2012), but in this study were only elevated in the Missisquoi in the July sampling time points. Concentrations of MeHg are not consistently related to river discharge (Riscassi et al., 2016), and may vary with landscape characteristics and connectivity under different flow conditions.

Dissolved concentrations of THg in the water column of the lake were elevated in June in the North Main Lake and in Missisquoi Bay following the storm (Table 8.4). The high North Main Lake Hg was also evident in the gravimetric particulate fraction (ng/g) (Table 8.5), though the cause is unknown. Concentrations of dissolved Hg were also elevated in the Missisquoi River following the storm. The highest particle loads of Hg (ng/L) occurred following the storm for the Missisquoi and Lamoille Rivers, and their adjoining basins (Missisquoi and Malletts) (Table 8.6).

The smaller Mill River did not have increased Hg concentrations in the July samples following the storm.

Concentrations of MeHg in plankton/ seston (>150 μm) in the epilimnion and hypolimnion of six Lake Champlain basins are shown in Figure 8.7 to 8.9. In the Main Lake basins (Figure 8.7), MeHg concentrations in plankton were similar between basins and between the hypolimnion and epilimnion of the lake in June. In early August, concentrations in the hypolimnion remained similar to June values in the North Main basin, and increased in the Main and South Main basin. By contrast, MeHg in the epilimnion decreased compared with the June concentrations. In August, concentrations in plankton in the hypolimnion increased in the North Main and Main basins, compared to July, and stayed constant in the South Main basin. In the epilimnion, concentrations increased from July to August, but remained much lower than in the hypolimnion. In Malletts Bay (Figure 8.8), MeHg concentrations in plankton were similar among the epilimnion and hypolimnion, and between June and July time points. In late August, MeHg concentrations increased in the hypolimnion and decreased in the epilimnion. In the two shallow bays, Missisquoi and St. Albans, MeHg in plankton had different temporal patterns, decreasing from June to early September in Missisquoi and increasing over the same timeframe in St. Albans (Figure 8.9).

Coarse plankton taxonomy in the epilimnion and hypolimnion of each basin was assessed and zooplankton versus phytoplankton ratios were determined based on cell area. Zooplankton Hg data was also compared with LCRI's zooplankton density data from May to October (Figure 8.10); this survey did not separate plankton by strata (eg. samples were taken from vertical tows across the entire water column at each basin).

Table 8.1 Filtered MeHg concentrations (ng/L) in epilimnion waters of Lake Champlain basins and tributaries.

SITE	DATE						
	6/5/2023	6/21/2023	7/17/2023	7/19/2023	8/2/2023	8/28/2023	9/01/2023
North Main		0.022 ± 0.004			0.023 ± 0.001	0.030 ± 0.007	
Main		0.049 ± 0.035			0.022 ± 0.003	0.026 ± 0.004	
South Main		0.027 ± 0.004			0.066 ± 0.012	0.031 ± 0.003	
Malletts		0.028 ± 0.006	0.033 ± 0.002			0.023 ± 0.006	
St. Albans	0.026 ± 0.001			0.029 ± 0.002			0.046 ± 0.024
Missisquoi	0.037 ± 0.010			0.085 ± 0.040			0.054 ± 0.000
Lamoille River		0.092 ± 0.005	0.101 ± 0.007			0.079 ± 0.001	
Mill River	0.169 ± 0.011			0.142 ± 0.019			0.096 ± 0.000
Missisquoi River	0.061 ± 0.003			0.124 ± 0.008			0.071 ± 0.002

Table 8.2 Gravimetric particulate MeHg concentrations (ng/g) in epilimnion waters of Lake Champlain basins and tributaries.

Site	DATE						
	6/5/2023	6/21/2023	7/17/2023	7/19/2023	8/2/2023	8/28/2023	09/01/2023
North Main		12.693 ± 2.353			3.892 ± 0.213	5.773 ± 0.830	
Main		11.345 ± 1.251			3.302 ± 1.353	4.987 ± 0.605	
South Main		14.116 ± 3.809			8.835 ± 0.840	7.423 ± 1.571	
Malletts		14.137 ± 1.501	2.225 ± 0.176			9.132 ± 0.799	
St. Albans	14.738 ± 7.823			6.334 ± 0.738			4.753 ± 0.059
Missisquoi	14.740 ± 2.910			11.235 ± 2.456			4.444 ± 0.491
Lamoille River		3.085 ± 0.111	0.997 ± 0.089			4.656 ± 0.351	

Mill River	15.053 ± 9.667			8.676 ± 0.708			5.020 ± 1.016
Missisquoi River	14.757 ± 6.852			2.463 ± 0.311			3.595 ± 0.783

Table 8.3 Volumetric particulate MeHg concentrations (ng/L) in epilimnion waters of Lake Champlain basins and tributaries.

Site	DATE						
	6/5/2023	6/21/2023	7/17/2023	7/19/2023	8/2/2023	8/28/2023	09/01/2023
North Main		0.007 ± 0.001			0.011 ± 0.001	0.009 ± 0.002	
Main		0.013 ± 0.002			0.012 ± 0.005	0.006 ± 0.001	
South Main		0.032 ± 0.010			0.039 ± 0.005	0.012 ± 0.003	
Malletts		0.016 ± 0.002	0.021 ± 0.002			0.011 ± 0.001	
St. Albans	0.018 ± 0.009			0.025 ± 0.003			0.052 ± 0.000
Missisquoi	0.043 ± 0.008			0.073 ± 0.010			0.047 ± 0.004
Lamoille River		0.047 ± 0.002	0.061 ± 0.008			0.024 ± 0.006	
Mill River	0.030 ± 0.005			0.017 ± 0.002			0.030 ± 0.003
Missisquoi River	0.036 ± 0.016			0.035 ± 0.006			0.028 ± 0.001

Table 8.4 Filtered THg concentrations (ng/L) in epilimnion waters of Lake Champlain basins and tributaries.

Site	DATE						
	6/5/2023	6/21/2023	7/17/2023	7/19/2023	8/2/2023	8/28/2023	9/01/2023
North Main		0.837 ± 0.325			0.195 ± 0.024	0.284 ± 0.021	
Main		0.341 ± 0.036			0.260 ± 0.045	0.258 ± 0.028	
South Main		0.279 ± 0.072			0.405 ± 0.033	0.253 ± 0.011	
Malletts		0.329 ± 0.020	0.272 ± 0.031			0.369 ± 0.027	
St. Albans	0.169 ± 0.029			0.214 ± 0.022			0.232 ± 0.001
Missisquoi	0.380 ± 0.101			1.366 ± 0.732			0.465 ± 0.180

Lamoille River		1.362 ± 0.114	0.682 ± 0.005			0.538 ± 0.022	
Mill River	0.605 ± 0.092			0.685 ± 0.078			0.818 ± 0.132
Missisquoi River	0.230 ± 0.011			1.313 ± 0.014			0.818 ± 0.109

Table 8.5 Gravimetric THg concentrations (ng/g) in the seston of epilimnion waters of Lake Champlain basins and tributaries.

Site	DATE						
	6/5/2023	6/21/2023	7/17/2023	7/19/2023	8/2/2023	8/28/2023	9/01/2023
North Main		184.9 ± 157.1			48.5 ± 22.4	53.7 ± 11.0	
Main		106.1 ± 14.4			67.9 ± 18.7	47.1 ± 10.1	
South Main		32.5 ± 18.4			51.0 ± 8.2	55.7 ± 4.5	
Malletts		86.8 ± 12.0	83.3 ± 19.1			85.3 ± 9.5	
St. Albans	120.9 ± 23.5			59.0 ± 11.2			55.6 ± 3.9
Missisquoi	81.9 ± 2.9			150.0 ± 14.4			38.0 ± 1.4
Lamoille River		73.4 ± 10.0	44.0 ± 2.34			86.3 ± 26.3	
Mill River	122.5 ± 96.0			124.0 ± 20.5			84.2 ± 11.4
Missisquoi River	120.1 ± 16.2			96.6 ± 0.9			95.6 ± 13.4

Table 8.6 Volumetric THg concentrations (ng/L) in the seston of epilimnion waters of Lake Champlain basins and tributaries.

Site	DATE						
	6/5/2023	6/21/2023	7/17/2023	7/19/2023	8/2/2023	8/28/2023	9/01/2023
North Main		0.101 ± 0.074			0.140 ± 0.067	0.084 ± 0.016	
Main		0.126 ± 0.014			0.248 ± 0.068	0.053 ± 0.015	
South Main		0.076 ± 0.050			0.226 ± 0.034	0.085 ± 0.006	
Malletts		0.101 ± 0.010	0.768 ± 0.156			0.105 ± 0.006	
St. Albans	0.144 ± 0.028			0.227 ± 0.039			0.611 ± 0.057
Missisquoi	0.240 ± 0.004			0.978 ± 0.017			0.405 ± 0.007
Lamoille River		1.107 ± 0.156	2.695 ± 0.245			0.420 ± 0.014	
Mill River	0.230 ± 0.006			0.247 ± 0.039			0.510 ± 0.014
Missisquoi River	0.294 ± 0.032			1.380 ± 0.060			0.754 ± 0.091

Table 8.7 TSS concentrations (mg/L) in the seston of epilimnion waters of Lake Champlain basins and tributaries.

Site	DATE						
	6/5/2023	6/21/2023	7/17/2023	7/19/2023	8/2/2023	8/28/2023	9/01/2023
North Main		0.583 ± 0.086			2.855 ± 0.186	1.571 ± 0.117	
Main		1.189 ± 0.113			3.655 ± 0.171	1.174 ± 0.113	
South Main		2.276 ± 0.225			4.456 ± 0.603	1.559 ± 0.083	
Malletts		1.166 ± 0.055	9.278 ± 0.509			1.237 ± 0.135	
St. Albans	1.195 ± 0.001			3.858 ± 0.065			11.013 ± 0.228
Missisquoi	2.937 ± 0.063			6.558 ± 0.518			10.653 ± 0.202
Lamoille River		15.080 ± 0.059	61.254 ± 2.237			5.153 ± 1.763	
Mill River	2.678 ± 2.047			1.992 ± 0.016			6.086 ± 0.675
Missisquoi River	2.454 ± 0.065			14.277 ± 0.486			8.021 ± 2.097

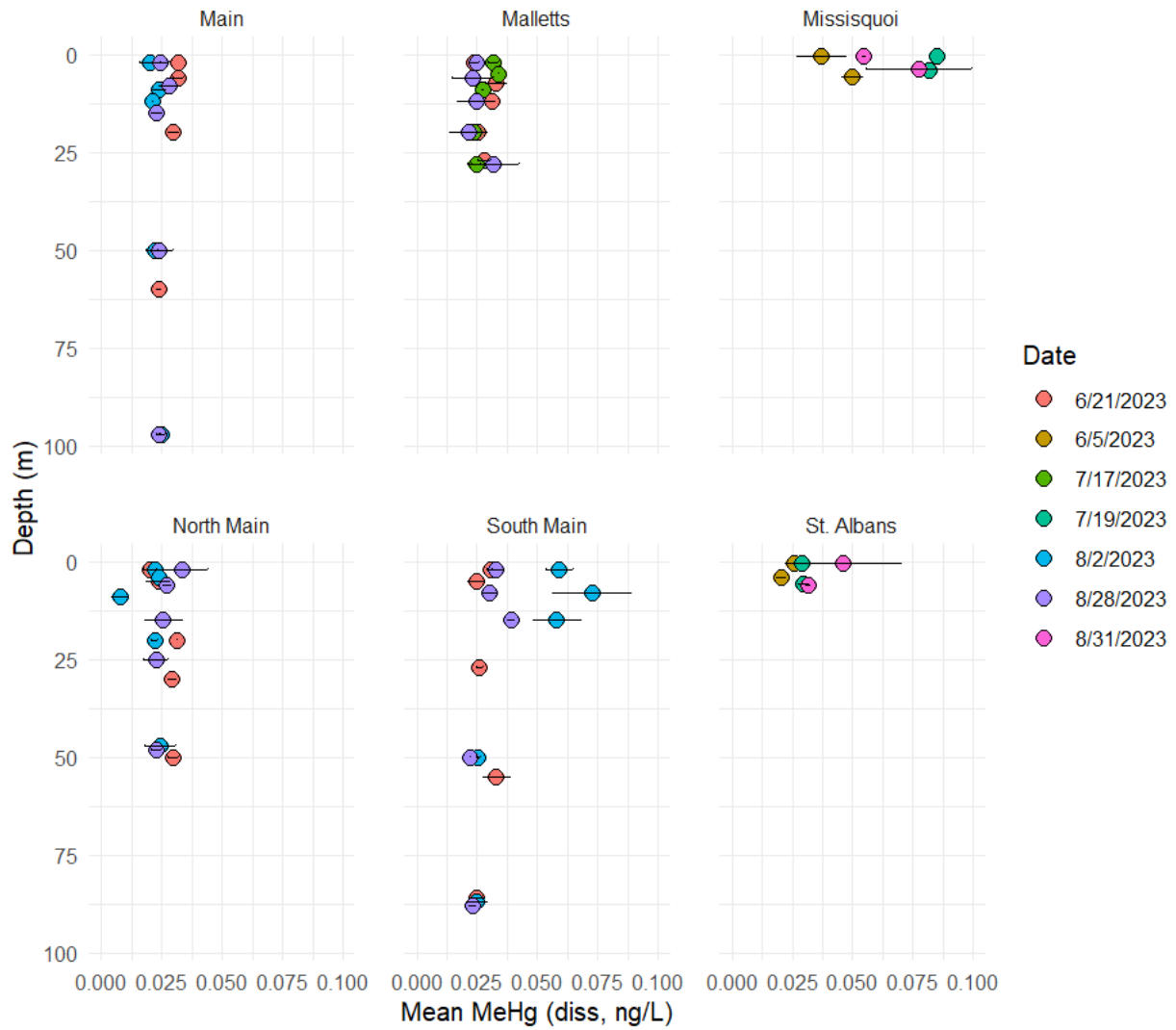


Figure 8.1 Depth profiles of MeHg concentration (ng/L; mean \pm SD) in filtered water samples from each lake site.

