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The effect of seasonality on yellow perch ecology and ecotoxicology within Lake Manganese

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THE EFFECT OF SEASONALITY ON YELLOW PERCH ECOLOGY AND ECOTOXICOLOGY WITHIN LAKE MANGANESE

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Abstract

Seasonality is a consistent component of aquatic ecosystems yet most fish biological and ecotoxicological studies commonly employ field sampling protocols focused during the warm open water season with minimal emphasis placed on winter sampling, especially for north-temperate latitude ecosystems. Such strategies limit our understanding of poikilotherm biology and ecology during the overwintering seasons. Here, I investigated seasonal changes in yellow perch (*Perca flavescens*) biology, ecology and ecotoxicology over a one-year period in Lake Manganese. Significant seasonality was observed for metrics including fish energy densities (kJ/g), gonadosomatic indices, whole-body lipid contents, and carbon stable isotope values ($\delta^{13}C$). Mercury concentrations quantified within a single yellow perch age class displayed significant seasonal and individual variability, with Hg concentrations for fall and winter collected fishes being higher than those for spring and summer fishes. Both fish protein mass and $\delta^{13}C$ were significant predictors of Hg bioaccumulation by Lake Manganese yellow perch. This study is among the few to demonstrate the role of seasonality on fish biology, ecology and pollutant bioaccumulation. Furthermore, these results demonstrate the need to include winter and establish entire growing season datasets under current climate change predictions.

1 Introduction

Winter and overwintering ecology represents a general knowledge gap for fisheries biology research and many other aquatic ecological sciences generally due to the logistical difficulties associated with sampling during this time of year [1]. It is important to study winter ecology due to the substantial differences in limnological factors that are anticipated relative to the warmer open water seasons. For example, cooler water temperatures in the fall through winter represent periods of slower growth [2] [3] and lower metabolic rates in fish [4]. Ice-covered lakes also differ substantially with respect to the availabilities of nitrogen and phosphorus relative to the open water season [5]. Due to seasonal differences in these limiting nutrient availabilities, winter zooplankton communities are of low biomass and phytoplankton community composition also differing from those present during the open water months [6] [5]. Winter dissolved oxygen levels can also differ from open water seasons due to changed oxygen solubility at cooler temperatures and ice-cover posing a barrier for atmospheric exchange [7]. Recent temporal variation and reductions in the duration of ice-cover have been attributed to climate change with ice-cover happening for shorter periods of time and on fewer bodies of water [8]. Such climate-induced changes in seasonal ice-cover could cause major shifts in temporal limnological factors with cascading effects on aquatic ecology. Due to a general paucity of winter ecology studies, the role of ice-cover on aquatic ecosystems is not well understood [1]. Additional winter ecological datasets such as generated by this research will help more accurately understand the importance of seasonality in aquatic environments and how climate change may act to reduce the magnitude of seasonality and associated ecosystem and species responses [1].

1.1 Yellow perch ecology

The yellow perch is a cool-warm water species found throughout much of Canada and the United States [9] and is of cultural and economic importance for multiple interest groups such as recreational and commercial fisherman and people living within the Great Lakes Basin. For example, approximately 70% of yellow perch sales in the United States occurs within and in close proximity to the Great Lakes basin [10]. At its peak in the 1950's and 1960's, yellow perch were harvested in excess of 15 million kg/year [10]. Although wild catch has decreased over the decades, this species still remains of economic importance, with the Department of Fisheries and Oceans Canada reporting a national yellow perch harvest of 8.5 million tonnes and worth $15 million in 2018 [11].

For yellow perch, seasonality plays and important role in their ecology and biology. For example, females require an extended chill period (> 185 days) of exposure to temperatures ≤ 6 °C for normal egg-yolk deposition and final maturation [10]. Both male and female yellow perch begin to spawn in the spring following ice-melt in northern latitudes. However, males and females reach sexual maturity at different ages. Males begin spawning after about one year while females typically take two years to reach sexual maturity [10] [9]. In preparation for spawning, females move into shallow weedy areas in early spring as water temperatures increase where they will lay egg ribbons on submerged vegetation which are then fertilized by broadcasting males [9, 12].

After hatching, larval yellow perch are planktivorous and congregate in littoral zones where they consume zooplankton until large enough to feed on larger macroinvertebrates, such as mayfly and caddisfly larva [9]. Individuals then transition to

a benthic diet within the littoral zone until they are large enough to make their final ontogenetic shift to include piscivory [13-15]. In order to compensate for reduced food availability during the winter, yellow perch tend to rely on lipid stores build up during the summer as an energy reserve [16]. During winter, yellow perch diets also shift to include larger proportions of benthic invertebrates and zooplankton relative to their summer diet [17, 18].

Yellow perch growth can vary among individuals and populations [19-23]. For example, yellow perch from Lake Erie [24] have demonstrated faster growth and larger maximum size relative to fish from Lake Gogebic located in Michigan's Upper Peninsula region [25]. Further, seasonal and age-related differences in growth patterns have also been observed, with summer representing a period of faster growth [26]. Growth rates have also been shown to change under situations when intra-specific resource competition increases due to a lack of predators, decreased fishing pressure, and/or decreased mortality rates within the population [23, 27, 28]. When these situations or combinations thereof are present, stunted or extremely slow growth can become manifested [20, 21]. Although this phenomenon is known to occur in yellow perch, it remains relatively unstudied and the broader ecological consequences of a stunted population are not fully understood.

1.2 Seasonality in aquatic ecosystems

Aquatic ecosystems display patterns of seasonal change wherein key abiotic factors, such as water temperature, dissolved oxygen, pH, and concentrations of nitrogen and phosphorus have been shown to exhibit noteworthy seasonal differences [5]. Within

the open-water season alone, there are substantial changes in limnological factors that greatly affect the ecology of a lake, but less is known about ecological changes that take place over the winter season. The development of ice-cover during the winter months at northern latitudes is one of the most visible seasonal changes, but is considered to pose a logistical challenge for studying winter-season limnology and aquatic ecology [1]. As a result, there is a general lack of data for the fall, winter, and early spring seasons. For example, at northern temperate latitudes, the cool-cold water seasons (i.e. < 10 °C) can span up to six months of the year. Therefore, studies that include winter sampling will greatly benefit the field of aquatic ecology by creating a more complete picture of how the ecology and biology of aquatic systems change throughout an entire year.

Distributions and assemblages of fishes also display patterns of seasonality [29] as different species, age classes, and sizes can respond differently to seasonality [30]. Winter also represents a period with important change in seasonal diet composition as many fish species experience shifts in available food resources, likely as associated with the decline primary productivity and availability of pelagic prey resources such as phytoplankton and zooplankton during the winter [18]. Individual movements within these aquatic systems also decreases in winter months, as fish find refuge and localize in deep, well-oxygenated areas [31]. This is a known adaption in fish species that have evolved to tolerate cold climates to help conserve energy and reduce overwinter mortality of susceptible age classes [17, 32].

Similar to fish, many invertebrates also display patterns related to seasonality. Notable differences in zooplankton and phytoplankton abundances and community

structures have been observed during the open water season and have been attributed to seasonal changes [5]. However, little research has been done to observe how these communities change under the ice [1]. Some studies have reported under ice phytoplankton blooms and subsequent increases in zooplankton populations [6]. Macroinvertebrates also experience significant seasonal change. For instance, temperature contributes very heavily to macroinvertebrate growth, with colder temperatures associated with much slower growth than warmer temperatures [33]. Ice-covered lakes also have lower available food resources for aquatic macroinvertebrates relative to the open water warmer months [34].

1.3 Mercury

Mercury in the form of methylmercury (MeHg) is an acute neurotoxin that possess the ability to cross the blood brain barrier. The primary source of human exposure to Hg is from the consumption of contaminated fish and seafood [35-37]. Mercury is found naturally in the environment and is released from igneous rocks [38, 39] that are often a component of the bedrock geology among north temperate latitude lakes, rivers and streams [38]. Elemental Hg generally does not pose a great risk to human health, as it is generally poorly absorbed from the diet by most consumer species, unlike MeHg which is easily absorbed by organisms [40]. The formation of MeHg typically occurs in anoxic environments when sulfur reducing bacteria indirectly assimilate inorganic Hg during metabolism and bind a methyl group to inorganic Hg [41]. In biological tissues, MeHg tends to bind strongly to sulfur containing amino acids with protein content generally describing the capacity of an animal to bioaccumulate Hg.

Mercury also exhibits the phenomenon of food-web biomagnification predominantly due to high efficiency of transfer from prey to consumer and generally slow rates of whole-body elimination relative to such dietary uptake [42]. The amount of protein content and the inherent capacity for Hg storage, increases as an organism increases in size and moves up in tropic position [43]. Because MeHg is slow to eliminate, it tends to increase with trophic position (i.e. biomagnification; [35, 44]). For this reason, consumption guidelines tend to exist for fish species across the Great Lakes basin, especially for top predator species that tend to be long-lived and large bodied [45].

The process of Hg methylation can be exacerbated by the development of anoxic conditions in aquatic. Although anoxic events occur naturally, anthropogenic activities such as eutrophication can increase the frequency and duration of anoxia [46-48]**.** Anthropogenic Hg inputs into aquatic systems can also increase the amount of Hg mobilized into the food web [49]. Historic and ongoing activities including chlor-alkali plant operation, fossil fuel combustion and artisanal gold mining activities continue to contribute to the global Hg cycle [50, 51]. Additionally, the flooding of land and creation of aquatic reservoirs as associated with the operation of hydro-electric dams and also by the activities of beavers (*Castor canadensis*) can contribute to Hg loading into aquatic ecosystems due to the mobilization of geologic Hg from previously unexposed soils and bedrock [35].

1.4 Summary

Yellow perch are an important ecological, economic and recreational fish. Examining the biological and ecological responses of this species as associated with

seasonal changes in limnology will help address knowledge gaps pertaining to winter ecology and biology. In this thesis, I investigated the general ecology of a yellow perch population over an entire growing season and also Hg bioaccumulation by these fish over this same duration. I first hypothesized that there will be significant differences in yellow perch biological and ecological metrics due to the range of variability in abiotic limnological parameters expected of a north temperate aquatic ecosystem over a one-year period. I also hypothesized that Hg concentrations in yellow perch will also demonstrate significant seasonality. This study was therefore undertaken in two parts; 1) an investigation of biological and ecological metrics for the Lake Manganese yellow perch over an annual growing season concomitant with the study of lake limnological parameters during this timeframe and; 2) monthly quantification of Hg concentrations within a single age class of Lake Manganese yellow perch over the same annual period. These results will contribute to the growing knowledge base focused on the roles of seasonality and our understanding of winter ecology and ecotoxicology.

2 Seasonal comparison of yellow perch (*Perca flavescens*) biology and ecology in Lake Manganese

2.1 Abstract

As poikilotherms, fish ecology and biology is predicted to be highly associated with ambient water temperatures. For example, fish species such as yellow perch (*Perca flavescens*) inhabit a wide latitudinal range across North America but relatively little is known of their biology and ecology across the seasonal range of temperatures associated with such distribution. In this study, yellow perch were collected monthly over one-year to evaluate seasonal changes in diet, proximate composition, condition indices, growth and also carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes. Fish average energy densities changed significantly ($P < 0.001$) from a high of 4.9 kJ/g during the summer to 3.9 kJ/g in the spring and generally reflected similar seasonal changes in fish lipid contents. Significant seasonal changes in fish $\delta^{13}C$ and $\delta^{15}N$ values were also observed. For $\delta^{13}C$, these changes were larger than the 1 ‰ fractionation described between predator and prey for aquatic ecosystems. However, for $\delta^{15}N$ this difference was less than the extent of change typically associated with one trophic position (± 3.4 ‰). An unexpected result of this study was the observation of extremely stunted growth for Lake Manganese yellow perch and individual fish ages up to 18 years. These results help demonstrate the empirical variability of fishery type research data and also contribute to the growing field of winter limnology and ecology.