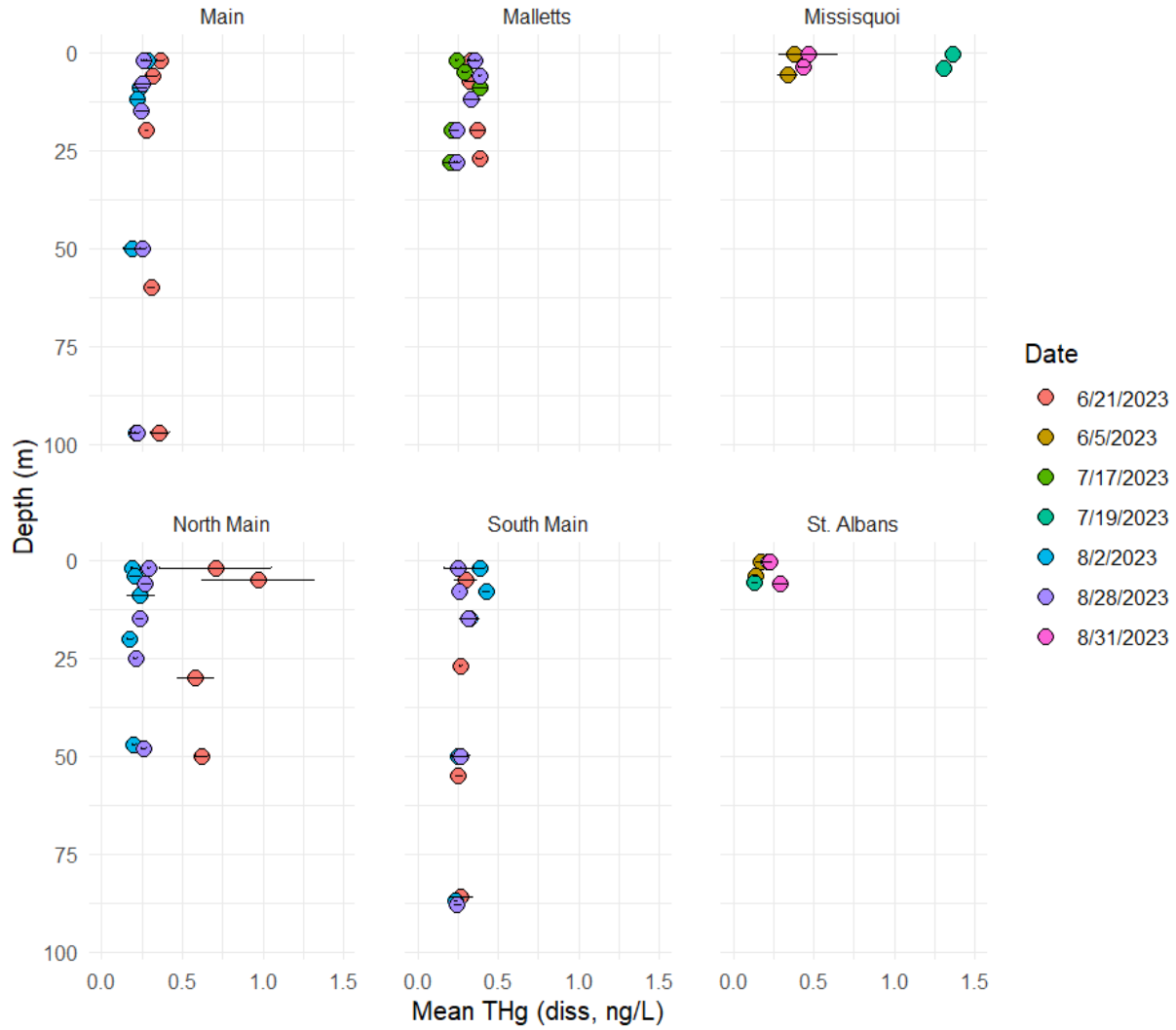


Figure 8.2 Depth profiles of THg concentration (ng/L; mean \pm SD) in filtered water samples from each lake site.



Figure 8.3 Depth profiles of MeHg concentration (ng/g; mean \pm SD) in particles from each lake site.



Figure 8.4 Depth profiles of THg concentration (ng/g; mean \pm SD) in particles from each lake site.

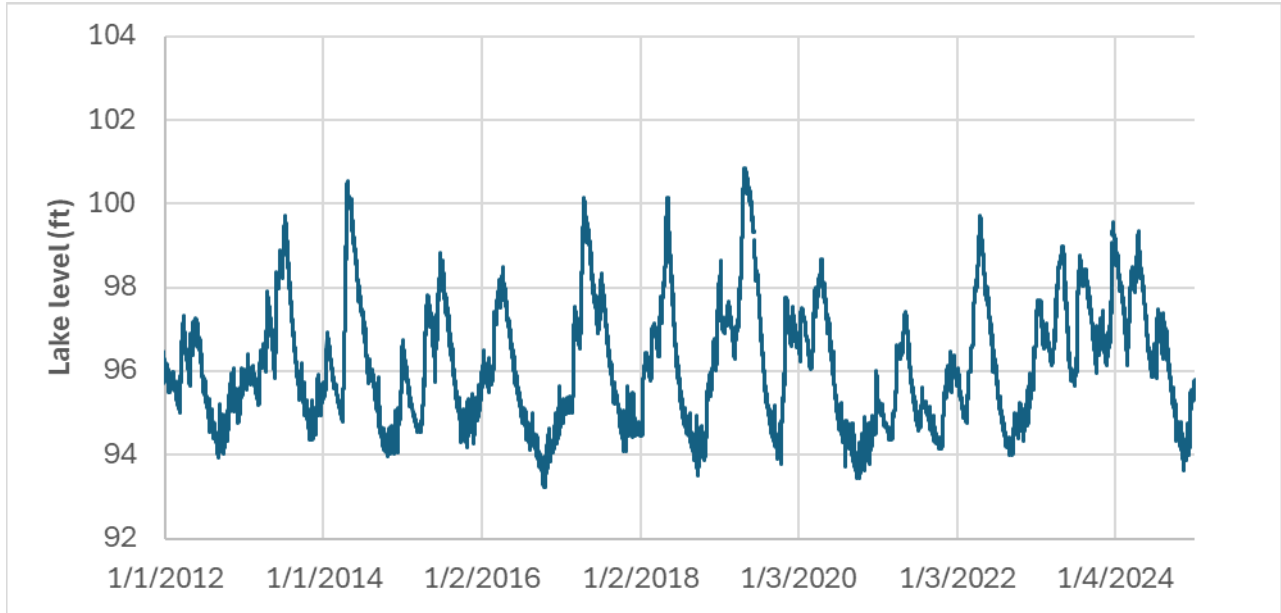


Figure 8.5 Lake levels (from Rouse's Point gauge) from 2012 to 2024, showing the unusually high levels throughout the summer in 2023.

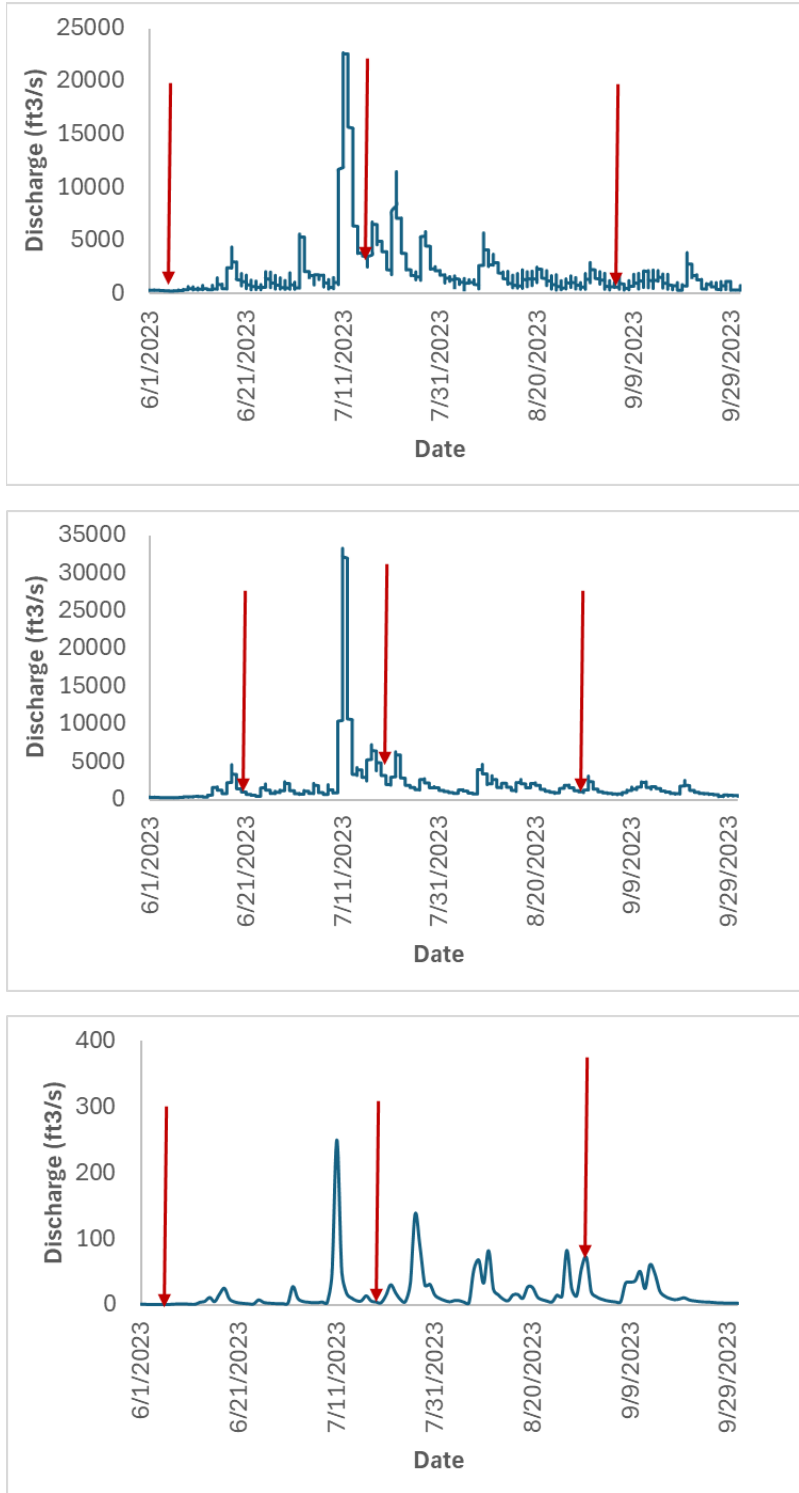


Figure 8.6 River discharge (ft³/s) in a) Missisquoi (from Swanton gauge), b) Lamoille (from East Georgia gauge, and c) Mill River (from Georgia Shore Rd gauge). Sampling time points are shown in red.

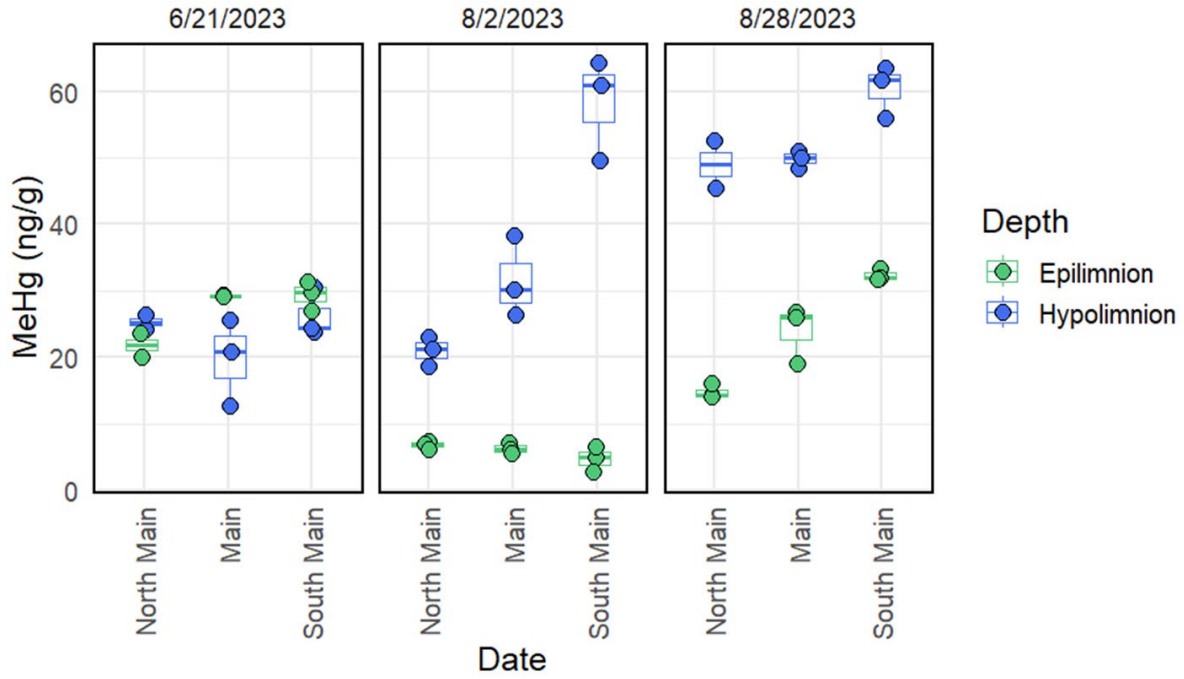


Figure 8.7 Methylmercury concentration (ng/g) in plankton from the epilimnion and hypolimnion of North Main, Main and South Main basins.

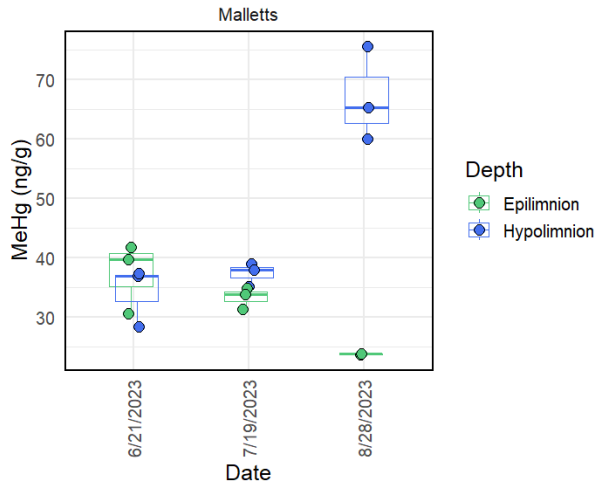


Figure 8.8 Methylmercury concentration (ng/g) in plankton from the epilimnion and hypolimnion of Malletts basin.

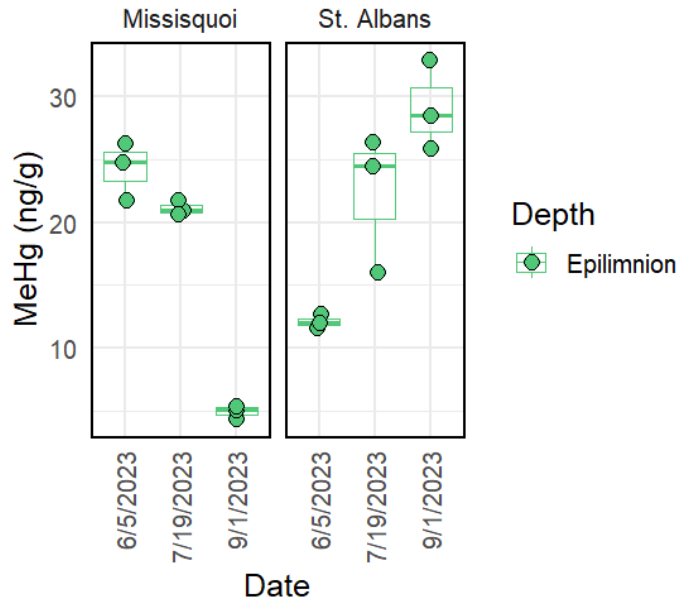


Figure 8.9 Methylmercury concentration (ng/g) in plankton from Missisquoi and Malletts Bays.

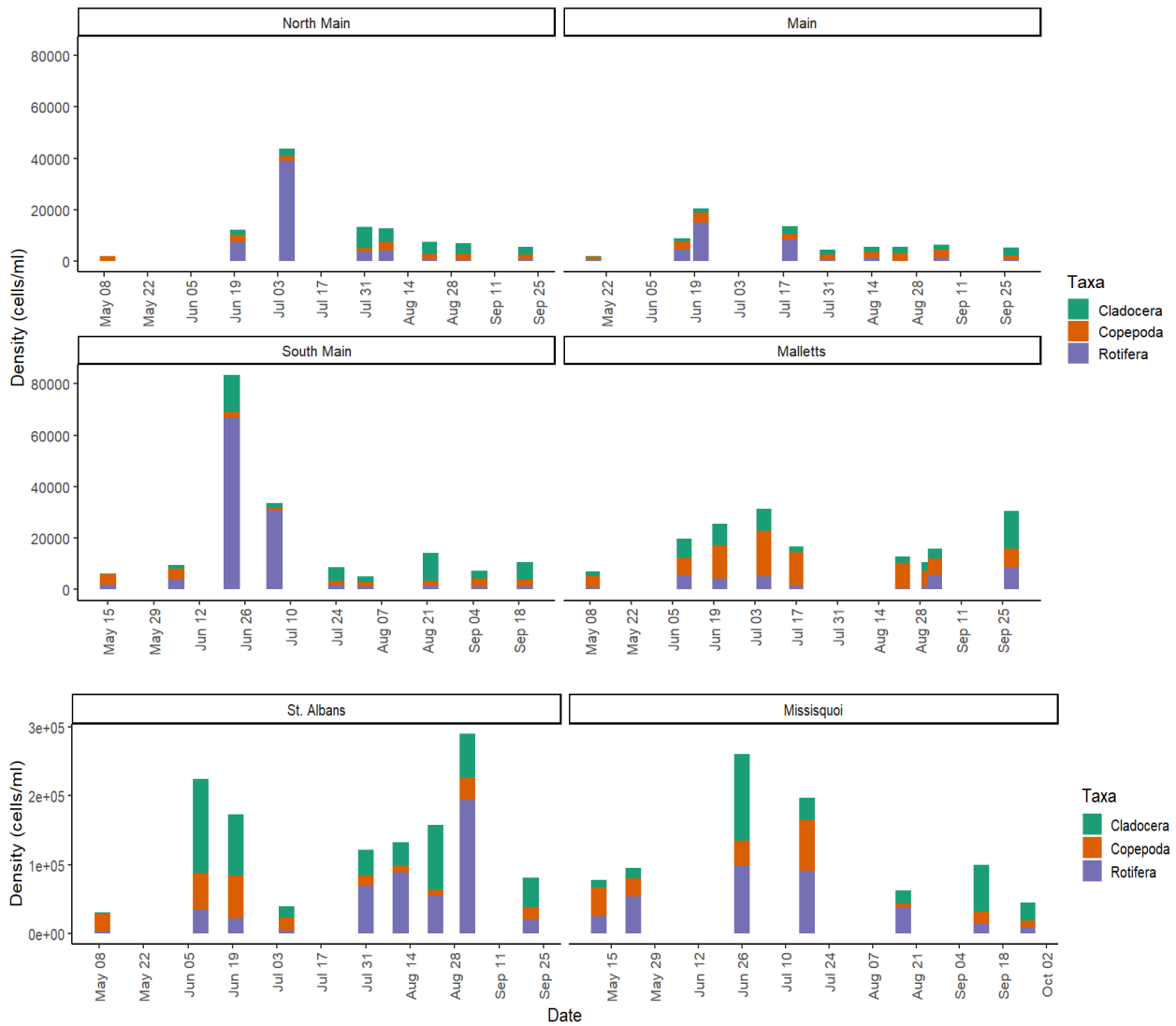


Figure 8.10 Zooplankton densities from the Lake Champlain Long-term Monitoring Program across basins in 2023 (from T. Mihuc).

Task 9: Evaluate water column drivers of Hg bioaccumulation.

Water, particulate and plankton sampling captured three timepoints: one in June, another in mid-July (rivers and nearshore basins) or early August (deep basins), and a third in late August/early September. The large storm on July 10-11 caused a major disturbance of water quality conditions, particularly in the nearshore basins that were sampled the following week; this introduced a confounding factor to trend analysis across time points. Correlation coefficients between Hg species and water quality parameters (chl-a, FDOM, temperature and TSS) across time points in the epilimnion are shown in Table 9.1, with samples grouped by deep Main Lake basins (North Main, Main, and South Main), Malletts Bay, the shallow bays (St. Albans and

Missisquoi) and the rivers (Mill, Missisquoi and Lamoille), to examine differences in drivers across these different regions of the lake and its tributaries.

Water and particulate: In the lake basins, dissolved MeHg (ng/L) concentrations were low with little variability across time points (Table 8.1), making it difficult to assess correlations with other parameters. In the deep basins, concentrations of dissolved MeHg were correlated to chl-a, though the relationship was driven by high values on 08/02/23 in the South Main basins (Figure 8.1). Particulate MeHg (ng/g) concentrations were strongly and negatively correlated with temperature, as concentrations decreased from June to late August. In the deep lake basins, particulate are predominantly phytoplankton, and a decline in MeHg in the seston in late summer is consistent with bloom dilution (Chen et al., 2012; Chen and Folt, 2005), where higher bloom densities associated with higher water temperatures in late summer reduce MeHg bioaccumulation from the water column. In this dataset the correlation is with temperature but not chlorophyll. Chlorophyll concentrations throughout the summer are plotted in Figure 9.1, showing peak blooms occurring just before the 08/02/23 and 08/28/23 sampling time points. The lack of correlation between particulate MeHg and Chl-a is driven by the outlying high concentrations of MeHg in the particulate in South Main on 08/02/23; elevated MeHg was also present in the dissolved phase at this time point, the cause of which is uncertain. A weak negative correlation between particulate MeHg and FDOM was also observed in the Main Lake basins, likely attributed to the increase in autochthonous DOM in the late summer (Figure 9.2). Chl-a concentrations from the Lake Champlain Long-term Monitoring Program survey show a dip in Chl-a on this date, and MeHg in the water column may be driven by a bloom die off. A weak negative correlation between particulate MeHg and FDOM was also observed in the Main Lake basins, likely attributed to the increase in autochthonous DOM in the late summer (Figure 9.2). Overall, the broad pattern of decreasing particulate MeHg with higher water temperatures in late summer when phytoplankton density is higher is consistent with our previous study (Chen et al., 2012; Miller et al., 2012).

In Malletts Bay, particulate MeHg concentrations and dissolved total Hg concentrations were strongly negatively associated with total suspended solids. Malletts Bay was evaluated separately from the Main Lake basins due to the strong influence of the Lamoille River following the storm. TSS in the July 17th samples were of terrestrial rather than algal origin, and a band of high turbidity was observed at ~12m depth from river water entering the basin. Terrestrial particles do not efficiently bioaccumulate MeHg and typically have much lower MeHg concentrations than algal particles, driving the negative relationship with TSS across time points. Dissolved THg concentrations were also lower at the July time point; the reason for this is unclear, it is possible the elevated particle load from the river sorbed Hg from the dissolved phase as it settled.

In the shallow bays, dissolved MeHg and THg were strongly correlated with FDOM. Concentrations of FDOM increased throughout the summer, driven by phytoplankton blooms and in Missisquoi Bay, inputs of terrestrial DOC. DOC serves to stabilize Hg and MeHg in the water column (Janssen et al., 2022; Lavoie et al., 2019), and these elements are often strongly linked. Dissolved MeHg was also related to Chl-a in the shallow basins, which may reflect MeHg from decaying algal cells. Particle MeHg concentrations were strongly, negatively associated with TSS in the shallow basins. These basins both experience dense algal blooms in late summer, and this relationship is likely due to bloom dilution.

In rivers, strong negative correlation coefficients were observed between particulate MeHg and THg (ng/g) concentrations and TSS, driven largely by the July time point. While the storm is associated with increased particle input to the basins, concentrations of Hg and particularly MeHg in those particles, which are predominantly glacial silt, are likely low (Goss et al., 2025) relative to algal particles.

Overall, bloom dynamics play a strong role in water column MeHg cycling. Across basins, there were higher concentrations of dissolved MeHg in the shallow, nearshore basins, particularly during the July time points. This pattern is attributed to DOC levels, which were elevated in the shallow basins due to eutrophication and river inputs. Particulate MeHg concentrations were similar across basins, and declined from June to August, consistent with bioaccumulation and bloom dilution of MeHg, with phytoplankton blooms increasing in late summer. Temporal correlations varied across basins, in part due to outliers caused by disturbances from site-specific responses to river discharge and bloom dynamics. In particular, Malletts Bay was highly turbid on the July sampling time point, whereas in other basins and time points, particles were mostly autochthonous. High dissolved and particulate MeHg concentrations in Otter Creek section of South Main Lake were elevated following a dense algal bloom.

Plankton: Concentrations of MeHg in plankton are shown in Figures 8.8 to 8.10. In the Main Lake basins, MeHg concentrations increase from June to late August in the hypolimnion samples, whereas concentrations decline from June to July in the epilimnion, and rebound in August (Figure 8.8). In Malletts Bay, MeHg concentrations were similar in the hypolimnion and epilimnion in June and July, then concentrations in the hypolimnion increased in August, whereas June concentrations declined (Figure 8.9). In the shallow bays where samples were taken from a single depth profile, concentrations declined across time points in the Missisquoi and increased in St. Albans Bay (Figure 8.10).