2.2 Introduction

Much of what is currently known of fish biology and ecology has been determined from field work commonly completed during the open-water warm-temperature growing season. The timing of such field efforts are associated with the perceived ease of sampling during the spring and summer open-water seasons and general schedules as associated with the academic calendar year [52-54]. However, such a single season or 'snapshot' approach limits our empirical knowledge regarding the biology, ecology, and physiology of fish populations to only these seasons when animal growth rates are likely to be optimal [14, 52, 55]. As ectotherms, much of fish biological and ecological characteristics are predicted to strongly correlate with seasonal temperature changes [4]. This becomes especially true across north temperate latitudes for cool-warm water species for which the preferred temperature growing season is limited from approximately late May through to early September and the cold-water fall and overwintering seasons can predominate the annual growth cycle. In addition, fish physiological responses to additional overwintering conditions such as dissolved oxygen concentrations, prey availability and even ice-cover represents a general knowledge gap in our understanding of fish populations and their responses to their ambient environment. Thus, an assessment of the importance of seasonality in fisheries biology will provide an important contribution for fisheries biologists, managers and stakeholders alike.

In addition to its role in regulating fish growth and metabolism, water temperature is also an important contributor to ecological mechanisms such as habitat and food

resource partitioning. For example, in north temperate latitude lakes, thermal stratification in the summer can restrict habitat availability to deeper waters below the thermocline especially for cold water species such as lake trout (*Salvelinus namaycush*). In contrast for cool-warm water species such as yellow perch (*Perca flavescens*), habitat selection is likely to be more restricted to meta- and epilimnetic waters where temperatures are closer to optimal and prey resources will be limited to those species that predominantly occupy similar habitats [56]. For these same ecosystems, inverse winter stratification has potentially contrasting effects on resource availability depending on the thermal preferences of the species under study. For a species such as lake trout with a thermal optimum between 8 – 12 °C, the loss of the thermocline and onset of colder fall, winter and spring water temperatures can maximize habitat availability and potentially increase prey choice selections during these seasons. In contrast, warmer water species such as yellow perch that have temperature optima > 20 °C are likely to select the marginally warmer water regions that tend to be limited to the greater depths of lentic ecosystems, especially during the ice-covered overwintering months [14, 56-58] . However, during winter months, ice- and snow-cover can reduce light penetration and limit nutrient availability thereby restricting the extent of primary production and overall food availability [1]. Such seasonal temperatures cycles can also play a role in oxygen availability throughout the water column. Summertime thermal stratification generally restricts oxygen mixing with the fall turnover period being required to replenish oxygen concentrations that can become reduced or even depleted during the warm water growing and high productivity seasons, especially in shallow and/or increasingly eutrophic lakes [59, 60]. Thus, for individual fish species, seasonal temperature cycles likely require

unique strategies for growth and survival that may be poorly understood if knowledge is derived primarily from the open water growing seasons only [1].

The yellow perch is a well-studied species with a depth of information available regarding this species' biology and ecology. For example, Kitchell et al. [4] developed a robust bioenergetics model for predicting characteristics such as consumption, respiration, egestion and growth rates across the life history of this species. However, limited research has been completed to evaluate yellow perch biological and ecological responses under natural conditions over an entire growing season. In North America, native populations of yellow perch range from approximately the state of Georgia to as far north as Great Slave Lake in Canada's Northwest Territories with their native western range limited by the Mississippi River within the contiguous United States [9]. Intentional stocking and accidental releases have expanded this range throughout the lower 48 United States and across most of the Canadian provinces [9]. Over such a broad latitudinal range, yellow perch will experience contrasting seasonal periods with respect to availability of optimal temperatures for growth. Generally considered a cool-warm water species, juvenile and adult yellow perch have an optimal temperature for growth at 23 °C which declines slowly as temperatures approach 10 °C [55, 61, 62] and reaches a zero/maintenance growth asymptote at temperatures less than 2 °C [4]. From these preferences, populations inhabiting aquatic ecosystems at the southern limit of their North American range will experience an extended summer growing season relative to populations occupying more northern latitude ecosystems where the open water season will be shorter. In contrast, for more northern populations, the cool-cold water fall and

winter non-growing seasons will persist for a longer duration relative to more southerly lakes. Such seasonal contrasts will also be important for prey availability especially given that the cold, darker overwintering periods are generally assumed to be a period of low productivity and dormancy [1].

Yellow perch are generally considered to be omnivorous with feeding habits typically displaying three distinct ontogenetic shifts during their life history. Perch spawn in the spring with post-hatch free swimming individuals being predominantly planktivorous and consume larger cladoceran and copepod species [63]. By the end of the first growing season, young of the year diets can switch to benthivory with macroinvertebrates species such as odonates, amphipods and caddisflies beginning to dominate the diet [14]. A final diet switch to piscivory can be facilitated as individuals grow sufficiently large in size (> 150 mm) to consumer other smaller fishes which can include fishes such as brook stickleback (*Cula inconstans*), young-of-the-year and juvenile bluegill sunfish (*Lepomis macrochirus*), and emerald shiners (*Notropis atherinoides*) with cannibalism also frequently observed among yellow perch populations [9]. Generally, however, and similar to feeding information for many fish species, yellow perch diets have been assumed from samples predominantly collected in the summer with less known for the cold-water periods when prey species such zooplankton and larger macroinvertebrates may be very low in abundance. Data is available describing seasonal changes in yellow perch energy density [26], and lipid content [64] that suggest food limitation during the winter. However, these studies have been completed without high frequency sampling over an annual temperature cycle within

wild populations, and as a result these changes and associated roles in yellow perch biology and ecology such as diet are generally unknown.

Lake Manganese located in Michigan's Keweenaw Peninsula provides habitat for one of the northernmost yellow perch populations within the contiguous US. This study was designed to investigate seasonal related changes in specific biological and ecological characteristics of the Lake Manganese yellow perch population. Specific objectives included: 1) comparing and contrasting yellow perch lipid and moisture contents, energy densities, gonado- and hepatosomatic indices, and diet preferences across the spring, summer, fall and winter seasons and; (2) evaluating differences in these characteristics between male and female fishes. It is predicted that there will be significant seasonal differences among these values and also that male and female fishes will contrast in their responses of these metrics to seasonality. These results will be valuable for understanding the overwintering ecology and biology of a commercially and recreationally valuable fish species inhabiting north temperate ecosystems.

2.3 Methods

Yellow perch for this study were collected monthly from Lake Manganese (Figure 2.1) using hook and line from April 2018 – March 2019. A maximum of 50 fish were collected each month with no fish collections being conducted in November 2018 due to adverse weather and poor ice-conditions on the lake. Bulk zooplankton were captured with a horizontal tow using a (64 μm) mesh zooplankton net in June 2018-August 2018. Excess water in samples was removed using 63 μm mesh metal sieve with samples stored frozen for stable isotope analysis. Unionid mussel samples were collected

by hand from shallow depths (< 1 m) for stable isotope analysis. Zooplankton and mussel samples were not collected in April, nor October 2018-March 2019 due to adverse sampling conditions for these taxa (e.g. ice cover and/or high turbidity). Fish collections for this research were authorized by the Michigan Department of Natural Resources DNR Scientific Collecting Permits obtained in 2018 and renewed in 2019. All samples were stored in ice filled coolers for transport back to the laboratory where they were subsequently stored frozen (-20°C) until ready for dissection or analysis. Handling and care of the fish was done under the approval of the Michigan Technological University Institutional Animal Care and Use Committee, project number 1414057-1.

Limnological data including temperature (°C), dissolved oxygen (mg/L), pH (unitless), specific conductivity (µS/cm), and oxidation-reduction potential (mV) were recorded during the open water months (May – October) using a Yellow Springs Instruments (YSI) Pro DSS Multiparameter Water Quality Meter. Water quality profiles were collected at the deepest point of the lake from the water surface to a depth just above the bottom (approx. 6.4m). A string of 7 Hobo Pendant™ (Model # UA-001-08) temperature loggers was deployed extending from the shallow littoral zone region of the lake (0.19 m) to approximately the deepest point of the lake with each logger corresponding to approximately 1 m increments in lake depth to the maximum depth. Temperature loggers were programmed to record water temperatures every 30 minutes and were in operation from April 2018 – March 2019.

2.3.1 Sample processing

Biological data collected from each fish included sex, total, fork and standard lengths (mm), gonad, liver and whole-body masses (g), with sagittal otoliths removed for aging purposes. Fish stomachs and intestinal tracts were also excised and prey items were identified during processing. Following dissection, fish were wrapped in in acetone/hexane (solvent) rinsed aluminum foil and stored at -20°C until ready for homogenization. Whole-body homogenates were prepared for moisture and lipid content determination and stable isotope analyses using a solvent rinsed stainless steel Waring 700S blender with up to a ~35 g subsample stored in a clean steel tin until ready for analysis. All dissection and homogenization equipment was thoroughly washed with soap and water and rinsed and rinsed between each fish. Gonadosomatic (*GSI*) and hepatosomatic (*HSI*) indices were calculated from the respective tissue weights following equations 1 and 2 below, respectively;

$$GSI = W_G/W_B \times 100 \quad (1)$$

$$HSI = W_L/W_B \times 100 \quad (2)$$

where W_G and W_L represent the masses of gonads or liver, respectively, and W_B represents whole body mass.

2.3.2 Gut contents

During fish collections, common earthworms (*Lumbricus terrestris*), wax worms (*Galleria mellonella*), and artificial lures were used to enhance fish collection. None of these items were identified as natural food items and were removed from prey enumerations to avoid any skew of results due to their inclusion. Invertebrate identifications followed the taxonomic keys outlined in [65]. Unidentifiable material including organic detritus were characterized as detritus. Prey species identified in individual gut contents were reported as the proportion of the total number of prey items quantified in each individual gut content.

2.3.3 Fish Aging

Perch ages were estimated from sagittal otoliths removed from each fish during dissection with aging method following the crack and burn procedure outlined by Christensen [113]. Otoliths were placed in Scotch™ brand lightweight mounting putty with their concave side facing up before being split in half with a scalpel through the core of the otolith and along the dorso-ventral axis. The otoliths were then held over an open flame for a few seconds until the otolith turned a light brown in color. The otolith was then inserted into a new piece of mounting putty with the cut surface facing up and then lightly painted with canola oil. Both halves of aged otoliths were imaged using an Olympus SZX9 dissecting microscope equipped with Imagingsource™ camera. Images were analyzed in FIJI/ImageJ image processing software. For quality control purposes, a subsample of 16 randomly selected otoliths were sent for aging by three individuals experienced in aging fish otoliths. Otolith identifications were blind to each reviewer

with only fish species information known. In the occurrence of discrepancies between readers, life-history tables and literature based yellow perch length at age ranges were consulted to provide a consensus age estimate for the otolith in question [58, 66] . The von Bertalanffy (VBL) outlined in equation (3) below was used to generate population and sex-specific growth rates for Lake Manganese yellow perch;

$$L_t = L_\infty\left(1 - e^{-K(t-t_0}\right) \quad (3)$$

where L_t represents estimated length (mm) at time t (yrs.), L_∞ asymptotic length when growth equals zero, K represents Brody growth rate coefficient (yr^{-1}) and t_0 is the theoretical age at length 0. For fish with missing or broken otoliths that could not be aged, the VBL model was rearranged to solve for age using the K, L_t and L_∞ estimates generated from otolith age and length data. Specific iterations of the VBL model included i) simulation for all fishes collected; ii) a subset of fishes aged 2 – 8 yrs.; iii) males only and; iv) females only.

2.3.4 Moisture content determination

Sample moisture contents were determined using approximately 1 g (± 0.0001 g) of whole-body homogenate, bulk zooplankton and homogenized mussel tissues. Aluminum weigh boats were pre-dried in an oven for 48 hours at 120 ℃ and allowed to cool to room temperature inside desiccators prior to sample addition. Weigh boats were

pre-weighed empty followed by addition of the sample material and subsequent drying for 48 hours at 60 ℃. Dried specimens were removed from the ovens and allowed to cool to room temperature inside desiccators with dried samples then reweighed (± 0.0001 g) and moisture contents determined as per equation (4);

$$\% \, Moisture = \left[1 - \frac{dry\ weight - boat\ weight}{wet\ weight}\right] \times 100 \qquad (4)$$

Where dry weight represents the mass of the dried sample and weigh boat after 48 hours at 60 °C (g); boat weight is the mass of the empty, pre-dried weigh boat (g); and wet weight is the wet mass (g) of sample added to the boat prior to oven drying.

2.3.5 Lipid content determination

Total lipid content for each sample was determined as a component of a paired pollutant extraction procedure conducted on each fish and outlined in [67]. Approximately 1.0 g of tissue homogenate was mixed with 10 g of pre-combusted sodium sulfate, (Granular 10-60 Mesh) added to a 20 mL glass syringe containing a glass wool plug, 10 mL of a 50:50 (vol:vol) mixture of a hexane and dichloromethane (DCM) extraction solvent attached to a VWR® syringe filter (25 mL 0.45 µm PTFE membrane). The mortar and pestle were then rinsed with an additional 5 mL of the extraction solvent with the 5 mL rinse transferred into the syringe. An additional 5.0g of sodium sulfate was added to the mortar and pestle, mixed in the mortar and quantitatively transferred into the

syringe, with a final 5.0 mL rinse of the mortar and pestle with the extraction solvent and transfer into the extraction syringe. Samples were then allowed to stand for one hour with eluents collected in 45 mL glass test-tube with an additional 15 mL of the 50:50 solvent added as a final rinse. Once samples had drained into test tubes, they were transferred into a 125 mL round-bottom flask with each test tubes rinsed with a small volume of hexane to remove any residual lipids in the test tube. The round-bottom flasks were evaporated to a volume < 10.0 mL using Heidolph® Roto Evaporators, and the extract was then transferred to 10 mL volumetric flasks and brought up to volume with hexane. A 1.0 mL subsample was then transferred from the 10 mL flask into a dried, pre-weighed and labelled aluminum weigh boat before being placed in the oven at 110 ℃ to dry for 1 hour. Following drying, weigh boats containing the lipid volume were removed and allowed to cool to room temperature in a desiccator before being weighed. Final % lipids were calculated using the following equation:

$$\% \, Lipid = \left[\frac{Dried\ mass - Boat\ mass}{Wet\ mass}\right] \times 1000 \qquad (5)$$

Where dried mass (g) is the mass of the dried weigh boat containing the lipid sample; boat mass (g) is the dried boat mass prior to sample additions; and wet mass represents the wet mass (g) of sample used for the extraction procedure.

2.3.6 Energy density and condition

Yellow perch energy densities (kJ/g) were estimated using the mass balance approach as outlined in [68]. Briefly, whole-body lipid and moisture masses (g) were determined from the product of sample lipid and moisture contents (% wet weight) and fish total masses. Ash content for fish tissues typically range between approximately 1 - 2 % [69] in [70] and the random number generator function of Excel software was used to estimate an ash content within this range. Fish tissues typically contain very little to no carbohydrates [71], thus protein mass was considered to represent the proportion of whole body tissue remaining following estimation of lipid, moisture and ash contents. Protein and lipids were assumed to contain caloric contents of 9.02 and 4.271 kcal/g [72], respectively, with a conversion factor of 4187 J/kcal used to estimate energy content [73] in [70].

Fulton's condition index (*K*) was used as a general fisheries metric of yellow perch growth and quality [74]. This value typically ranges between 0.8 – 2.0 for fishes and was calculated as per equation (6):

$$K = \left(\frac{W \times 100}{TL^3}\right) \quad (6)$$

Where *W* represents fish total mass (g) and *TL* is fish total length (cm).

2.3.7 Stable isotope analysis

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes values were quantified in samples to provide indicators of yellow perch, zooplankton and mussel trophic structure in Lake Manganese. Analysis of $\delta^{13}C$ and $\delta^{15}N$ was completed using the individual fish whole body homogenate that was previously dried at 60 ℃ for 48 hrs and ground to a fine powder using a glass mortar and pestle. Between 0.04 - 0.06 mg of the dried powder was added to a 3.0 x 5.5 mm tin capsule, which was then folded and placed into sample trays. Zooplankton and mussel samples for stable isotope analysis were prepared in the same manner as fish samples. Sample stable isotope analyses were completed by the Stable Isotope Laboratory at Cornell University (Ithaca, New York) using a Finnigan MAT Delta Plus isotope ratio mass spectrometer. Stable isotope results are calculated relative to a reference standard and expressed as a delta (δ) notation:

$$\delta^{15}\text{N or } \delta^{13}\text{C (‰)} = \left[\left(R_{sample}/R_{standard}\right) - 1\right] \times 1000 \qquad (7)$$

Where *R* represents the ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) in the sample materials relative to the reference standard. For $\delta^{13}C$ measurements, samples were quantified against a Pee Dee Belemnite standard with atmospheric nitrogen being used for $\delta^{15}N$ quantification. An internal animal standard (Deer) was run along with all samples for accuracy and precision (± 0.03 ‰ for $\delta^{15}N$, and ± 0.05 ‰ for $\delta^{13}C$) and a chemical standard (methionine) used to test the accuracy across a range of amplitudes.

Isotope corrections were performed at the Stable Isotope Laboratory using two-point normalization on (linear regression) of all $\delta^{15}N$ and $\delta^{13}C$ using two additional in-house standards (KCRN and CBT).

2.3.8 Data Analysis

All statistical analyses were completed using the software package JMP Pro version 14.0 for Windows (SAS Institute, Cary, North Carolina, USA) with a criterion for significance of $\alpha = 0.05$ ($p < 0.05$) used in all cases. All data were tested for normality using normal probability plots. Energy density, length, mass, lipid contents, $\delta^{13}C$, $\delta^{15}N$, and condition data were all determined to be normally distributed. However, *GSI* values required log(x+1) transformation to meet the assumption of normality. Statistical comparisons of the biological variables across seasons and between male and female fishes were completed using one-way analysis of variance (ANOVA) with analyses corrected for age, total length or body mass covariates where necessary. To increase statistical power, monthly sampling data were combined to represent spring (April – June), summer (July – August), fall (September – December) and winter (January – March) seasonal collections. A Tukey's highly significantly different (HSD) post-hoc test was used for all pairwise comparisons. For yellow perch data, ANOVA comparisons were conducted on the population level sampling data and also within 8-year-old fishes only. This age class was predominant cohort within the population and thus used as a proxy for evaluating cohort level responses to seasonal changes. Seasonal comparisons of the limnological variables were completed using one-way ANOVAs followed by Tukey's post-hoc tests.

A one-way ANOVA was used to determine if there were significant differences between male (n = 236) and female (n = 209) total length and mass results for the population level sample collections and were also used to determine if there were significant differences between total length and mass between 8 year-old male (n = 51) and female (n = 46). A one-way ANOVA model was used to test whether 'taxa' (mussels n = 3; zooplankton n = 5; and yellow perch n = 117) significantly differed in $\delta^{13}C$ and $\delta^{15}N$ values. A Bonferroni correction ($\alpha = 0.017$) was used when comparing stable isotopes among species to account for uneven sample sizes. Unless indicated otherwise, all results are reported as arithmetic mean ± 1 standard error. Full summary statistics are provided in supplementary tables S1-S5 at the end of this chapter.

2.4 Results

2.4.1 Water Quality

A summary of the water quality parameters recorded from Lake Manganese over the duration of this study are provided in Table 2.1. Daily water temperatures recorded by the temperature loggers ranged from 0.2 – 24.4 °C over the course of this study with these minimum and maximum values being recorded during the months of March and August, respectively. The month of August 2018 also had the highest recorded mean temperature (16.7 ± <0.1 °C) with December 2018 being the coldest on average (2.5 ± <0.1 °C; Figure 2.2). Water temperatures differed significantly across the seasons with averages of 6.8 ± < 0.1 °C (spring), 16.0 ± < 0.1 °C (summer), 4.4 ± < 0.1 °C (fall) and 2.7 ± < 0.1 °C (winter) recorded over the study using HOBO data loggers ($F = 590.0$, $P < 0.001$; Table 2.1; Table S2.1). Mean seasonal water temperature depth profiles for Lake

Manganese are presented in Figure 2.3. No distinct thermocline was observed during the summer but water temperatures declined from 19.0 ± 0.2 °C at the lake surface to a minimum of 11.2 °C approaching the lake bottom (6.2 m). Lake Manganese was nearly isothermal across all depths during the fall with a surface water temperature of 3.7 °C and an average temperature of 6.1 °C recorded at the lake bottom (6.4 m). A degree of inverse stratification was observed during the winter with an average water temperature of 5.0 °C measured at the lake bottom and an average surface temperature of 0.5 °C recorded during this period.

Lake Manganese did not demonstrate any periods of anoxia with dissolved oxygen (DO) concentrations ranging between 3.6 - 10.8 mg/L from spring through fall seasons (Table 2.1). YSI readings were taken monthly from May - October 2019. Highest dissolved oxygen levers were all recorded in October, ranging from 10.8 mg/L at 0.1 m to 10.4 mg/L at 7 m. Average DO was highest in fall (10.5 ± 0.4 mg/L) which was significantly different than spring (7.9 ± 0.3 mg/L) and summer (7.9 ± 0.2 mg/L). The lowest overall DO was measured on May 18, 2018 at 7m and was 3.6 mg/L compared to 10.1 mg/L at 0.1m during this sampling event. Mean seasonal water DO depth profiles are presented in Figure 2.4. Dissolved oxygen levels gradually decreased with depth during the spring and summer but the fall profile demonstrating evenly mixed DO concentrations throughout the water column. Specific conductivities ranged from 129.2 µS/cm in summer to 69.7 µS/cm in fall. Total dissolved solids ranged from 84.0 mg/L in summer to 45.4 mg/L in fall. pH ranged from 8.2 in summer to 7.6 in spring. Oxidation-reduction potential values (ORP) did not display any significant seasonal variation with

depth (F = 2.626, P > 0.100; Table S2.4.) or with season (F = 3.041, P > 0.050; Table S2.4).

2.4.2 Seasonal biological, ecological and stable isotope data

Summary seasonal biological and stable isotope data for population level sampling of Lake Manganese yellow perch are provided in Table 2.2. Male yellow perch collected for this study ranged in age from 2 – 18 years old with females ranging from 2 – 14 years old. No fish ≤ 1 year of age were collected. Across the seasons and between the sexes, females were consistently large than males in both total length and body mass (Table S2). Von Bertalanffy (VBL) growth curves also demonstrated females to have substantially different growth relative to males (Figure 2.5). For females, VBL growth model estimates indicated a maximum asymptotic length (L_∞) of 17.1 cm relative to a value of 14.2 cm for males. Also, Brody growth coefficients (k) of 0.5 yr^{-1} and 0.6 yr^{-1} were estimated for females and males, respectively.

Lipid content and energy density both significantly differed based on season (Table 2.1). Whole body lipid contents were highest in winter (1.9 ± 0.2 %) and was significantly different from fall (1.1 ± 0.1 %) and spring (0.8± 0.1 %). Summer (1.5 ± 0.1 %) was significantly different than spring (0.8 ± 0.1 %) (F = 22.3, P = 0.001 S1.) (Table 2.2). There was no significant difference between winter and summer lipid content. Lipids also significantly decreased with age (F = 8.3, P < 0.005 S1.) Energy density was highest in summer (4.7 ± 0.1 kJ/g) and decreased through fall (4.5 ± 0.1 kJ/g), winter (4.5 ± 0.2 kJ/g) and into the spring (3.9 ± 0.1 kJ/g) (F= 16.6, P < 0.001 Table S2.1.)

Highest *GSI* values for Lake Manganese yellow perch occurred in winter collections (4.2 ± 0.3 %) and was lower in fall (3.2 ± 0.3 %), both of which were significantly greater than spring (2.2 ± 0.3 %) and summer (1.5 ± 0.4 %; $F = 22.0$, $P < 0.0001$; Table S2.1.) There was no significant difference in *GSI* values between males and females ($F = 2.3$, $P > 0.100$). Gonadosomoatic indices decreased significantly with age ($F = 6.0$, $P = 0.015$), and with length ($F = 17.1$, $P < 0.001$). In contrast, *GSI* values increased significantly with mass ($F = 23.4$, $P < 0.001$). Hepatosomatic indices were highest in spring (1.2 ± 0.1 %) and was significantly greater than winter (0.9 ± 0.1 %), which was significantly greater than fall (0.6 ± 0.1 %) and summer (0.5 ± 0.1 %) ($F = 21.1$, $P < 0.001$). Female *HSI* were significantly higher (1.1 ± 0.1 %) than those for males (0.6 ± 0.1 %; $F = 19.8$, $P < 0.001$). There was a significant decrease in *HSI* with increasing length ($F = 17.1$, $P < 0.001$), and a significant increase with both age ($F = 4.6$, $P = 0.033$), and mass ($F = 16.2$ $P < 0.001$). Condition indices demonstrated significant seasonality with summer (0.9 ± <0.1 g/cm^3) 0.1% smaller than the peak condition in winter ($F = 3.9$, $P = 0.0091$). Condition also demonstrated a significant increase with age ($F = 5.4$, $P < 0.021$).