Plankton samples were collected with vertical tows using a 150 μm closing net. Sample composition can incorporate both phytoplankton and zooplankton species at this mesh size, which can confound interpretation of MeHg concentrations across trophic levels. To further interpret results, the approximate ratio of zooplankton to phytoplankton was estimated by microscopy (based on cell area) in samples from the Main Lake basins and Malletts Bay. The percent of zooplankton (relative to zooplankton + phytoplankton cells) in the epilimnion samples was between 1 and 8%, with the exception of the July sample from Malletts Bay, which contained no phytoplankton. By contrast, the hypolimnion samples were 100% zooplankton, with the exception of the 08/02/23 time point where the North Main, Main and South Main samples were 72%, 68% and 57% respectively. While this method was not applied to the shallow bay samples, in early September, plankton samples from Missisquoi were predominantly cyanobacteria with very few zooplankton cells present. The September 1 sample from St. Albans contained both zooplankton and cyanobacteria.

Ratios of $\delta^{15}\text{N}$, which reflects trophic level, are in general agreement with the taxonomy data. Plankton from the epilimnion were 2-3‰ higher in $\delta^{15}\text{N}$ ratio than those from the hypolimnion in the Main Lake samples (Figure 9.3), indicating a higher trophic level in the hypolimnion. The hypolimnion samples across time points had similar $\delta^{15}\text{N}$ ratios; if a significant portion of phytoplankton were present as suggested by the taxonomy data, there would likely be a decline in $\delta^{15}\text{N}$ in early August. The same pattern in $\delta^{15}\text{N}$ between the hypolimnion and epilimnion was evident in Malletts Bay (Figure 9.4), though this differed from the taxonomy data for the July

time point, which found no phytoplankton present. In the shallow basins, $\delta^{15}\text{N}$ varied across time points, with the lowest values in June in the Missisquoi Basin, and in late August in St. Albans.

In zooplankton, large differences in MeHg concentrations between the hypolimnion and epilimnion of the deep basins are likely explained by the higher proportion of zooplankton in the deeper samples. Samples from the photic zone incorporate a large percentage of phytoplankton, which have lower MeHg concentrations due to their lower trophic level. This is a limitation of sampling by mesh size (Armstrong et al., 2025a), though the need for sufficient biomass to analyze MeHg precludes other sampling methods (eg. picking). The lower MeHg concentrations in the epilimnion than hypolimnion appear to be largely driven by differences in sample composition between the two compartments, however, it is noted that bioaccumulation in the hypolimnion may be more efficient due to negligible phytoplankton densities below the photic zone, and lower concentrations of DOC. These effects are difficult to distinguish as hypolimnion particulate samples likely include particles deposited from the epilimnion, and many zooplankton species migrate between compartments.

However, the temporal patterns in zooplankton MeHg are not explained by sample composition. The percentage of zooplankton was consistently much lower in the epilimnion, yet in the June time point, there is little difference between the hypolimnion and epilimnion MeHg concentrations, and in late August, hypolimnion concentrations increase, despite decreasing MeHg concentrations in the particles between June and August (Table 8.2). This suggests that while MeHg concentrations in the phytoplankton are declining due to biodilution, concentrations are increasing at higher trophic levels. A possible explanation lies in the zooplankton taxonomy across months. From the Lake Champlain Long-term Monitoring Program surveys (Figure 8.10), zooplankton densities in the Main Lake basins have a large fraction of rotifera, which declines later in the summer along with overall zooplankton densities, making cladocera the dominant sub-class of zooplankton. Higher uptake of MeHg has been observed in cladoceran species relative to rotifera (Stewart et al., 2008) and copepods (Pickhardt et al., 2005). Furthermore, zooplankton biomass can be negatively correlated with zooplankton MeHg concentration (Back et al., 2003; Kidd et al., 1999; Miller et al., 2012). The shift from high total zooplankton densities dominated by small-bodied rotifera in the early summer, to lower total densities and fewer rotifera in the mid to late summer may in part explain the pattern in MeHg concentrations across the Main Lake basin. In a long-term study of Onandaga Lake in New York, a shift in zooplankton assemblage toward more large-bodied cladoceran species coincided with an increase in zooplankton MeHg concentrations, despite an increase in eutrophication and chl-a concentrations over the same period (Todorova et al., 2015). A similar pattern may explain the seasonal trend in the Main Lakes.

In contrast to the Main Lake, zooplankton community in Malletts Bay have only a small percentage of rotifera, and a larger proportion of copepoda than the Main Lake basins across all time points. Total zooplankton densities are also relatively consistent throughout the season. Here and across basins, the increasing MeHg in zooplankton in spite of declining MeHg in the phytoplankton over time may reflect bioaccumulation of MeHg in these longer-lived higher trophic level organisms. Zooplankton efficiently absorb MeHg from ingestion and excrete MeHg slowly (Tsui and Wang, 2004) leading to bioaccumulation over the lifespan (which varies between species but typically 1-2 months). Changes in zooplankton MeHg concentrations may therefore respond slowly to changing phytoplankton MeHg concentrations and may continue to rise despite lower concentrations in food sources.

The opposing patterns of MeHg in plankton from the shallow basins may reflect both community composition and conditions. The low concentrations in September from the Missisquoi are likely due to the sample being predominantly cyanobacteria, with few zooplankton present. The higher concentrations of MeHg in the St. Albans Bay samples, which were taken during a time with higher zooplankton densities as well as elevated chl-a, may be explained by bioaccumulation over time, despite the biodiluting effects of eutrophication in the phytoplankton.

Overall, the concentrations of MeHg in plankton followed a pattern of increasing throughout the summer in the hypolimnion. These samples were found to be predominantly zooplankton (100% by microscopic methods) and a higher trophic than the epilimnion samples (by $\delta^{15}\text{N}$). In the Main Lake, the increases in MeHg concentrations may be influenced by decreases in zooplankton density and a shift from rotifera to cladocera being the major taxonomic group. However, bioaccumulation of MeHg in zooplankton over timescales of multiple weeks may explain the diverging patterns of MeHg in zooplankton and phytoplankton over the summer season in all basins. These observations are important to understanding bioaccumulation in the lake food web, where MeHg concentrations in fish particularly from deep basins frequently reach high levels.

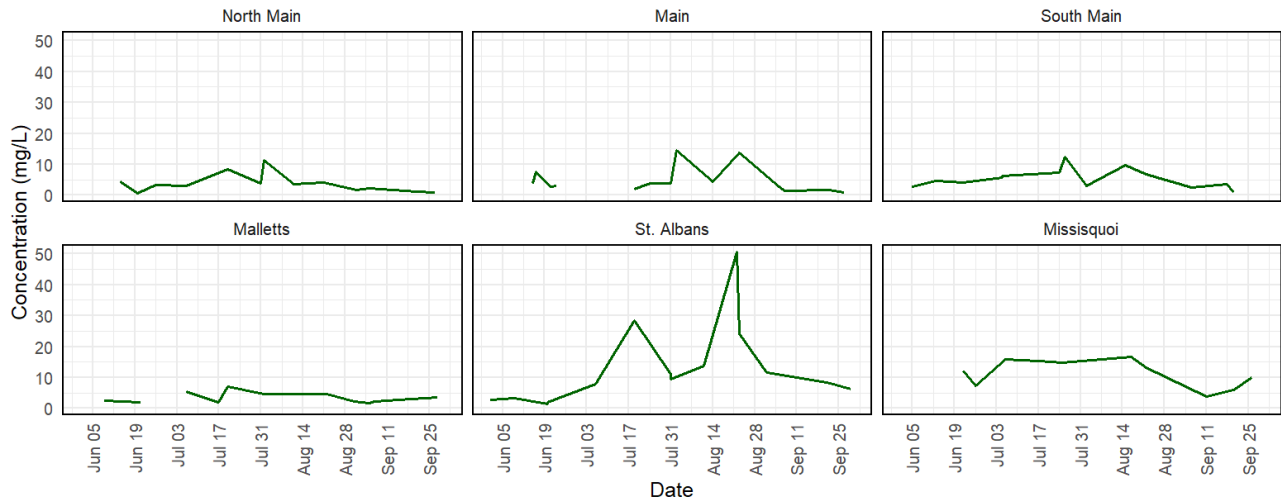


Figure 9.1 Chl-a concentration (mg/L) from June to October by basins, from the Lake Champlain Long-term Monitoring Program (2023).

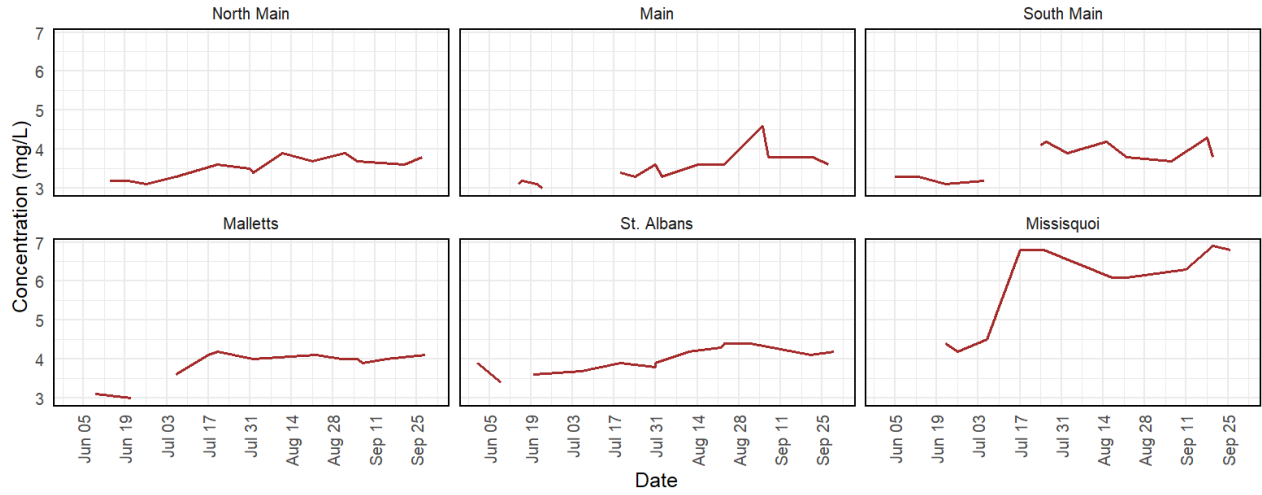


Figure 9.2 DOC concentration (mg/L) in the epilimnion, from June to October in by basin, from the Lake Champlain Long-term Monitoring Program (2023).

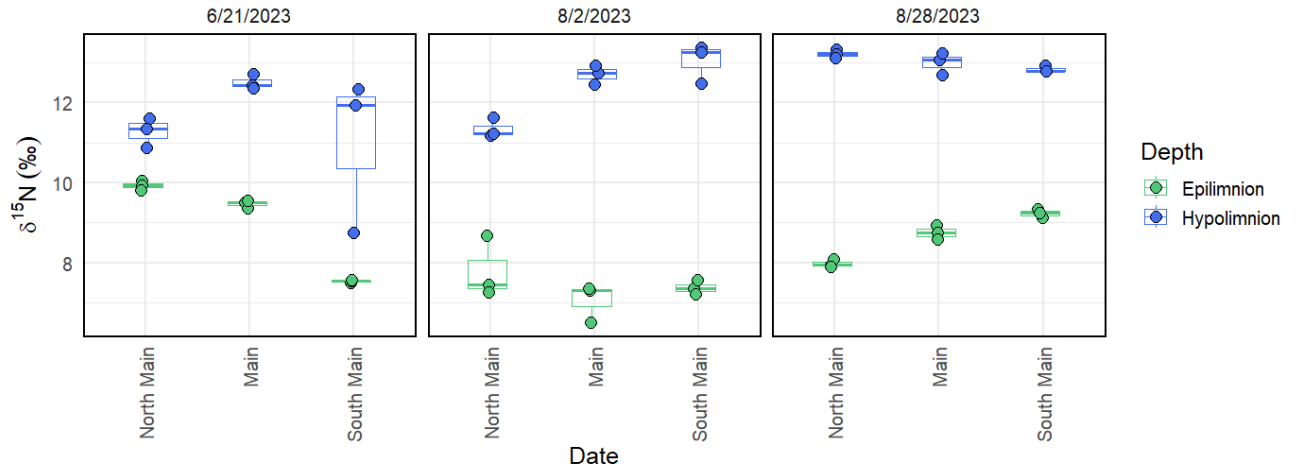


Figure 9.3 $\delta^{15}\text{N}$ ratios in plankton samples from the epilimnion and hypolimnion of the Main Lake Basins.

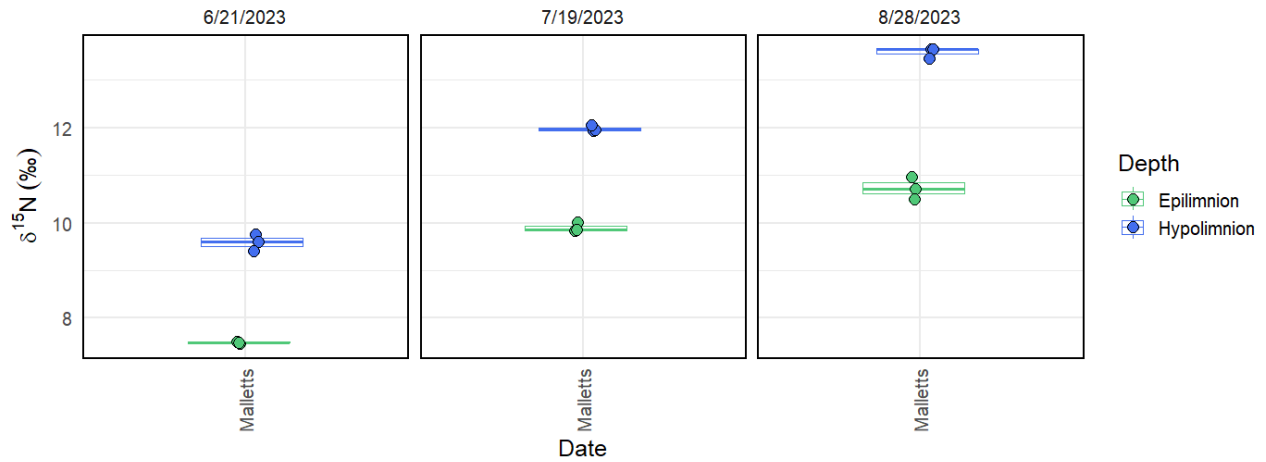


Figure 9.4 $\delta^{15}\text{N}$ ratios in plankton samples from the epilimnion and hypolimnion of the Malletts Basins.

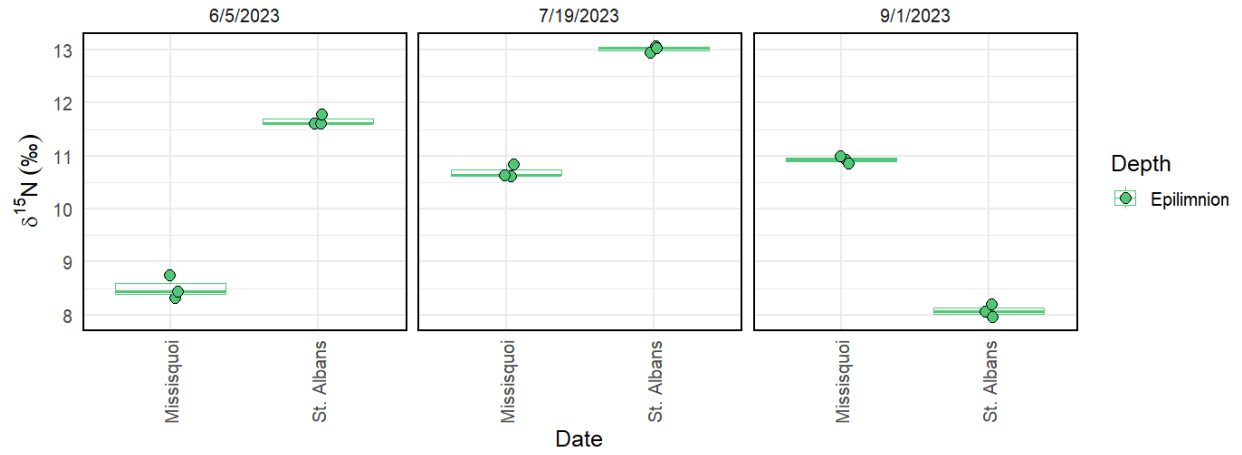


Figure 9.5 $\delta^{15}\text{N}$ ratios in plankton samples Missisquoi and St. Albans Basins.

Task 10: Analyze changes in MeHg relative to environmental stressors.

Submitted 08/05/2025.

Over the course of the Hg record in Lake Champlain game fish, Hg concentrations declined sharply in the 1980s to 2000's, in response to effective national and international regulation of Hg emissions, resulting in a decline in atmospheric Hg deposition documented in sediment records throughout the Northeast (Roberts et al., 2021; Taylor et al., 2022). In recent years, trends in fish Hg concentrations in Lake Champlain are no longer tracking atmospheric Hg deposition (Swinton and Nierzwicki-Bauer, 2019). A similar trend has been observed in the Great Lakes and in lakes throughout New York state (Richter and Skinner, 2020), and suggests that with declining atmospheric Hg concentrations, Hg concentrations in fish are predominantly driven by other processes. In the Great Lakes, this has been attributed to industrial and urban releases of Hg, and to the impacts of invasive species on Hg bioaccumulation (Lepak et al., 2019; Richter and Skinner, 2020). For Lake Champlain, we explore the recent trends in fish Hg concentrations and patterns of bioaccumulation, to try to better understand and predict their response to environmental change.

Long-term weather changes are expected to cause shifts in Hg bioaccumulation impacting Hg concentrations in top trophic level fish (Pinkney et al., 2015; Schartup et al., 2019). The Lake Champlain region is experiencing increases in temperature and precipitation (Guilbert et al., 2014), predicted to cause a higher number of growing degree days and longer periods of stratification, and higher levels of watershed runoff (Isles et al., 2017). The impacts of long-term weather changes are complex and can vary between systems. Our data was collected during a year with high levels of precipitation, and did not capture oxygen depletion with stratification, which has been associated with increased MeHg in the hypolimnion and in some cases a peak in MeHg in the epilimnion with fall turnover (Armstrong et al., 2025b; Todorova et al., 2009). Oxygen depletion in the Main Lake basins is rare, though it may play a role in Hg cycling in the polymictic shallow basins. However, we did observe an increase in MeHg in deep water zooplankton over the course of the summer, suggesting longer growing seasons may lead to an increase in MeHg in the food web. In a study of six small Wisconsin lakes, growing degree days were negatively correlated with interannual changes in Hg concentration in young-of-year yellow perch, attributed to biodilution (Kolka et al., 2019). Our data suggests that bioaccumulation in the deep offshore compartments of the lake is less susceptible to the dampening effects of biodilution on Hg bioaccumulation.

Lake Champlain's large and steep watershed and the predicted increase in storm frequency and intensity is a major cause of concern for the lake's water quality and ecosystem health. Storm events predominantly drive transport of particle bound Hg in rivers, though MeHg was correlated with discharge in a study of the Lake Champlain tributaries (Shanley and Chalmers, 2012). In our study, concentrations of Hg and MeHg in the particulate from rivers were low following the flood (though particulate concentrations were elevated). Much of this particle-bound Hg from river export is deposited in the river mouth (Janssen et al., 2024), and is not readily bioavailable. The impact of high discharge events on Hg cycling and bioaccumulation is more likely to come from shifts in in-lake processes from increasing turbidity and associated nutrient inputs, than direct inputs. The influence of river discharge in the deep main lake basins is difficult to discern: photodemethylation is a major sink for MeHg in the epilimnion of the Main Lake basins, and is driven by light penetration, though the effects of short-term change in turbidity are difficult to assess. Increased water temperature (Bockwoldt et al., 2017) and turbidity from storm

events have caused shifts in zooplankton communities (Mihuc et al., 2014), though again, long-term effects on Hg bioaccumulation are unclear. Direct evidence of changes to water quality from river discharge are most evident in the near shore regions of the lake, where eutrophication is associated with a decrease in Hg bioaccumulation and trophic transfer, responsible for the lower Hg levels in Missisquoi fish compared to the deeper basins. Changes in lake level may also drive Hg bioaccumulation in the nearshore reaches of the lake. In a thirty year study of two Wisconsin lakes, oscillations in lake level were found to drive temporal patterns in Hg concentrations in water and perch (Watras et al., 2020). This pattern is associated wetting and drying of the shoreline region, which promotes Hg methylation and transfer to littoral zones.

Invasive species have been linked with temporal changes in Hg bioaccumulation in large lakes, and are a concern for Lake Champlain due to the possible invasion of quagga mussels in the future. In the Great Lakes, invasive species are thought to be the major cause of temporal shifts in Hg concentrations that are unrelated to declines in Hg deposition through emissions reduction (Lepak et al., 2025, 2019). In small lakes, Hg concentrations often decrease with invasives, due to shortening of the food web and shifting of energy pathways to littoral zones where Hg concentrations are higher. However, trends in the Great Lakes, and in oligotrophic lakes in Minnesota, are the opposite, with increasing Hg concentrations in pelagic fish following benthic invasions (Blinick et al., 2024; Lepak et al., 2025, 2019). This is due to the energy-poor food webs in the littoral zones of these lakes, which result in poorer food quality and fish body condition, causing Hg to concentrate in fish tissues. The effects of invasive species on Hg concentrations in Lake Champlain fish are difficult to discern to date. Zebra mussels invaded Lake Champlain in 1993, and alewives entered the lake in 2003, altering the food webs supporting Lake Trout (Lesser et al., 2024). These invasions occurred during a time in the Lake Champlain Hg game fish record when sample numbers were low, and Hg concentrations had declined from higher values in the 1980s. More recently, spiny waterfleas (*Bythotrephes longimanus*) have been detected in the lake beginning in 2014, though their numbers are low. These invasive plankton inhabit the cold, deep waters of the lake. The timing of this invasion suggests it may play a role in the higher Hg bioaccumulation in 2016-17, though the effects of their invasion on the deep lake food webs are not yet understood. The lack of major shifts in the lake food webs from invasions suggests a different cause of the variation in mercury concentrations in fish over the past ~15 years. Invasion of Lake Champlain by the quagga mussel may be imminent, and would likely cause shifts in energy pathways in the lake (Chiapella et al., 2023). Quagga mussel invasions in the Great Lakes have been associated with long-term changes to lake food webs and to Hg bioaccumulation and Hg isotope composition. The current dataset on Lake Champlain provides a baseline for the lake prior to the potential arrival of this species.

6. PROJECT METRICS

<u>Metric</u>	<u>Final value</u>
Engagement metrics: # people engaged in the products of grant	<p>1000 people received rack cards about Hg in game fish distributed by Boat Ramp Stewards, bait shops, websites and fishing derbies.</p> <p>70 anglers contributed fish samples to the survey</p> <p>20,000 viewers of “Across the Fence” were informed of trends in Hg in Lake Champlain game fish</p> <p>20 state legislators learned about Hg in the lake on an outreach cruise on the Melosira</p> <p>1000 Fish advisory cards were produced and distributed to grocery stores around the state</p> <p>Data from the study was used in the new VT safe eating guidelines, relevant to all of the state’s population who consume fish.</p>
Total number of volunteers engaged in project	<p>25 volunteers were engaged at the weighing stations of the LCI and Rotary fishing derbies in 2022 and 2023.</p> <p>2 fishing boat captains helped with fish collection.</p>
Total number of volunteer hours	200
Value of volunteer hours (Hourly rates: NY: \$33.17; VT: \$26.85; QC: \$30.01)	VT: \$3222; NY: \$2653.6
Number of grant-funded events	Three workshops for regional government stakeholders.
Number of people attending grant-funded events	10 per workshop
Education: Number of students involved	2 undergraduate students were trained at Dartmouth.

7. CONCLUSIONS

This project completed the LCBP fish survey and leveraged these samples for an isotopic study of Hg bioaccumulation; conducted a study of Hg and MeHg cycling and bioaccumulation at the base of the food web; and communicated with researchers, government stakeholders, and anglers throughout the region.

The project developed a new working group of scientists and government stakeholders in the Lake Champlain region to address the differences in fish consumption guidelines among the province and states bordering the lake, and to share information on current research, and communication strategies. These meetings have been highly beneficial and are planned to continue. Presenters have shared new information on fishing practices and knowledge of contaminants for angling communities, which has led to better strategies for prioritizing and communicating with groups who fish for sustenance. Changes to fish consumption guidelines due to PFAS is identified as an increasing concern; PFAS bioaccumulate via different pathways than Hg, and contamination tends to be more localized. New avenues for sharing information, including recommendations on where to fish, reaching anglers through social media and through community group leaders, have also been discussed. Based on some of these discussions, our new Lake Champlain Sea Grant project is analyzing PFAS in some of the 2022-23 dataset from this project, as well as collecting and analyzing nearshore species (carp, suckers, sunfish, eels) that are reportedly consumed by sustenance anglers, and for which we have very little data. These fish will be analyzed for Hg, PFAS and omega-3 fatty acids.

The project completed the LCBP game fish survey for 2022-23, providing data for VT's new fish consumption guidelines. The survey showed Hg concentrations in yellow and white perch and smallmouth bass have declined in the past 5-6 years. For yellow perch and smallmouth bass, Hg concentrations have returned to 2011 levels and for white perch, were the lowest on record. Lake trout Hg concentrations have also declined, though the trend was not quite statistically significant. Concentrations of Hg in yellow perch from Malletts Bay were higher than in the other basins, suggesting that trophic status plays an important role in bioaccumulation across basins, as reported previously (Chen et al., 2012; Miller et al., 2012).

The fish survey is conducted through opportunistic sampling, though it relies heavily on the VT DEC community and lake trout surveys. One of the issues with opportunistic sampling is the large range of fish sizes collected, particularly for walleye and bass, which were mostly sampled at fishing derbies. If continued, the VT DEC lake trout surveys offer an opportunity to target a specific size range of fish (eg. 600-700mm), which reduces the uncertainty caused by length correction on mean fish Hg. This would also allow for alignment of Hg concentrations in these whole lake sentinel species with data from the Great Lakes (GLFSMP) surveys, which are ongoing – though it is noted that the Great Lakes program analyzes whole fish, not filet.

Our isotopic Hg study found precipitation is a less important source of Hg to pelagic fish in Lake Champlain than in the Great Lakes, inferring that watershed Hg is delivered to the offshore basins from the watershed. Photodemethylation, a critical process by which MeHg is removed from surface waters, was found to correlate strongly with Secchi depth (water clarity) across Lake Champlain and the Great Lakes, despite different limnological conditions driving water clarity between lakes. Changes in water quality, both from algal blooms or increasing turbidity, will decrease the extent of photodemethylation in the epilimnion. Across shallow regions of the

lake, Hg and N isotope varied between basins, in part reflecting the effect of trophic status on bioaccumulation across basins.