There was significant seasonal variation in $\delta^{15}N$, which peaked in fall (7.5 ± 0.1 ‰) and decreased in spring (7.1 ± 0.1 ‰) ($F = 3.0$, $P = 0.032$; Table S2.1). There was also a significant increase in $\delta^{15}N$ with both age ($F = 6.9$, $P < 0.010$; Table S2.1) and length ($F = 6.0$, $P = 0.016$; Table S2.1). Also, fish $\delta^{13}C$ demonstrated significant seasonal variation with values peaking in spring (-31.3 ± 0.2‰), decreasing in summer (-31.7 ± 0.4‰), fall (-32.8 ± 0.4‰), and winter (-32.9 ± 0.5‰) ($F = 7.0$, $P < 0.001$; Table S2.1).

There was also a significant increase in $\delta^{13}C$ with fish length ($F = 13.7$, $P < 0.001$: Table S2.1). Mussels, yellow perch, and zooplankton $\delta^{15}N$ values differed significantly ($F = 135.6$, $P < 0.001$, Table S2.3) with values averaging 2.7 ± 0.3 ‰, 7.3 ± < 0.1 ‰, and 5.3 ± 0.4 ‰, respectively. Yellow perch were significantly different from mussels and zooplankton $\delta^{13}C$ differed significantly ($F = 13.7$, $P < 0.001$; Table 2.3) with yellow perch $\delta^{13}C$ values averaging -31.9 ± 0.2 ‰ and zooplankton averaging -37.8 ± 1.2 ‰.

Sex

Lipid contents were significantly different ($F = 18.0$, $P < 0.001$; Table 2.1) between males and females. Males had significantly greater lipid content (1.6 ± 0.1%) than females (1.1 ± 0.1%). Condition indices were significantly different ($F = 256.0$, $P < 0.001$; Table 2.1) between males (-0.1 ± 0.1 g/mm^3) and females (-1.5 ± 0.1 g/mm^3).

Summary seasonal biological and stable isotope data for 8-year-old Lake Manganese yellow perch are provided in Table 2.3. Male fish within this age class were significantly smaller in both total length ($F = 109.2$, $P < 0.001$; Table S2.4) and body mass ($F = 53.3$, $P < 0.0001$; S4) relative to 8-year-old females. Lipid content for male and female 8-year-olds peaked in winter (2.0 ± 0.2%) and was significantly larger than fall (1.0 ± 0.1%) and spring (0.8 ± 0.1%) ($F = 10.5$, $P < 0.001$; Table S3). Energy densities within this age class were also greatest in the summer (4.7 ± 0.1 kJ/g), and decreased in fall (4.5 ± 0.1 kJ/g), winter (4.4 ± 0.2 kJ/g), and reached its lowest value in the spring (4.0 ± 0.1 kJ/g). Spring collected fishes had significantly lower energy density relative to individuals collected in the summer and fall ($F = 69$, $P < 0.001$, Table S3).

There was a significant seasonal difference for *GSI* which had the highest values in fall (4.0 ± 0.8 %) and winter (4.6 ± 0.8 %) and were significantly different from summer (1.8 ± 0.8 %) and spring (1.8 ± 0.6 %) ($F = 6.9$, $P < 0.001$). Hepatosomatic indices peaked in spring (1.6 ± 0.1%) and were significantly different from winter (0.8 ± 0.2 %), both of which are significantly different than summer (0.4 ± 0.1%) ($F = 12.0$, $P < 0.001$). Condition indices did not demonstrate any significant differences across seasons ($F= 1.6$, $P = 0.2$, Table S2.3), but was significantly different between sex ($F = 7.2$, $P = 0.009$). No significant seasonal patterns in $\delta^{15}N$ or $\delta^{13}C$ were evident among 8-year-old fishes (Table S2.4).

2.4.3 Diet

The greatest total number of prey taxa identified from fish diets occurred in the summer with 13 different prey types identified from the gut contents of fish sampled during this season (Figure 2.8). This was followed by spring (n = 12), winter (n = 9), and fall (n = 6) collections. Female and male diets differed seasonally with respect to the diversity of prey items identified within gut contents. For example, females consumed a wider diversity of prey species in the spring (n = 11), summer (n = 12) and fall (n =7) relative to males (spring n = 5; summer n = 9; fall 3; winter n = 9). For males, snails (*Physa spp.*), mayflies (*Hexagenia limbata*) and detritus were consistently observed in diets each season. For females, *Physa spp.,* crayfish (*Orconectes propinquus)*, dragonflies (*Anisoptera spp.*), caddisflies (*Trichoptera spp.*) and unidentifiable fish remains and detritus remains were consistently observed in the diet. Spring peeper (*Pseudacris crucifer*), aquatic and terrestrial *Coleptera spp*., and amphipods were found

only in female diets. The only prey item unique to male diets included fingernail clams (*Sphaerium spp.*) with the diets of male fishes consisting of 11 different prey species over the one-year sampling period. Three of the prey items; *Isopoda*, *Sphaerium*, and *Anisoptera*, were only found during one season and each contributed < 3.0% to prey counts in the diet. *Physa* constituted the largest proportion of male diets in spring (46.4 %), fall (48.2 %), and winter (60.0 %), whereas females consumed the most *Physa* in spring (62.1 %) but dropped to 5.9 % in summer, 0 % in fall, and 13.0 % in winter. Within male diets, *Trichoptera* were present in spring (28.8 %), summer (2.7 %), fall (25.0 %) and winter (16.3 %). In summer detritus (51.4 %) made up the largest proportion of the diet, along with *Chironomids* (8.1 %) and *Orconectes propinquus* (5.4 %) reaching peak abundance in male diets. Fish remains occurred infrequently in the gut contents of males (< 3.8%).

2.5 Discussion

Traditional snapshot sampling of fish populations has typically been conducted during the summer during which water temperatures, nutrient levels, primary productivity, and prey availability are generally highest and conditions for fish growth are optimized relative to the remainder of the year [7, 75]. However, such single season sampling will not permit the ability to empirically capture the extent of annual variability for common biological metrics frequently quantified by fisheries monitoring programs. In this study, significant seasonality was observed across a range of biological variables for a yellow perch population at both the population- and age-class levels of sampling. Significantly different seasonal responses were also determined between male and female

fishes that could be associated with the unique growth of the Lake Manganese perch population. Such seasonal variation contributes to our knowledge of fish ecology and also our understanding of population responses to natural forcing factors such as seasonality.

Yellow perch lipid contents demonstrated a significant seasonal pattern with highest lipid levels occurring in the winter and prior to spring reproduction. This pattern agrees well with that described for yellow perch and other cool and cold-water fish species that spawn in the spring [16]. Due to the high caloric content of lipid tissues, it would be expected that energy density would follow a similar seasonal pattern as lipid content. Instead, energy density peaked in the summer contrasting the winter lipid peak. A possible explanation for this contrast could be associated with the specific seasonality of yellow perch reproduction. For example, the lowest *GSI* values were recorded in the summer and reproductive tissues generally have low water content but require a large energy investment to create [12, 26] . Condition was also lowest in the summer months, which would indicate that yellow perch in Lake Manganese have less body mass per unit length in the summer than the rest of the year when *GSI* increases. Increases in *HSI* are also associated with the production of reproductive tissues and this metric demonstrated a seasonal increase from fall through to the spring, before a rapid decline following the spring spawning period [26, 70]. Therefore, seasonal changes observed in lipid content and energy density are all potentially correlated with the seasonal nature of reproductive physiology. Further, moisture content for fishes were highest for winter collected fishes and this component of proximate composition does not contribute to whole body energy

density. This trend also agrees well seasonal changes in *GSI* and *HSI* as associated with yellow perch reproduction and is a characteristic of the population that would not be captured during the open water non-reproductive seasons for this species and other similar percids such as walleye (*Sander vitreum*) that also spawn in the spring.

The stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are considered to represent longer-term spatial integrations of animal trophic ecology with $\delta^{13}C$ providing insight into habitat and food resource exploitation strategies and $\delta^{15}N$ provide a measure of organismal trophic position [71, 76-79]. In this study, yellow perch $\delta^{13}C$ values differed by up to 2.2 ‰ among seasons for the fishes collected here which is greater than the < 1 ‰ generally assumed for the fractionation of $\delta^{13}C$ between prey and predator [80]. Importantly, such differences in fish $\delta^{13}C$ occurred between sampling events that were conducted within a few months apart suggesting that this particular ecological tracer may integrate shorter time frames of animal resource exploitation relative to general assumptions of a life-history integration for this particular tracer [77]. Yellow perch $\delta^{13}C$ values in this study were generally quite negative for this species and also encompassed a wide range (-27.3 to -35.3 ‰) suggesting a wide variety of habitat and food resource exploitation. In comparison, Happel et al. [81] reported $\delta^{13}C$ values ranging from -18 to -24 ‰ for Lake Michigan yellow perch collected from across a variety of rocky and sandy substrate locations across the lake. Lake Manganese receives inflow from Aetna and French Annie Creeks which drain through flooded marsh habitat before emptying into the lake. Anaerobic habitats within such marshes can result in highly negative $\delta^{13}C$ signatures approaching -40.0 ‰ due to high levels of bacterial respiration that alters

carbon isotope fractionation [82]. Hecky and Hesslein [83] also described similarly highly negative $\delta^{13}C$ values for Arctic lakes where phytoplankton carbon production resources predominate to overall consumer production and these trends could contribute to the $\delta^{13}C$ profiles determined for yellow perch in this study.

Carbon stable isotopes in lentic ecosystems tend to follow a general pattern of becoming increasingly negative along a transect from nearshore littoral zones, to offshore pelagic areas, and typically being the most negative in deeper profundal regions [83-85]. Gut contents data collected for this study demonstrated a total of 14 different diet items and agree with the generally omnivorous diet described for yellow perch [9]. Male diets encompassed a broader range of prey items relative to females with snails (*Physa* spp), detritus and mayflies representing the predominant biomass ingested by males across the seasons. Hecky and Hesslein [83] reported an average $\delta^{13}C$ of -30.8 ‰ for benthic grazing snails (*Physa jennessii jennessii*) in an Arctic lake that were highly similar to zooplankton values (-32.7 ‰). This similarity was attributed to increased turbidity that inhibits light penetration and the development of benthic algae that generally produce isotopically heavier (less negative) $\delta^{13}C$ values as associated with photosynthesizing in the undisturbed boundary layer proximate to the lake bottom [83]. Lake Manganese is a small (21.2 ha) and relatively shallow (z_{max} = 7.6 m) ecosystem that was frequently highly turbid during sampling with total dissolved solids values averaging 84.0 mg/L during summer sampling. Carbon stable isotope values for male yellow perch in Lake Manganese were consistently more negative relative to those for females suggesting that males are selecting deeper regions of the lake where phytoplankton and zooplankton

resources support consumer production. In contrast, the less negative $\delta^{13}C$ values characterized for female yellow perch in Lake Manganese Sport conclusions of shallower nearshore habitat selection by this sex [86] where light penetration can better support the growth of benthic algal resources and produce the less negative $\delta^{13}C$ values characteristic of this habitat [83].