Our study of Hg and MeHg cycling in the water column of the deep and shallow basins of the lake found an overall decrease in MeHg in particulates (predominantly phytoplankton) over the course of the summer. This decline is consistent with biodilution, as bloom densities increase from early to late summer. However, we also found an increase in MeHg in the zooplankton from the hypolimnion of the deep lake basins. This increase could be in part due to changes in plankton assemblage, and to efficient bioaccumulation below the photic zone. The efficient assimilation and slow excretion of MeHg by zooplankton also suggests that MeHg continues to bioaccumulate over the growing season despite declining concentrations in phytoplankton. These increasing concentrations of Hg in zooplankton throughout the growing season are important to our overall understanding of bioaccumulation in the deep basins of the lake. We infer that with increasing numbers of growing degree days Hg concentrations in the base of the food web may continue to increase.

Lake Champlain is an important system to study Hg, as it supports a large number of anglers, including those who fish for subsistence. The lake has properties of both the Great Lakes, where in the deep clear basins, Hg is efficiently bioaccumulated by plankton, and of individual small lakes, where in the shallow basins, nutrients from river inputs influence trophic status and Hg bioaccumulation. This study provides new insights into the sources of Hg to the lake's fish, and the pathways by which they are accumulated. Our ongoing research will make available PFAS and omega-3 concentrations on a subset of the fish from this work.

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8. APPENDICES

Appendix 5_6

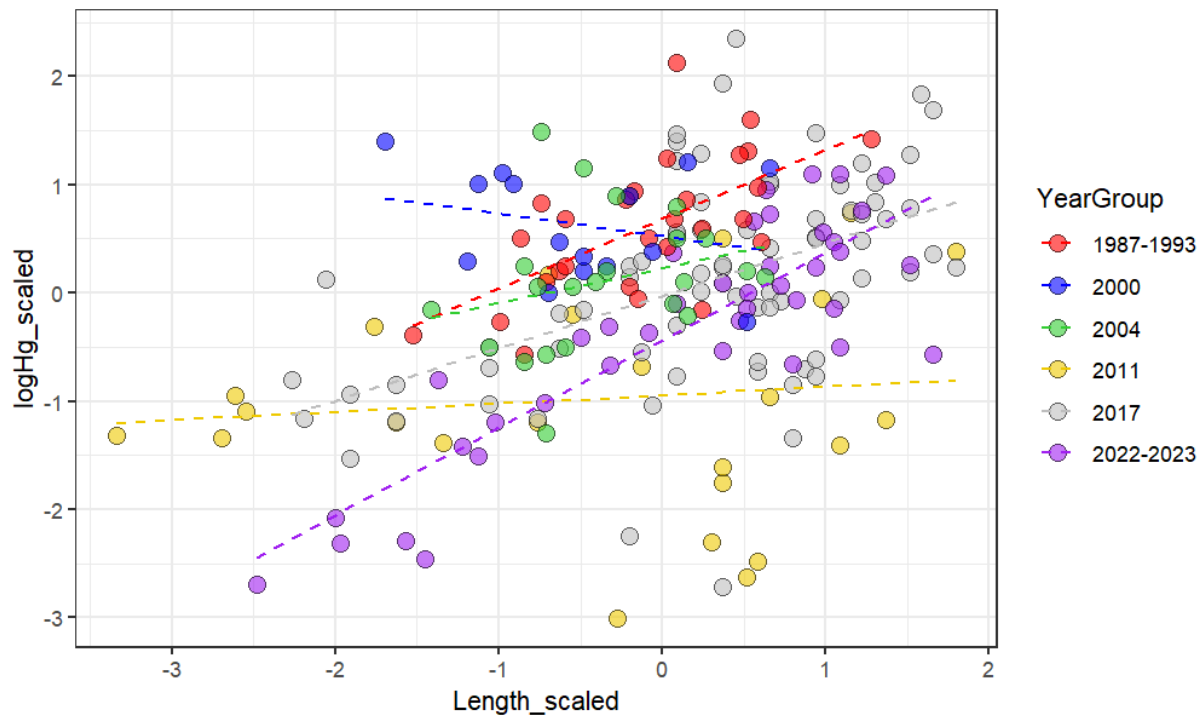


Figure 5_6.1S) Covariate model of logHg (scaled) in lake trout by length (scaled) and year, with an interaction term between length and year.

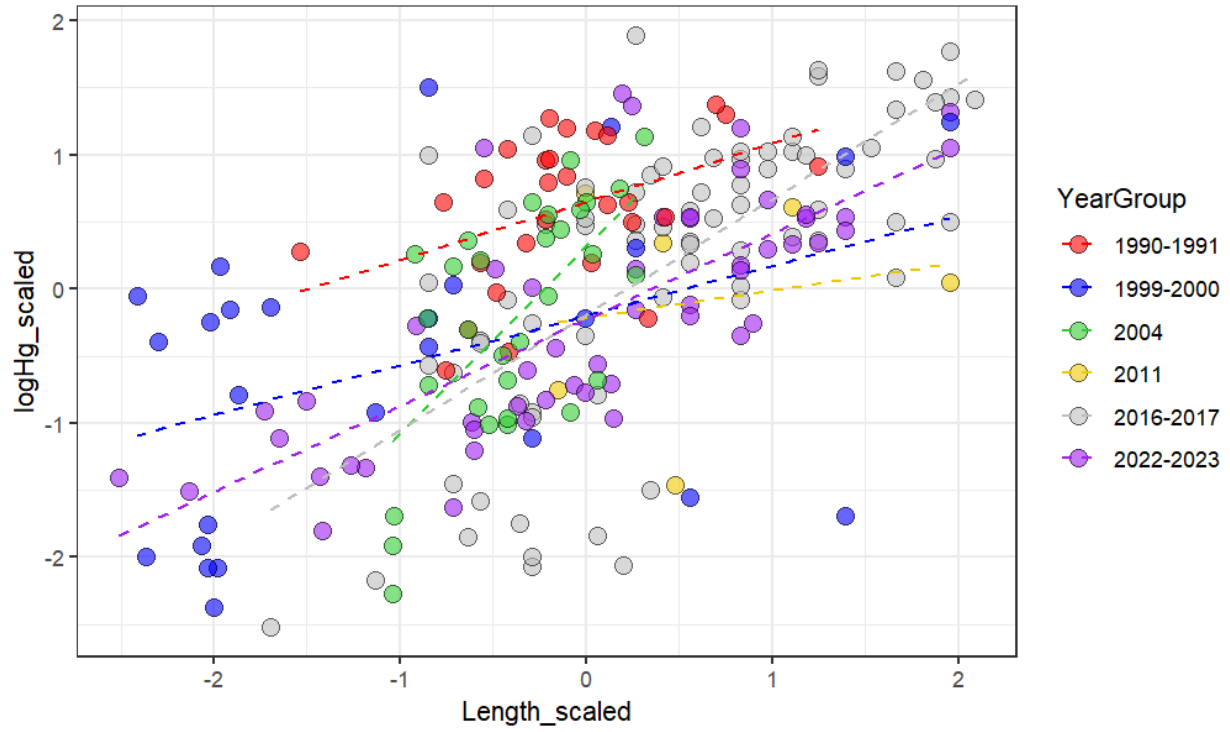


Figure 5_6.2S) Covariate model of logHg (scaled) in walleye by length (scaled) and year, with an interaction term between length and year.

Table 5_6.1S) Random and fixed effects for Hg concentrations in yellow perch.

Yellow perch: logHg_scaled ~ Length_scaled + YearGroup + (Length_scaled | Basin)

Random effects:	Groups	Name	Variance	Std.Dev.	Corr.
	Basin	(Intercept)	0.1021	0.3195	
		Length_scaled	0.0267	0.1635	-0.84
	Residual		0.4284	0.6545	
Number of obs:	439	Groups:	Basin, 7		

Fixed effects:	Estimate	Std. Error	df	t value	Pr(> t)	Signif
(Intercept)	0.53266	0.17202	20.4979	3.097	0.005574	**
Length_scaled	0.5782	0.07104	4.68394	8.139	0.000616	***
YearGroup2003-2004	-0.1724	0.21871	425.8	-0.788	0.431035	
YearGroup2011	-0.8888	0.13881	428.983	-6.403	4.01E-10	***
YearGroup2016-2017	-0.3911	0.13325	428.722	-2.935	0.003516	**
YearGroup2022-2023	-0.7159	0.14286	424.349	-5.011	7.97E-07	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

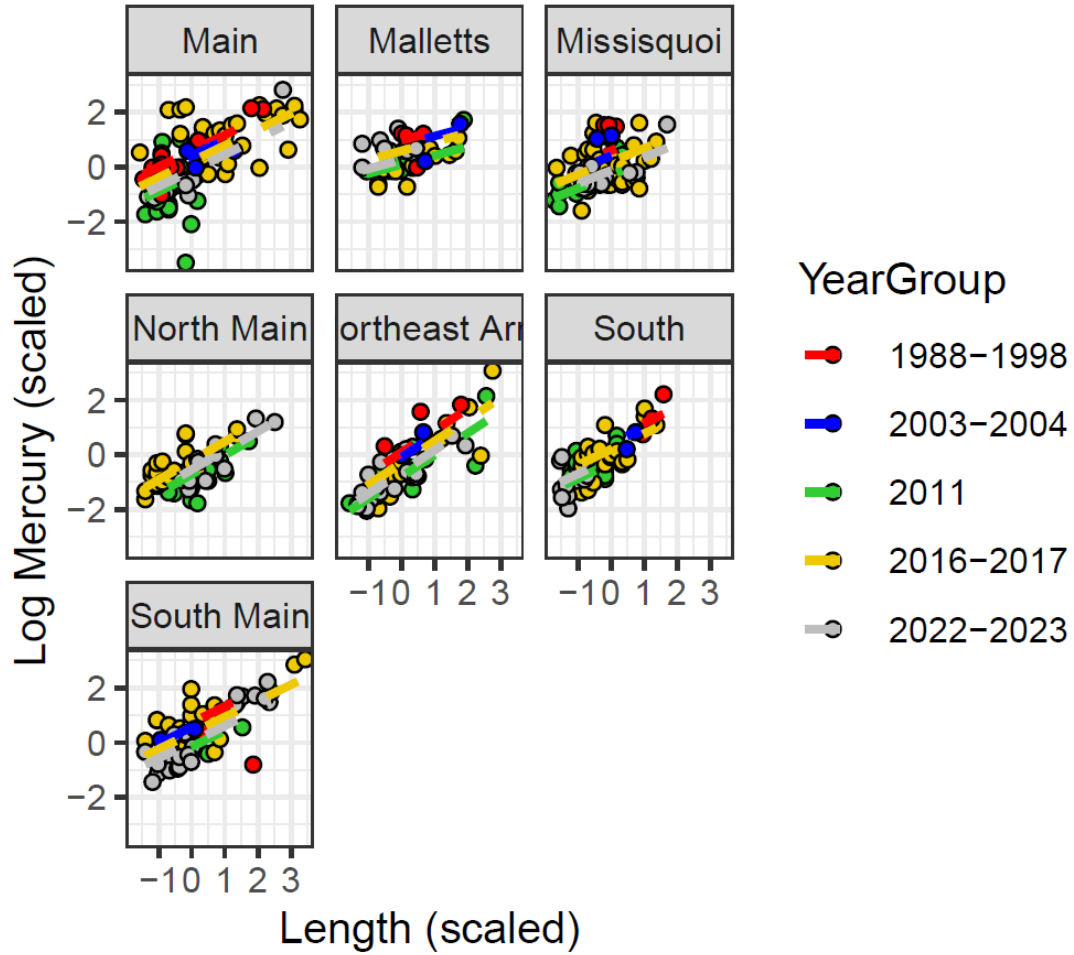


Figure 5_6.3S) Mixed linear model for logHg (scaled) in yellow perch where length (scaled) is a covariate, year is the independent variable, and there is a random slope effect between length and lake basin.

Table 5_6.2S) Random and fixed effects for Hg concentrations in white perch.

White perch: logHg_scaled ~ Length_scaled + YearGroup + (1 | Basin)

Random effects:	Groups	Name	Variance	Std.Dev.
	Basin	(Intercept)	0.1353	0.3679
	Residual		0.2929	0.5412
Number of obs:	280	groups:	Basin, 7	
R2m:	0.6271	R2c:	0.745	

Fixed effects:	Estimate	Std. Error	df	t value	Pr(> t)	Significance
(Intercept)	0.26483	0.253	52.491	1.047	0.3	
Length_scaled	0.83567	0.0373	273.65	22.412	<2.00E-16	***
2003-2004	-0.25164	0.2544	269.8	-0.989	0.3234	
2011	-0.16299	0.2219	270.6	-0.735	0.4632	
2016-2017	-0.12428	0.2181	270.13	-0.57	0.5692	
2022-2023	-0.74736	0.2207	269.44	-3.386	0.0008	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

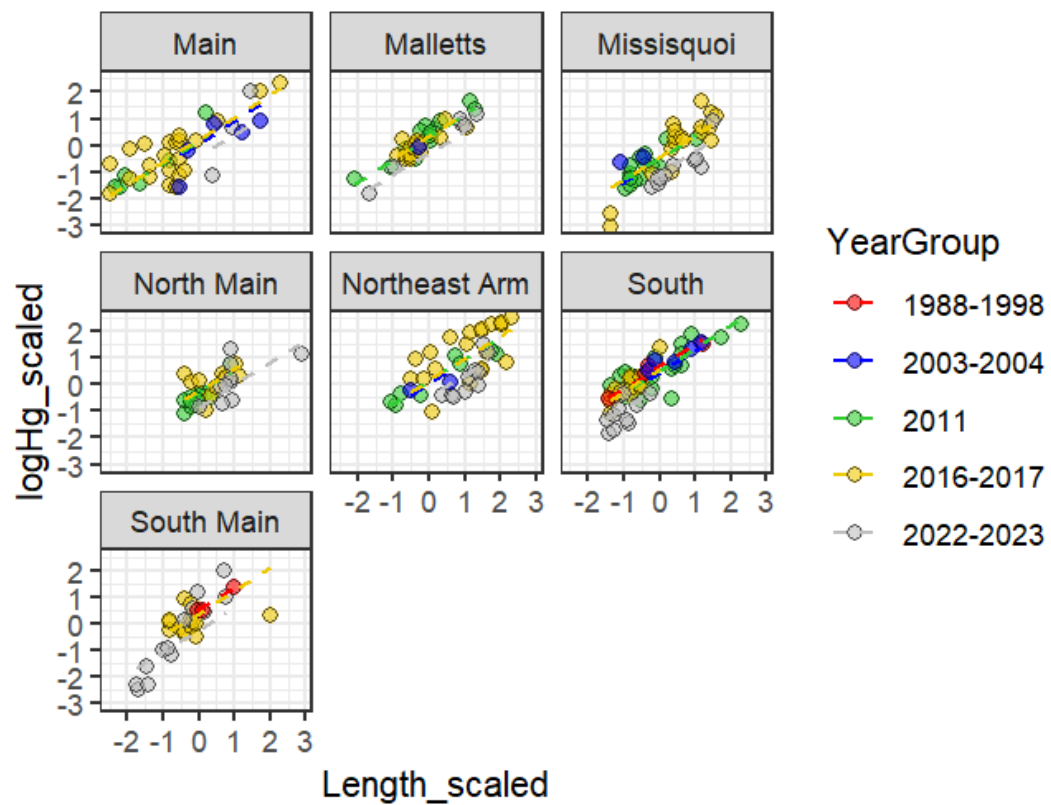


Figure 5_6.4S: Mixed linear model for logHg (scaled) in white perch where length (scaled) is a covariate, year is the independent variable, and there is a random slope effect between length and lake basin.

Table 5_6.3S) Random and fixed effects for Hg concentrations in smallmouth bass across years.

Smallmouth Bass: logHg_scaled ~ Length_scaled + YearGroup + (1 | Basin)

Random effects:	Groups	Name	Variance	Std.Dev.
	Basin	(Intercept)	0.00522	0.07223
	Residual		0.35247	0.59369
Number of obs:	394	groups:	Basin, 7	
R2m	0.6452	R2c	0.6503	

Fixed effects:	Estimate	Std. Error	df	t value	Pr(> t)	Significance
(Intercept)	0.28884	0.19358	344.813	1.492	0.1366	
Length_scaled	0.78926	0.03256	338.897	24.244	<2e-16	***
2003-2004	-0.2847	0.22351	392.827	-1.274	0.2036	
2011	-0.4422	0.20391	393.861	-2.168	0.0307	*
2016-2017	-0.1222	0.19723	393.982	-0.62	0.5358	
2022-2023	-0.421	0.19804	393.832	-2.126	0.0341	*

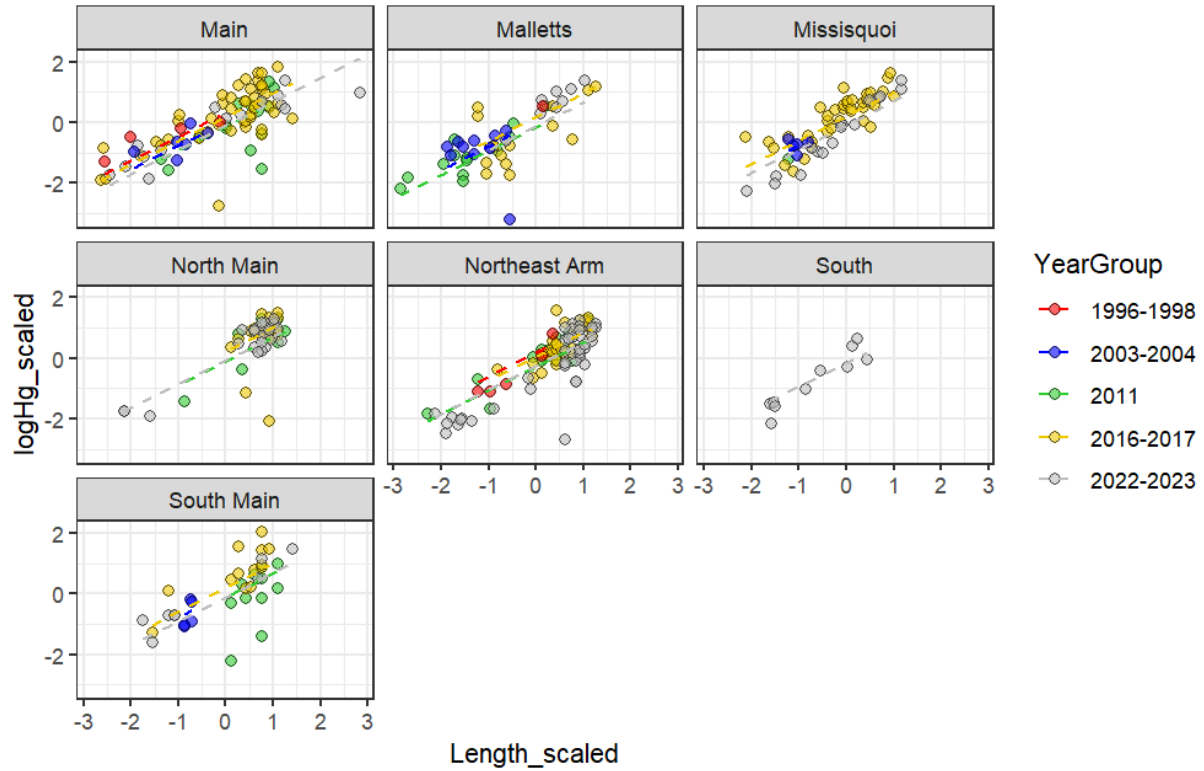


Figure 5_6.5S: Mixed linear model for logHg (scaled) in smallmouth bass were length (scaled) is a covariate, year is the independent variable, and there is a random slope effect between length and lake basin.

Table 5_6.4S) Random and fixed effects for Hg concentrations in yellow perch across basins and years (from 2011 to 2023).

$$\log\text{Hgppb_scaled} \sim \text{Length_scaled} + \text{YearGroup} * \text{Basin} + (0 + \text{Length_scaled} | \text{YearGroup:Basin})$$

Random effects:	Groups	Name	Variance	Std.Dev.	
	YearGroup:Basin	Length_scaled	0.0508	0.2254	
		Residual	0.3569	0.5974	
		Number of obs.	394	groups:	YearGroup:Basin, 21
		R2m	0.6487	R2c	0.6924

Fixed effects:	Estimate	Std. Error	df	t value	Pr(> t)	Significance
(Intercept)	-0.74605	0.19154	82.8571	-3.895	0.0002	***
Length_scaled	0.62744	0.06651	19.6368	9.433	9.89E-09	***
YearGroup2016-2017	1.611	0.23367	143.287	6.894	1.59E-10	***
YearGroup2022-2023	0.59793	0.28509	242.21	2.097	0.037	*
BasinMalletts	1.09285	0.24588	176.342	4.445	1.55E-05	***
BasinMissisquoi	0.80805	0.29466	133.756	2.742	0.00694	**
BasinNorth Main	-0.23457	0.25644	167.41	-0.915	0.36166	
BasinNortheast Arm	0.12574	0.24122	167.545	0.521	0.60286	
BasinSouth	0.70343	0.25654	76.6938	2.742	0.0076	**
BasinSouth Main	0.53001	0.35711	157.264	1.484	0.13977	
YearGroup2016-2017:BasinMalletts	-1.98267	0.33844	240.501	-5.858	1.53E-08	***
YearGroup2022-2023:BasinMalletts	-0.03264	0.38018	316.159	-0.086	0.93163	
YearGroup2016-2017:BasinMissisquoi	-1.558	0.33257	174.25	-4.685	5.62E-06	***
YearGroup2022-2023:BasinMissisquoi	-0.72775	0.40932	273.156	-1.778	0.07653	.
YearGroup2016-2017:BasinNorth Main	-0.61511	0.32953	249.849	-1.867	0.06313	.
YearGroup2022-2023:BasinNorth Main	-0.25184	0.38397	278.9	-0.656	0.51243	
YearGroup2016-2017:BasinNortheast Arm	-1.55067	0.31938	262.289	-4.855	2.07E-06	***
YearGroup2022-2023:BasinNortheast Arm	-0.66739	0.34799	313.196	-1.918	0.05604	.

YearGroup2016-2017:BasinSouth	-1.38719	0.31576	138.938	-4.393	2.20E-05	***
YearGroup2022-2023:BasinSouth	-0.82416	0.47052	50.3178	-1.752	0.08594	.
YearGroup2016-2017:BasinSouth Main	-0.79414	0.39605	198.7	-2.005	0.0463	*
YearGroup2022-2023:BasinSouth Main	-0.36669	0.4346	241.015	-0.844	0.39964	

Basin	YearGroup	emmean	SE	df	lower.CL	upper.CL	.group
North Main	2011	-0.9806	0.17	360.4	-1.498	-0.4628	a
Main	2011	-0.7461	0.196	73.8	-1.363	-0.1294	abc
Northeast Arm	2022-2023	-0.6898	0.135	367.5	-1.101	-0.279	ab
North Main	2022-2023	-0.6345	0.2	292.4	-1.245	-0.0237	abcde
Northeast Arm	2011	-0.6203	0.146	358.9	-1.066	-0.1742	abc
Northeast Arm	2016-2017	-0.56	0.164	371.3	-1.059	-0.0605	abcd
South	2022-2023	-0.2689	0.339	19.7	-1.448	0.9099	abcdefghi
South Main	2011	-0.216	0.301	195.7	-1.139	0.7068	abcdefghi
Main	2022-2023	-0.1481	0.211	354.8	-0.793	0.4968	abcdefg
Missisquoi	2022-2023	-0.0678	0.19	359.8	-0.648	0.5123	abcdefgh
South	2011	-0.0426	0.182	69.8	-0.616	0.5309	bcdefgh
Malletts	2016-2017	-0.0249	0.197	298.4	-0.626	0.5767	bcdefgh
South Main	2022-2023	0.0152	0.132	363.8	-0.387	0.4178	cdefg
North Main	2016-2017	0.0153	0.155	326.3	-0.459	0.4895	bcdefgh
Missisquoi	2011	0.062	0.233	195.4	-0.652	0.7764	bcdefghi
Missisquoi	2016-2017	0.115	0.080	364.9	-0.128	0.3577	ef
South	2016-2017	0.1812	0.128	360.3	-0.211	0.573	defgh
Malletts	2011	0.3468	0.154	354.7	-0.124	0.8178	fghi
South Main	2016-2017	0.6008	0.105	357.9	0.28	0.9219	ghi
Main	2016-2017	0.8649	0.133	362.6	0.458	1.2723	i
Malletts	2022-2023	0.9121	0.198	362.1	0.308	1.5158	hi

Table 5_6.5S) Random and fixed effects for Hg concentrations in white perch across basins and years (from 2011 to 2023).

White perch

$$\log\text{Hgppb_scaled} \sim \text{Length_scaled} + \text{YearGroup} * \text{Basin} + (0 + \text{Length_scaled} | \text{YearGroup:Basin})$$

Random effects:				
Groups	Name	Variance	Std.Dev.	
YearGroup:Basin	Length_scaled	0.111	0.3331	
Residual		0.2336	0.4833	
Number of obs.	258,	groups:	YearGroup:Basin, 20	
	R2m	0.7085647	R2c	0.8022

Fixed effects:	Estimate	Std. Error	df	t value	Pr(> t)	Significance
(Intercept)	0.63329	0.33436	151.173	1.894	0.06013	.
Length_scaled	0.90895	0.09164	15.2002	9.919	4.93E-08	***
YearGroup2016-2017	-0.44918	0.35405	164.262	-1.269	0.20635	
YearGroup2022-2023	-1.32747	0.52562	80.8143	-2.526	0.01351	*
BasinMalletts	-0.2173	0.35703	169.48	-0.609	0.5436	
BasinMissisquoi	-1.07391	0.36484	157.235	-2.944	0.00374	**
BasinNorth Main	-1.08324	0.36229	174.708	-2.99	0.00319	**
BasinNortheast Arm	-0.56504	0.37813	186.289	-1.494	0.13679	
BasinSouth	-0.06495	0.3492	163.971	-0.186	0.85269	
BasinSouth Main	1.09806	0.43116	64.3391	2.547	0.01328	*
YearGroup2016-2017:BasinMalletts	0.24682	0.39916	191.166	0.618	0.53708	
YearGroup2022-2023:BasinMalletts	0.8534	0.5755	104.259	1.483	0.14112	
YearGroup2016-2017:BasinMissisquoi	0.2309	0.40588	179.155	0.569	0.57014	
YearGroup2022-2023:BasinMissisquoi	0.4796	0.57198	90.6017	0.838	0.40396	
YearGroup2016-2017:BasinNorth Main	0.75765	0.41836	179.996	1.811	0.07181	.
YearGroup2022-2023:BasinNorth Main	1.22911	0.58718	93.6337	2.093	0.03903	*
YearGroup2016-2017:BasinNortheast Arm	0.88088	0.42734	198.14	2.061	0.04058	*
YearGroup2022-2023:BasinNortheast Arm	0.27416	0.63151	72.181	0.434	0.66548	