An unexpected result from this study was distinct evidence of highly stunted growth for the Lake Manganese yellow perch population. For example, male yellow perch collected from Lake Manganese for this study averaged only 141 mm in length with females averaging 165 mm. Additionally, fish up to 18 years of age were collected for this study. For comparison, Ridgway and Chapleau [28] described average lengths of 172 mm and 241 mm for stunted male and female yellow perch, respectively, collected from Lac du Printemps in Quebec. These results compare to the highly productive Lake Erie yellow perch population for which average sizes of 7-year-old male and female fish can range up to 233 and 374 mm, respectively [87]. Fish collected from Lac du Printemps had a maximum age of 10 years which is not atypical for yellow perch and lifespans up to 15 years have been reported for this species [28, 88]. However, the 18-year-old male yellow perch collected in the current study appear to be atypical and rare for this species and have remained undocumented for the Lake Manganese population until now.

Stunting in fish populations is indicated by earlier sexual maturation, smaller sizes at age, longer lifespans, and exceedingly slow growth rates relative to those described for typical population [20-23, 27, 28]. This phenomenon can be attributed to a variety of

factors including competition for limited food resources [21, 23, 28] which in turn can be associated with additional factors such as including a loss of fishing pressure, introductions of non-native species, a shortage of forage fish, and a lack of benthic invertebrates [23, 27]. Decreased mortality within a population can also result in stunting or exacerbate it within an already stunted or at-risk population [23]. Stunting is, however, a plastic growth response to environmental conditions with transplantation studies demonstrating rapid increases in growth observed for stunted individuals under laboratory settings and *ad-libitum* feeding conditions [21]. The relatively high north temperate latitude location and limnological conditions within Lake Manganese may also contribute to the degree of stunting observed for this yellow perch population. For the 6 months from November through May, water temperatures were consistently within one degree of the 2 ºC temperature at which yellow perch growth is predicted to approach a zero growth or maintenance level [4]. Further, there was no single month of the year during which water temperatures achieved the 22 – 23 ºC range for optimum yellow perch growth. Northern latitudes are associated with longer periods of cooler temperatures and a decreased number of growing degree days [53, 89]. For Lake Manganese, only the top 4 meters of the water column consistently averaged water temperatures sufficiently high (> 13.5 ºC) for yellow perch to maintain consistent positive growth during the summer growing season. Furthermore, prolonged exposure to cold temperatures typically also results in longer fish lifespans [16]. The slow growth rates within Lake Manganese which, when combined with longer-lived individuals, could further increase resource limitation and also contribute to stunting in this yellow perch population.

In addition to abiotic factors, highly abundant golden shiner (B. Duxbury, *pers. obs.*) may also attribute to the stunting of Lake Manganese yellow perch. Juvenile yellow perch feed on a diet of zooplankton [9], which are also the main food source of golden shiner. There has also been a diversity of successful and unsuccessful introductions of several non-native species into Lake Manganese species such as; largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), splake (*Salvelinus namaycush* x *Salvelinus fontinalis*), artic grayling (*Thymallus arcticus*), brook trout (*Salvelinus fontinalis*) and the now extirpated strain of Lake Medora lake whitefish (*Coregonus clupeaformis medorae*) [90, 91]. Of these introductions, only largemouth bass appear to have been successful based on collections completed for the current study. Historical survey data also indicate a diversity of smaller forage fishes including lake chub (*Couesius plumbeus*), fine-scale dace (*Chrosomus neogaeus*), red-bellied dace (*Chrosomus eos*), Johnny darter (*Etheostoma nigrum*), Iowa darter (*Etheostoma exile*), stickleback (*Gasterosteus* spp.) and brassy minnow (*Hybognathus hankinsoni*) in Lake Manganese [41]. The introductions of larger-bodied predators such as largemouth bass and splake likely rapidly depleted these forage fish species as none were collected during sampling efforts here. Subsequently, for yellow perch in Lake Manganese, competition with golden shiner for zooplankton prey, and a general absence of larger more energy dense prey such as small bodied forage fish likely prohibits progression through the ontogenetic shifts that permit increased growth and greater body sizes.

Overall the stunted Lake Manganese yellow perch population provides a demonstration of the effects of central ecological concepts such as predation,

competition, and resource limitation. It is important to note that only yellow perch and largemouth bass have successfully established within Lake Manganese, and the Michigan Department of Natural Resources is currently attempting another bluegill introduction into Lake Manganese (G. Madison MiDNR *pers. comm.*). Bluegill sunfish are a benthic omnivore and such introductions may only increase competition for benthic prey and the extent of stunting evident in this yellow perch population. Future studies to evaluate such a potential consequence would prove valuable to identify the extent to which growth stunting can be manifested within a population and ecosystem. Lake Manganese also allows for the study of extremely old age and slow growth rates and the effects these may have on food-web ecology and biology in Lake Manganese. The high competition, and lack of predators have the possibility to influence stunting by altering energy density and $\delta^{15}N$ values. Stunted yellow perch in Lake Manganese generally occupy a single trophic level as indicated by $\delta^{15}N$. A seasonal comparison of stable isotope values among the other fishes inhabiting this ecosystem could help demonstrate the extent of competition occurring in this ecosystem. This would prove beneficial for identifying populations at risk in the lake, or possibly identify seasonal periods of habitat and resource limitation that could contribute to stunting.

2.5.1 Conclusion

This study is among the few that have comprehensively examined a range of biological, ecological and limnological parameters for a single species and from an individual waterbody over an entire annual cycle. For some of the biological parameters studied here such as *HSI*, *GSI* and fish condition, seasonal changes in these metrics were

not entirely unexpected as associated with our general knowledge of poikilothermy, fish ecology and aquatic ecology. However, such seasonal variability has rarely been quantified empirically. Of particular interest from this study was the seasonal variability observed for yellow perch $\delta^{13}C$ values. The stable isotopes of carbon and nitrogen are considered to integrate long-term and spatial variability in the trophic ecology of aquatic and terrestrial consumer species [77]. The results of this study suggest that, for yellow perch, $\delta^{13}C$ values may reflect more recent habitat and food resource exploitation behaviors and that general assumptions derived from single season sampling may not fully capture long-term variability. Fishes collected for this study also demonstrated significant seasonal variability in proximate composition and energy density which is important for our understanding of pollutant bioaccumulation in fish species. For example, pollutants such as polychlorinated biphenyls (PCBs) and mercury (Hg) accumulate in animal lipid and protein tissues, respectively [92, 93]. These two pollutants also contribute to the majority of fish consumption advisories across the Great Lakes basin. Thus, understanding seasonal variability in the ability of aquatic species to bioaccumulate pollutants is likely important for furthering our knowledge of aquatic ecotoxicology, and also for managing human risks associated with the consumption of contaminated fish which is also not limited to a single season.

2.6 Chapter 2 Tables and Figures

Table 2.1 Seasonal water quality monitoring data including average temperature (°C), dissolved oxygen (DO; mg/L), specific conductivity (Sp. Cond.; µS/cm), total dissolved solids (TDS; mg/L), pH, and oxidation reduction potential (ORP; mV) for Lake Manganese collected with a YSI® Professional Plus Multi-Parameter Instrument and HOBO data loggers* between May 2018 and June 2019.

Variable	Spring 18	Summer 18	Fall 18	Winter 19	Spring 19
Temperature	11.1 (0.7)	16.3 (0.4)	6.2 (1.0)	N/A	N/A
Temperature*	N/A	16.0 (<0.1)	4.4 (<0.1)	2.7 (<0.1)	6.8 (<0.1)
DO	7.9 (0.3)	7.9 (0.2)	10.5 (0.4)	N/A	N/A
Sp. Cond.	115.7 (6.4)	129.2 (4.1)	69.7 (10.2)	N/A	N/A
TDS	75.2 (4.2)	84.0 (2.7)	45.4 (6.6)	N/A	N/A
pH	7.6 (0.1)	8.2 (0.1)	8.4 (0.1)	N/A	N/A
ORP	126.6 (16)	86.0 (10.2)	128.0 (25.3)	N/A	N/A

*HOBO data loggers collected temperature measurements every 30 minutes from 8/17/18 - 6/24/19 where YSI was only collected once per month 5/18/18 – 10/13/18

Table 2.2 Summary biological data including length (mm), mass (g), lipid content (%), *GSI* (%), *HSI* (%), Fulton's condition (*K*; g/mm^3), energy density (kJ/g), and stable nitrogen ($\delta^{15}N$; ‰), and carbon ($\delta^{13}C$; ‰) isotope data for yellow perch collected from Lake Manganese, Grant Township, Michigan. Collections occurred April 11, 2018 through March 2, 2019. Data for female fish represent individuals ranging from 2 – 14 years in age and from 2 – 18 years for males. Values represent arithmetic means with 1 standard error in parentheses.

Variable	Sex	Spring	Summer	Fall	Winter
Length	F	168 (1.8)	165.5 (2.7)	160.7 (3.2)	158.1 (3)
	M	139.5 (1.3)	145.2 (1.4)	140 (1.5)	140.1 (0.9)
	All	159.1 (1.3)	157.6 (1.6)	150.2 (2)	145.9 (1.5)
Mass	F	46.4 (2.4)	42.2 (3.5)	40.7 (4.3)	43.1 (3.9)
	M	25.8 (0.9)	28.3 (1)	24.6 (1)	25.2 (0.6)
	All	40.0 (1.7)	36.7 (2)	32.5 (2.5)	31 (1.9)
Lipid content	F	0.8 (0.1)	1.2 (0.2)	0.8 (0.2)	1.7 (0.3)
	M	0.9 (0.1)	1.8 (0.2)	1.4 (0.2)	2.0 (0.2)
	ALL	0.8 (0.1)	1.5 (0.1)	1.1 (0.1)	1.9 (0.2)
GSI	F	1.9 (0.3)	1.2 (0.5)	3.4 (0.6)	7.4 (0.6)
	M	2.6 (0.4)	2.3 (0.6)	3.0 (0.4)	3.0 (0.2)
	All	2.2 (0.3)	1.5 (0.5)	3.2 (0.4)	4.2 (0.3)
HSI	F	1.4 (0.1)	0.5 (0.1)	0.7 (0.1)	1 (0.1)
	M	0.6 (0.1)	0.5 (0.1)	0.3 (0.1)	0.8 (0.1)
	All	1.2 (0.1)	0.5 (0.1)	0.6 (0.1)	0.9 (0.1)
K	F	0.9 (<0.1)	0.9 (<0.1)	0.9 (<0.1)	0.9 (<0.1)
	M	0.9 (<0.1)	0.9 (<0.1)	0.9 (<0.1)	0.9 (<0.1)
	All	0.9 (<0.1)	0.9 (<0.1)	0.9 (<0.1)	0.9 (<0.1)
Energy density	F	3.9 (0.1)	4.6 (0.2)	4.5 (0.2)	4.4 (0.3)
	M	3.9 (0.1)	4.8 (0.2)	4.6 (0.2)	4.5 (0.2)
	All	3.9 (0.1)	4.7 (0.1)	4.5 (0.1)	4.5 (0.2)
$\delta^{15}N$	F	7.2 (0.1)	7.5 (0.2)	7.8 (0.2)	7.8 (0.3)
	M	7.0 (0.1)	7.4 (0.1)	7.3 (0.2)	7.1 (0.2)
	All	7.1 (0.1)	7.4 (0.1)	7.5 (0.1)	7.3 (0.1)
$\delta^{13}C$	F	-30.6 (0.2)	-31.9 (0.5)	-32.7 (0.7)	-32.2 (0.8)
	M	-32.3 (0.3)	-31.3 (0.5)	-33 (0.6)	-33.6 (0.5)
	All	-31.4 (0.2)	-31.6 (0.4)	-32.9 (0.4)	-33.2 (0.5)

Table 2.3 Summary biological data including length (mm), mass (g), lipid content (%), *GSI* (%), *HSI* (%), Fulton's condition (*K*; g/mm^3), energy density (kJ/g), and stable nitrogen ($\delta^{15}N$; ‰), and carbon ($\delta^{13}C$; ‰) isotope data for 8-year-old yellow perch collected from Lake Manganese, Grant Township, Michigan. Collections occurred April 11, 2018 through March 2, 2019. Values represent arithmetic means with 1 standard error in parentheses.

Variable	Sex	Spring	Summer	Fall	Winter
Length	F	180.3 (4.0)	166 (4.5)	174.7 (7.4)	170.0 (9.0)
	M	139.3 (2.3)	144.2 (2.2)	138.4 (2.6)	139.1 (1.6)
	All	166.6 (2.6)	157.1 (2.6)	153.9 (3.6)	143.8 (3.7)
Mass	F	59.5 (4.6)	40.9 (5.1)	52.9 (8.4)	51 (10.2)
	M	25.7 (1.8)	29.1 (1.7)	23.4 (2)	24.9 (1.2)
	All	48.2 (2.8)	36.1 (2.9)	36.0 (4.0)	28.9 (4.0)
Lipid	F	0.9 (0.2)	1.1 (0.2)	0.6 (0.2)	1.7 (0.3)
	M	0.6 (0.2)	1.8 (0.2)	1.3 (0.2)	2.1 (0.2)
	All	0.8 (0.1)	1.4 (0.1)	1 (0.1)	2 (0.2)
GSI	F	1.5 (0.4)	1.3 (0.5)	4.3 (0.8)	9.8 (0.9)
	M	2.5 (1.3)	2.7 (1.6)	3.8 (1.3)	3.6 (0.8)
	All	1.8 (0.6)	1.8 (0.8)	4 (0.8)	4.6 (0.8)
HSI	F	2.0 (0.2)	0.4 (0.2)	0.9 (0.4)	0.9 (0.3)
	M	0.7 (0.1)	0.4 (0.1)	0.5 (0.2)	0.8 (0.1)
	All	1.6 (0.1)	0.4 (0.1)	0.7 (0.2)	0.8 (0.2)
K	F	1.0 (<0.1)	0.9 (<0.1)	0.9 (<0.1)	1.0 (<0.1)
	M	0.9 (0.1)	0.8 (0.1)	1.2 (0.1)	1.0 (0.1)
	All	1.0 (<0.1)	0.9 (<0.1)	0.9 (<0.1)	0.9 (<0.1)
Energy density	F	4.0 (0.2)	4.6 (0.2)	4.5 (0.2)	4.3 (0.3)
	M	4.0 (0.2)	4.8 (0.2)	4.5 (0.2)	4.5 (0.1)
	All	4.0 (0.1)	4.7 (0.1)	4.5 (0.1)	4.4 (0.1)
$\delta^{15}N$	F	7.4 (0.1)	7.5 (0.2)	7.9 (0.2)	7.8 (0.2)
	M	7.3 (0.2)	7.3 (0.2)	7.4 (0.2)	6.9 (0.2)
	All	7.4 (0.1)	7.4 (0.1)	7.6 (0.1)	7.2 (0.1)
$\delta^{13}C$	F	-30.3 (0.5)	-31.7 (0.5)	-32.5 (0.7)	-32.5 (0.8)
	M	-33.4 (0.7)	-31.4 (0.5)	-33.2 (0.6)	-33.4 (0.5)
	All	-31.5 (0.4)	-31.6 (0.4)	-32.9 (0.5)	-33.1 (0.5)

Table 2.4 Averages of: length (mm), mass (g), lipid content (%), *GSI* (%), *HSI* (%), condition K, (g/mm^3), Energy density (kJ/g), δ^{15}N (‰), and δ^{13}C (‰) with standard error, yellow perch collected from Lake Manganese, Grant Township, Michigan. Collections occurred April 11, 2018 through March 2, 2019.

	Female	Male	Female	Male
Variable	(2 – 14 yrs.)	(2 – 18 yrs.)	(8-year-old fish only)	
Length	164.5 (1.1)	140.9 (1.2)	173.7 (2.5)	140.1 (2.0)
Mass	44.0 (1.4)	25.8 (1.5)	51.4 (2.7)	25.7 (2.2)
Lipid content	1.0 (0.1)	1.3 (0.1)	1.0 (0.1)	1.5 (0.1)
GSI	2.9 (0.2)	2.8 (0.3)	2.6 (0.5)	3.3 (0.5)
HSI	1.1 (0.1)	0.6 (0.1)	1.3 (0.1)	0.7 (0.1)
K	0.9 (0)	0.9 (0)	0.9 (<0.1)	0.9 (<0.1)
Energy density	4.1 (0.1)	4.3 (0.1)	4.4 (0.1)	4.4 (0.1)
δ^{15}N	7.4 (0.1)	7.2 (0.1)	7.6 (0.1)	7.2 (0.1)
δ^{13}C	-31.2 (0.3)	-32.5 (0.2)	-31.5 (0.3)	-32.8 (0.3)

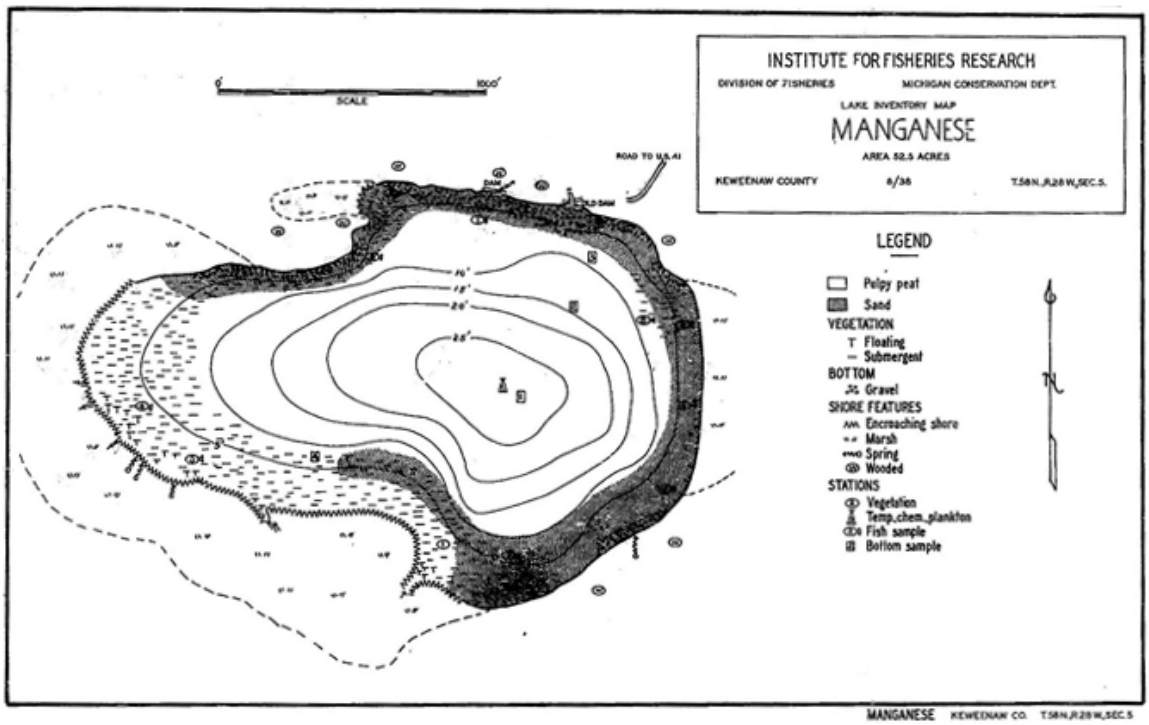

Figure 2.1: Lake Manganese bathymetry. Map information collected by the Michgian Conservation Department's Institute for Fisheries Research (August 1938).

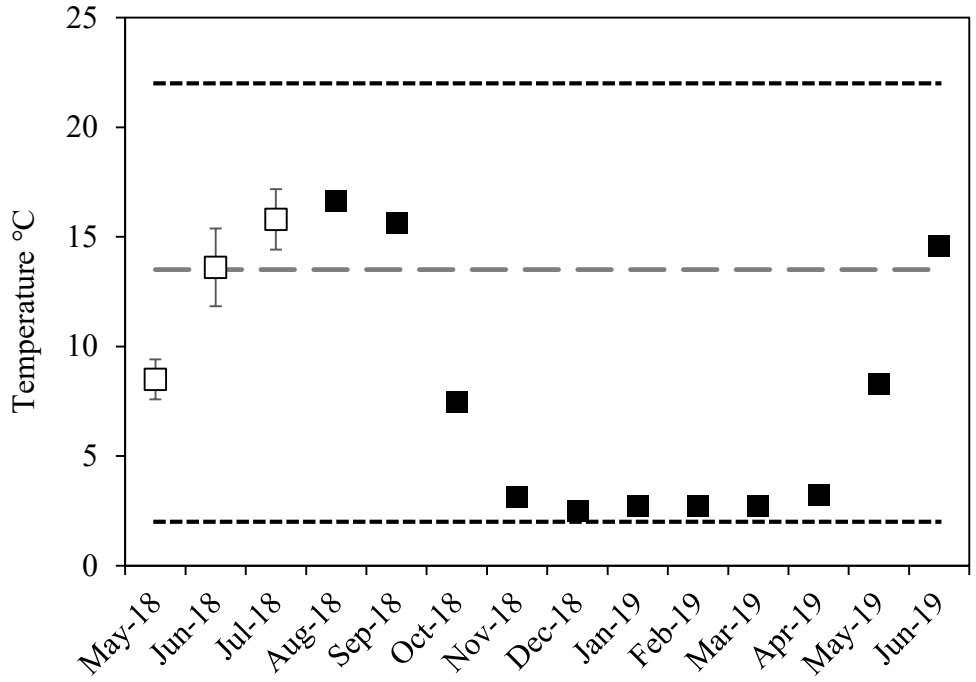

Figure 2.2: Average monthly water temperatures for Lake Manganese from May 2018-July 2019 with standard error in brackets, white squares represent YSI data with error bars representing ± standard error. Black squares represent temperature data collected by remote HOBO data loggers. Minimum temperature (13.5 ℃) required for yellow perch growth is represented by the shaded dashed line. Upper and lower dotted lines represent the temperatures for optimum (22 °C) and zero or maintenance growth (2 ℃) asymptotes for yellow perch growth.

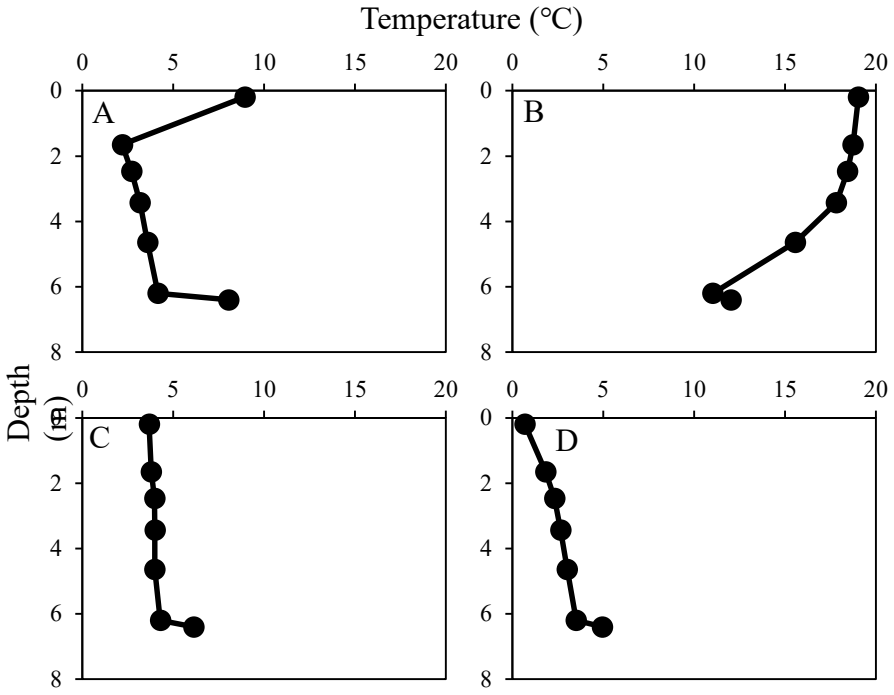

Figure 2.3: Average water temperature profiles for Lake Manganese collected via Hobo data loggers from 8/17/18 - 6/24/19. Profiles represent spring (A), summer (B), fall (C), and winter (D).