YearGroup2016-2017:BasinSouth	0.69968	0.44345	138.565	1.578	0.11689	
YearGroup2022-2023:BasinSouth	0.57829	0.62938	71.0419	0.919	0.36129	
YearGroup2016-2017:BasinSouth Main	-1.14634	0.46653	82.5726	-2.457	0.0161	*

Basin	YearGroup	emmean	SE	df	lower.CL	upper.CL	.group
Missisquoi	2022-2023	-1.2885	0.19	209.8	-1.8683	-0.7086	a
Northeast Arm	2022-2023	-0.9851	0.336	49.4	-2.0532	0.0831	abc
Main	2022-2023	-0.6942	0.407	55.7	-1.9793	0.5909	abcde
Missisquoi	2016-2017	-0.6589	0.136	235.7	-1.0745	-0.2433	ab
North Main	2022-2023	-0.5483	0.241	176	-1.2842	0.1876	abcd
North Main	2011	-0.45	0.14	219.5	-0.8756	-0.0243	abc
Missisquoi	2011	-0.4406	0.152	221.3	-0.9045	0.0232	abc
South	2022-2023	-0.1808	0.324	40.3	-1.2242	0.8625	abcde
North Main	2016-2017	-0.1415	0.181	189.9	-0.6948	0.4118	bcde
Malletts	2022-2023	-0.0581	0.202	224.4	-0.6738	0.5577	bcde
Northeast Arm	2011	0.0683	0.176	224.4	-0.4681	0.6046	bcde
South Main	2016-2017	0.1358	0.134	228.5	-0.2738	0.5454	cde
Main	2016-2017	0.1841	0.118	228.8	-0.1771	0.5453	cde
Malletts	2016-2017	0.2136	0.134	236.7	-0.1952	0.6224	cde
South Main	2022-2023	0.4039	0.161	237	-0.0878	0.8956	de
Malletts	2011	0.416	0.126	221.2	0.03191	0.8001	de
Northeast Arm	2016-2017	0.5	0.167	236.1	-0.0086	1.0085	de
South	2011	0.5683	0.101	219.7	0.26043	0.8762	e
Main	2011	0.6333	0.345	158.5	-0.4229	1.6894	bcde
South	2016-2017	0.8188	0.251	73.6	0.0335	1.6042	e
South Main	2011	nonEst	NA	NA	NA	NA	

Table 5_6.6S) Random and fixed effects for Hg concentrations in smallmouth bass across basins and years (from 2011 to 2023).

Smallmouth Bass:

$$\log\text{Hgppb_scaled} \sim \text{Length_scaled} + \text{YearGroup} * \text{Basin} + (0 + \text{Length_scaled} | \text{YearGroup}:\text{Basin})$$

Random effects:			
Groups	Name	Varianc	
YearGroup:Basin	Length_scaled	0.0126	
	Residual	0.3162	
Number of obs:	357,	groups:	YearGroup

Fixed effects:	Estimate	Std. Error	df	t value	Pr(> t)	
(Intercept)	-0.30433	0.1463	336.8477	-2.08	0.03832	*
Length_scaled	0.83448	0.0469	16.61493	17.776	3.04E-12	***
YearGroup2016-2017	0.49834	0.1674	335.5765	2.977	0.00312	**
YearGroup2022-2023	0.28854	0.2073	335.5414	1.392	0.16495	
BasinMalletts	0.43048	0.2682	54.99611	1.605	0.11418	
BasinMissisquoi	-0.13216	0.2026	337.7456	-0.652	0.51454	
BasinNorth Main	0.28315	0.2289	236.8364	1.237	0.2173	
BasinNortheast Arm	0.25846	0.2029	333.8899	1.274	0.20365	
BasinSouth	-0.10056	0.2452	209.2837	-0.41	0.68213	
BasinSouth Main	-0.31797	0.2323	255.2056	-1.369	0.17218	
YearGroup2016-2017:BasinMalletts	-0.81028	0.3159	84.35086	-2.565	0.01209	*
YearGroup2022-2023:BasinMalletts	-0.13916	0.3547	136.703	-0.392	0.69538	
YearGroup2016-2017:BasinMissisquoi	0.2702	0.2358	337.9877	1.146	0.25273	
YearGroup2016-2017:BasinNorth Main	-0.47813	0.2886	158.9444	-1.657	0.09949	.
YearGroup2022-2023:BasinNorth Main	-0.21118	0.2977	295.2153	-0.709	0.47858	
YearGroup2016-2017:BasinNortheast Arm	-0.446	0.2457	331.1301	-1.815	0.07039	.
YearGroup2022-2023:BasinNortheast Arm	-0.71091	0.2653	333.4572	-2.679	0.00774	**
YearGroup2016-2017:BasinSouth Main	0.62869	0.2915	306.1953	2.157	0.03178	*

YearGroup2022-2023:BasinSouth Main	0.55364	0.3445	294.5672	1.607	0.10907	
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Basin	YearGroup	emmean	SE	df	lower.CL	upper.CL	.group
South Main	2011	-0.6223	0.182	143.6	-1.1755	-0.0691	a
Northeast Arm	2022-2023	-0.4682	0.0874	334.4	-0.7309	-0.2056	a
Main	2011	-0.3043	0.147	336.4	-0.7454	0.1367	ab
Malletts	2016-2017	-0.1858	0.154	285.2	-0.6487	0.2771	abc
Missisquoi	2022-2023	-0.148	0.142	329.9	-0.5734	0.2775	abc
South	2022-2023	-0.1164	0.203	108.5	-0.7352	0.5025	abc
Northeast Arm	2011	-0.0459	0.141	325.2	-0.4686	0.3768	abc
North Main	2011	-0.0212	0.18	120.6	-0.5693	0.527	abc
Main	2022-2023	-0.0158	0.147	333.5	-0.4583	0.4267	abc
North Main	2016-2017	-0.001	0.162	40.3	-0.5154	0.5134	abc
Northeast Arm	2016-2017	0.0065	0.114	115.3	-0.3408	0.3538	abc
North Main	2022-2023	0.0562	0.124	335.7	-0.3171	0.4294	abc
Malletts	2011	0.1261	0.242	20.7	-0.6927	0.945	abc
Main	2016-2017	0.194	0.0812	325.5	-0.0502	0.4382	bc
South Main	2022-2023	0.2199	0.21	291	-0.4124	0.8522	abc
Malletts	2022-2023	0.2755	0.178	325.3	-0.2592	0.8102	bc
Missisquoi	2016-2017	0.332	0.0905	337.8	0.0602	0.6039	c
South Main	2016-2017	0.5047	0.157	335.6	0.0318	0.9777	c
Missisquoi	2011	nonEst	NA	NA	NA	NA	
South	2011	nonEst	NA	NA	NA	NA	
South	2016-2017	nonEst	NA	NA	NA	NA	

APPENDIX 8.1 Ratios of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, Molar C:N ratio in zooplankton

Basin	Date	Depth	N	Mean_ $\delta^{13}\text{C}$ V_PDB	SD_ $\delta^{13}\text{C}$ V_PDB	Mean_ $\delta^{15}\text{N}$ Air	SD_ $\delta^{15}\text{N}$ Air	Molar C:N	SD_Molar C:N
				‰	‰	‰	‰		
Main	6/21/2023	Hypolimnion	3	-32.4	1.0	12.5	0.2	4.77	0.79
Main	6/21/2023	Epilimnion	3	-30.8	0.0	9.5	0.1	3.96	0.03
Main	8/2/2023	Hypolimnion	3	-31.5	0.4	12.7	0.2	5.19	0.36
Main	8/2/2023	Epilimnion	3	-27.0	0.3	7.1	0.5	6.51	0.19
Main	8/28/2023	Hypolimnion	3	-30.4	0.3	13.0	0.3	5.49	0.50
Main	8/28/2023	Epilimnion	3	-27.8	0.2	8.8	0.2	4.90	0.71
Malletts	6/21/2023	Hypolimnion	3	-31.2	0.7	9.6	0.2	4.60	0.79
Malletts	6/21/2023	Epilimnion	3	-31.0	0.1	7.5	0.0	4.06	0.01
Malletts	7/17/2023	Hypolimnion	3	-30.2	0.1	12.0	0.1	3.45	0.05
Malletts	7/17/2023	Epilimnion	3	-28.5	0.0	9.9	0.1	3.25	0.02
Malletts	8/28/2023	Hypolimnion	3	-32.1	0.3	13.6	0.1	3.57	0.06
Malletts	8/28/2023	Epilimnion	3	-28.7	0.7	10.7	0.2	5.34	
Missisquoi	6/5/2023	Epilimnion	3	-30.3	0.6	8.5	0.2	3.44	0.21
Missisquoi	7/19/2023	Epilimnion	3	-29.4	0.4	10.7	0.1	3.48	0.18
Missisquoi	9/1/2023	Epilimnion	3	-25.3	0.2	10.9	0.1	5.07	0.04
North Main	6/21/2023	Hypolimnion	3	-29.8	1.7	11.3	0.4	5.39	0.58
North Main	6/21/2023	Epilimnion	3	-29.9	0.4	9.9	0.1	4.55	0.47
North Main	8/2/2023	Hypolimnion	3	-29.4	0.2	11.3	0.2	4.15	0.06
North Main	8/2/2023	Epilimnion	3	-26.9	0.0	7.8	0.8	5.49	0.08
North Main	8/28/2023	Hypolimnion	3	-30.0	0.1	13.2	0.1	4.92	0.05
North Main	8/28/2023	Epilimnion	3	-27.8	0.2	8.0	0.1	5.06	0.46
South Main	6/21/2023	Hypolimnion	3	-30.2	2.6	11.0	2.0	5.59	1.09
South Main	6/21/2023	Epilimnion	3	-31.0	0.1	7.5	0.0	3.85	0.08
South Main	8/2/2023	Hypolimnion	3	-31.9	1.4	13.0	0.5	5.64	2.13
South Main	8/2/2023	Epilimnion	3	-26.8	0.2	7.4	0.2	5.91	0.07
South Main	8/28/2023	Hypolimnion	3	-30.5	0.2	12.8	0.1	4.72	0.29
South Main	8/28/2023	Epilimnion	3	-29.0	0.2	9.2	0.1	4.03	0.09

St. Albans	6/5/2023	Epilimnion	3	-29.0	0.1	11.7	0.1	3.80	0.02
St. Albans	7/19/2023	Epilimnion	3	-20.5	0.9	13.0	0.1	3.76	0.21
St. Albans	9/1/2023	Green	3	-22.3	0.1	7.9	0.0	4.72	0.05
St. Albans	9/1/2023	Epilimnion	3	-22.2	0.2	8.1	0.1	3.75	0.02

Appendix 8.2 Concentration of MeHg, THg and % zooplankton (from microscopy) in seston (>150µm mesh)

Depth	N	Mean_MeHg_ng.g	SD_MeHg_ng.g	Mean_THg_ng.g	SD_THg_ng.g	%MeHg	Zoop/Seston (%)
Hypolimnion	3	19.8	6.6	58.1	4.1	34%	100%
Epilimnion	3	29.3	0.1	52.5	1.8	56%	4%
Hypolimnion	3	31.6	6.1	68.5	17.4	46%	68%
Epilimnion	3	6.3	0.9	11.3	2.3	56%	8%
Hypolimnion	3	49.8	1.3	52.7	2.3	95%	100%
Epilimnion	3	24.0	4.2	35.6	0.8	67%	4%
Hypolimnion	3	34.2	5.0	75.3	7.7	45%	100%
Epilimnion	3	37.4	6.0	58.4	4.6	64%	4%
Hypolimnion	3	37.3	1.9	98.6	0.1	38%	100%
Epilimnion	3	33.3	1.8	62.5	3.7	53%	100%
Hypolimnion	3	66.9	8.0	61.7	4.5	108%	100%
Epilimnion	3	23.8	0.1	37.2	2.5	64%	4%
Epilimnion	3	24.3	2.3	55.5	1.9	44%	
Epilimnion	3	21.1	0.6	41.4	1.4	51%	
Epilimnion	3	4.9	0.6	6.9	0.5	71%	
Hypolimnion	3	25.3	1.6	40.4	1.1	63%	100%
Epilimnion	3	21.9	2.5	48.9	0.8	45%	1%
Hypolimnion	3	21.0	2.2	30.8	1.9	68%	72%
Epilimnion	3	6.8	0.7	18.8	0.7	36%	5%
Hypolimnion	3	49.0	5.0	50.9	3.0	96%	100%
Epilimnion	3	14.8	1.1	19.9	2.1	75%	5%
Hypolimnion	3	26.3	3.7	47.7	0.7	55%	100%
Epilimnion	3	29.4	2.2	52.1	1.0	56%	4%
Hypolimnion	3	58.3	7.7	66.8	0.5	87%	57%
Epilimnion	3	4.7	1.9	11.3	2.5	42%	6%
Hypolimnion	3	60.4	3.9	78.7	1.0	77%	100%
Epilimnion	3	32.4	0.9	37.3	0.9	87%	3%
Epilimnion	3	12.1	0.5	31.6	3.2	38%	

Epilimnion	3	22.3	5.5	47.7	3.3	47%
Green	3	8.6	3.6	12.2	1.2	71%
Epilimnion	3	29.1	3.6	31.2	2.8	93%