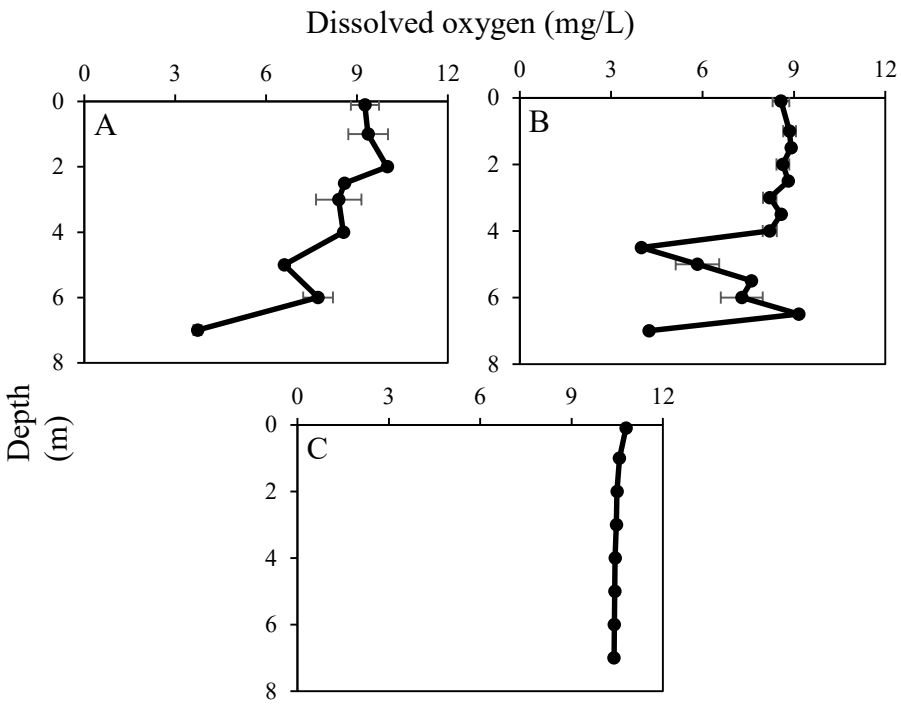

Figure 2.4: Average dissolved oxygen profiles for Lake Manganese collected via YSI from once per month 5/18/18 – 10/13/18. Profiles represent spring (A), summer (B), and fall (C) with bars representing standard error. No standard error bars are available for fall as there was only one measurement.

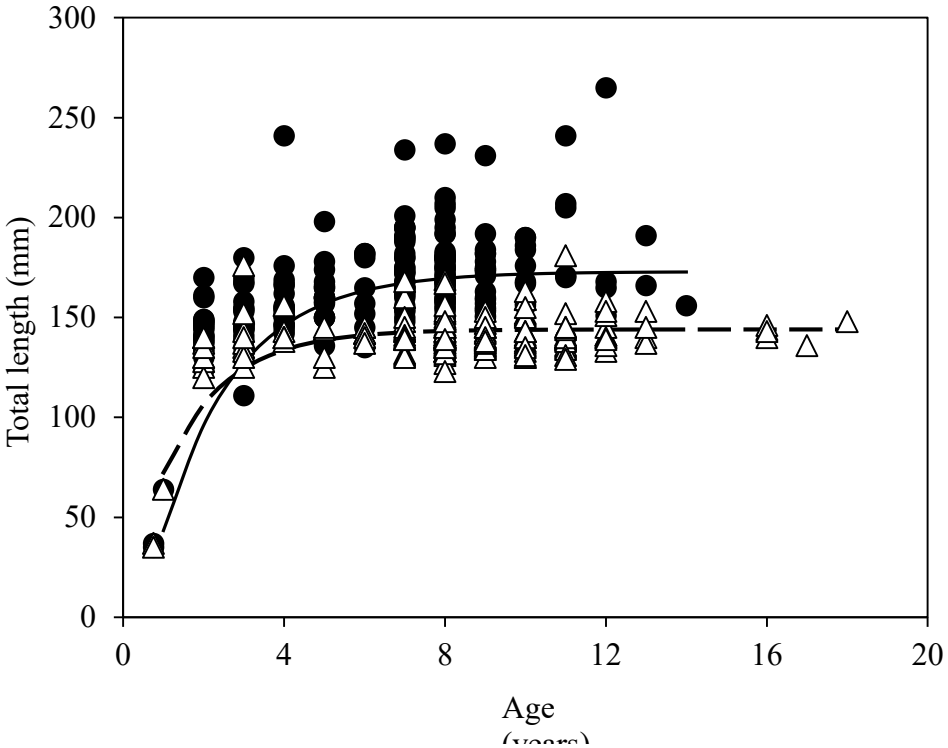

Figure 2.5: Von Bertalanffy (VBL) growth curve for male (△; n = 210) and female (●; n = 236) yellow perch collected from Lake Manganese in Keweenaw County, MI between April 11, 2018 and March 17, 2019. Solid and dashed lines represent von Bertalanffy growth models for female and male perch, respectively.

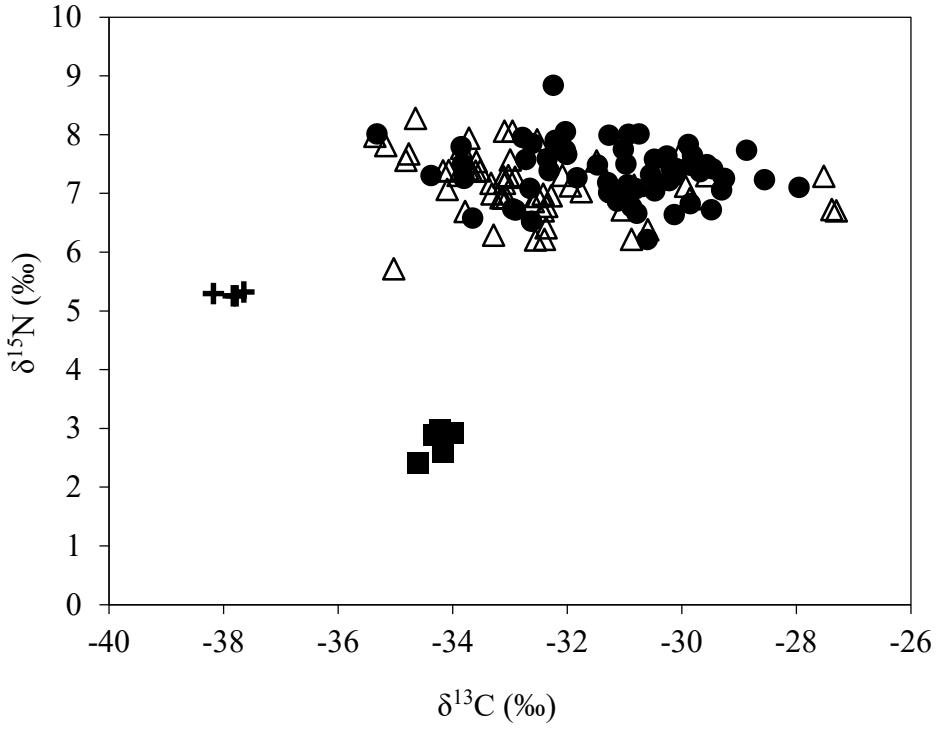

Figure 2.6: Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values for zooplankton (+), mussels (■), and male (△ , n = 37) and female (●, n = 32) yellow perch. Samples were collected from April 2018 – March 2019

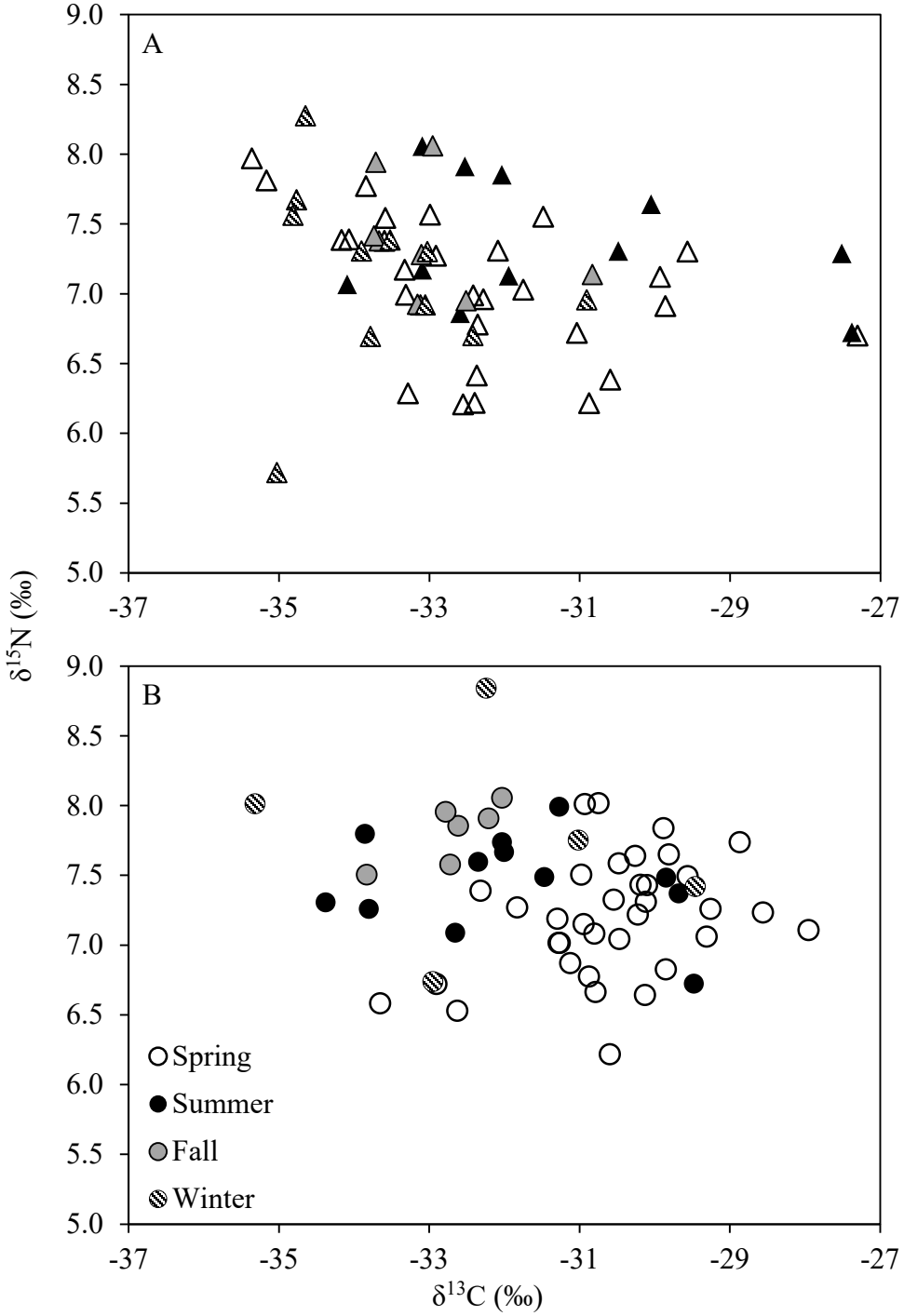

Figure 2.7: Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values for all male (△) and female (●) yellow perch collected from Lake Manganese, Grant Township, Michigan. Collections occurred April 11, 2018 through March 2, 2019 ranging in ages from 2 – 18.

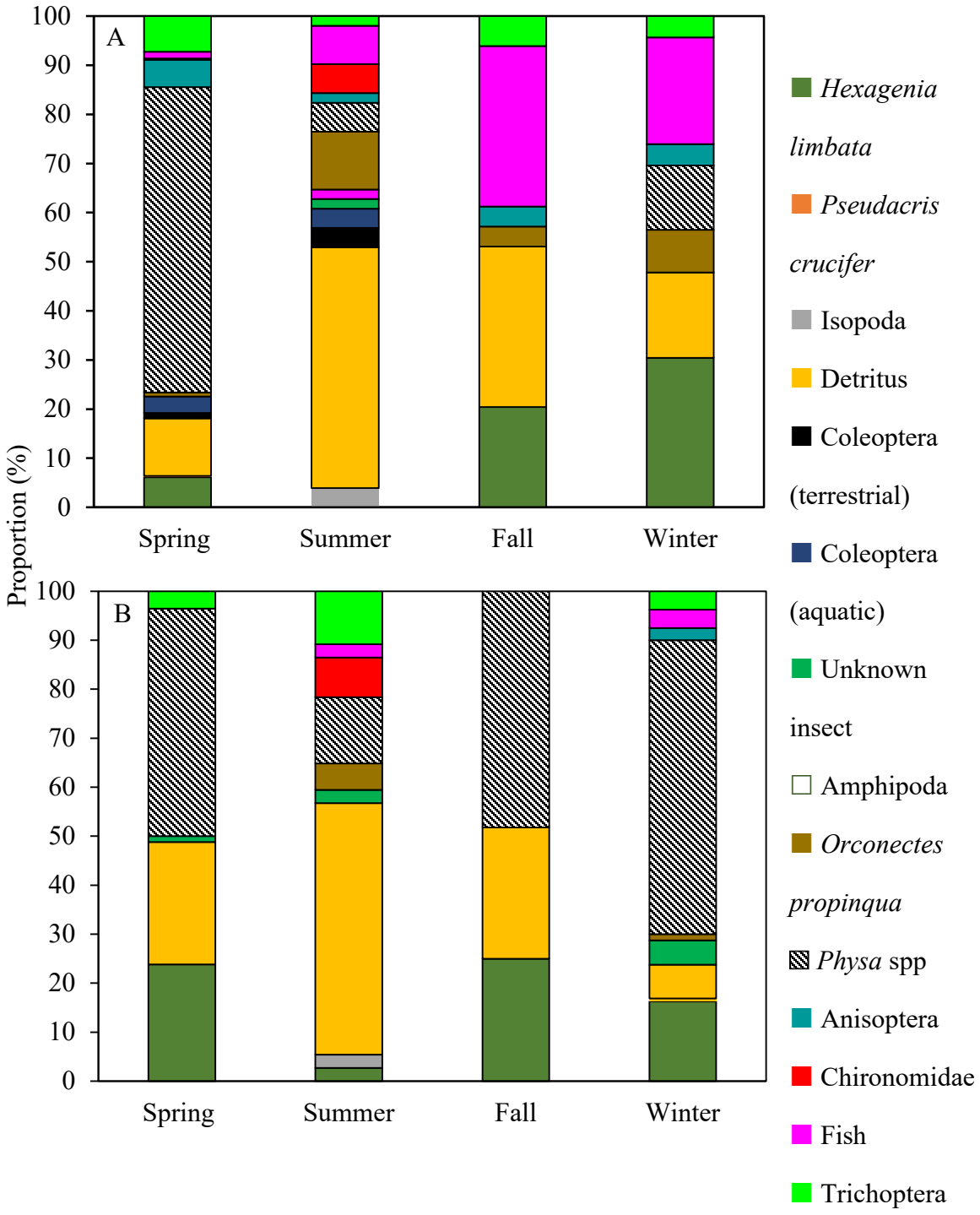

Figure 2.8: Diet composition data for female (A; n = 236), and male (B; n = 209) yellow perch collected from Lake Manganese, Grant Township, Michigan. Collections occurred April 11, 2018 through March 2, 2019.

2.7 Supplemental information

Table S2.1 Results of the one-way ANOVA model for the effects of season (Spring [April-June], Summer [July-September], Fall [October, December], Winter [January-March]), and the covariates: Age (2-18), Sex (Male, Female), length (mm), and mass (g) on the lipid content (%), log(GSI+1) (%), HSI (%), Fulton's K (g/mm^3), δ^{15}N (‰), and δ^{13}C (‰) on Lake Manganese yellow perch (*Perca flavescens*). All factors were treated as fixed effects with bold values indicating a significant effect at $\alpha = 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
(A) Lipid content (%)				
Season	**3.0**	**5.8**	**22.3**	**<0.001***
Age	**1.0**	**2.1**	**8.3**	**0.005***
Sex	1.0	0.8	3.0	0.1
Total (mm)	1.0	0.2	0.8	0.4
Total (g)	1.0	0.3	1.2	0.3
Model Error	111.0	0.3		
(B) log (GSI+1) (%)				
Season	**3.0**	**6.4**	**22.0**	**<.0001***
Age	**1.0**	**1.8**	**6.0**	**0.0145***
Sex	1.0	0.7	2.3	0.1
Total (mm)	**1.0**	**5.0**	**17.1**	**<.0001***
Total (g)	**1.0**	**6.9**	**23.4**	**<.0001***
Model Error	351.0	0.3		
(C) HSI (%)				
Season	**3.0**	**7.1**	**21.1**	**<.0001***
Age	**1.0**	**1.5**	**4.6**	**0.0331***
Sex	**1.0**	**6.6**	**19.8**	**<.0001***
Total (mm)	**1.0**	**2.1**	**6.2**	**0.0136***

Total (g)	**1.0**	**5.4**	**16.2**	**<.0001***
Model Error	281.0	0.3		
(D) Condition (g/mm^3)				
Season	**3.0**	**0.0**	**3.9**	**0.0091***
Age	**1.0**	**0.0**	**5.4**	**0.0208***
Sex	1.0	0.0	1.8	0.2
Total (mm)	**1.0**	**1.0**	**192.4**	**<.0001***
Total (g)	**1.0**	**2.1**	**411.7**	**<.0001***
Model Error	426.0	<0.1		
(E) Energy density (kJ/g)				
Season	**3.0**	**3.8**	**16.6**	**<.0001***
Age	1.0	0.0	0.0	1.0
Sex	1.0	0.7	3.1	0.1
Total (mm)	1.0	0.5	2.3	0.1
Total (g)	1.0	0.2	0.8	0.4
Model Error	110.0	0.2		
(F) $\delta^{15}N$ (‰)				
Season	**3.0**	**0.6**	**3.0**	**0.0317***
Age	**1.0**	**1.4**	**6.9**	**0.0099***
Sex	1.0	0.0	0.0	0.9
Total (mm)	**1.0**	**1.2**	**6.0**	**0.0162***
Total (g)	1.0	0.2	1.1	0.3
Model Error	109.0	0.2		

(G) $\delta^{13}C$ (‰)

Season	**3.0**	**15.1**	**7.0**	**0.0002***
Age	1.0	0.5	0.2	0.6
Sex	1.0	1.6	0.8	0.4
Total (mm)	**1.0**	**29.5**	**13.7**	**0.0003***
Total (g)	1.0	5.5	2.6	0.1
Model Error	109.0	2.2		

(A) Overall model for lipid content (%): N =119, $R^2 = 0.439$, $P < 0.0001$
(B) Overall model for GSI (%): N = 359, $R^2 = 0.262$, $P < 0.0001$
(C) Overall model for HSI (%): N = 289, $R^2 = 0.316$, $P < 0.0001$
(D) Overall model for condition (g/mm^3): N = 434, $R^2 = 0.533$, $P < 0.0001$
(E) Overall model for energy density (kJ/g): N = 118, $R^2 = 0.350$, $P < 0.0001$
(F) Overall model for $\delta^{15}N$ (‰): N = 177, $R^2 = 0.281$, $P < 0.0011$
(G) Overall model for $\delta^{13}C$ (‰): N = 177, $R^2 = 0.367$, $P < 0.0011$

Table S2.2 Results of the one-way ANOVA model for the effects of age (2-18), and sex (M/F) on total length (mm) and mass (g) on Lake Manganese yellow perch (*Perca flavescens*). All factors were treated as fixed effects with bold values indicating a significant effect at $\alpha = 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
(A) Length				
Season	**3**	**749.8**	**3.1**	**0.0263***
Age	**1**	**18106.4**	**75.1**	**<.0001***
Sex	**1**	**62903.4**	**260.9**	**<.0001***
Model Error	428	241.1		
(B) Mass				
Season	3	508.7	1.3	0.2660
Age	**1**	**17960.9**	**46.8**	**<.0001***
Sex	**1**	**42534.7**	**110.7**	**<.0001***
Model error	428	384.2		

(A) Overall model for total length (mm) : N =434, $R^2 = 0.440$, $P < 0.0001$
(B) Overall model for mass (g): N = 434, $R^2 = 0.249$, $P < 0.0001$

Table S2.3. Results of the one-way ANOVA model for the effects of Season (Spring [April-June], Summer [July-September], Fall [October, December], Winter [January-March]), and the covariates: , Sex (Male, Female), length(mm), mass, (g) on the Lipid Content (%), GSI (%), HSI (%), Fulton's K (g/mm^3), δ^{15}N (‰), and δ^{13}C (‰) on Lake Manganese 8-year-old yellow perch (*Perca flavescens*). All factors were treated as fixed effects with bold values indicating a significant effect at $\alpha = 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
(A) Lipid content%				
Season	**3.0**	**3.2**	**10.5**	**<.0001***
Sex	1.0	0.5	1.7	0.2
Total (mm)	1.0	0.5	1.5	0.2
Total (g)	1.0	0.6	2.0	0.2
Model Error	51.0	0.3		
(B) GSI (%)				
Season	**3.0**	**2.0**	**7.7**	**0.0001***
Sex	1.0	0.1	0.4	0.5
Total (mm)	1.0	0.3	1.0	0.3
Total (g)	1.0	0.2	0.8	0.4
Model Error	76.0	0.3		
(C) HSI (%)				
Season	**3.0**	**4.2**	**12.0**	**<.0001***
Sex	**1.0**	**3.3**	**9.5**	**0.0030***
Total (mm)	**1.0**	**1.5**	**4.4**	**0.0405***
Total (g)	**1.0**	**1.4**	**4.2**	**0.0458***
Model Error	60.0	0.3		

(D) Condition (g/mm^3)				
Season	3.0	0.0	1.6	0.2
Sex	**1.0**	**0.0**	**7.2**	**0.0089***
Total (mm)	**1.0**	**0.3**	**69.5**	**<.0001***
Total (g)	**1.0**	**0.4**	**101.9**	**<.0001***
Model Error	90.0	<0.1		
(E) Energy density (kJ/g)				
Season	**3.0**	**1.6**	**6.9**	**0.0005***
Sex	1.0	0.4	1.6	0.2
Total (mm)	1.0	0.1	0.5	0.5
Total (g)	1.0	0.0	0.1	0.8
Model Error	52.0	0.2		
(F) $\delta^{15}N$ (‰)				
Season	3.0	0.3	1.3	0.3
Sex	1.0	0.0	0.0	1.0
Total (mm)	1.0	0.0	0.2	0.7
Total (g)	1.0	0.1	0.3	0.6
Model Error	52.0	0.2		
(G) $\delta^{13}C$ (‰)				
Season	3.0	6.1	2.5	0.1
Sex	1.0	2.3	0.9	0.3

Total (mm)	1.0	9.4	3.9	0.1
Total (g)	1.0	2.1	0.9	0.4
Model Error	52.0	2.4		

(A) Overall model for lipid content (%): N =58, $R^2 = 0.482$, $P < 0.0001$
(B) Overall model for GSI (%): N = 83, $R^2 = 0.288$, $P < 0.0002$
(C) Overall model for HSI (%): N = 67, $R^2 = 0.520$, $P < 0.0001$
(D) Overall model for condition (g/mm^3): N = 97, $R^2 = 0.581$, $P < 0.0001$
(E) Overall model for energy density (kJ/g): N = 59, $R^2 = 0.293$, $P < 0.0047$
(F) Overall model for $\delta^{15}N$ (‰): N = 59, $R^2 = 0.277$, $P < 0.0075$
(G) Overall model for $\delta^{13}C$ (‰): N = 59, $R^2 = 0.363$, $P < 0.0005$

Table S2.4 Results of the one-way ANOVA model for the effects of age (2-18), and sex (M/F) on total length (mm) and mass (g) on Lake Manganese yellow perch (*Perca flavescens*). All factors were treated as fixed effects with bold values indicating a significant effect at $\alpha = 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
(A) Length (mm)				
Season	3	275.6	1.5	0.2333
Sex	**1**	**20755.6**	**109.2**	**<.0001***
Model Error	92	190.0		
(B) Mass				
Season	3	510.9	2.2515	0.0876
Sex	**1**	**12085.3**	**53.2570**	**<.0001***
Model error	92	226.9		

(A) Overall model for total length (mm) : N =97, $R^2 = 0.616$, $P < 0.0001$
(B) Overall model for mass (g): N =97, $R^2 = 0.456$, $P < 0.0001$

Table S2.5 Results of the one-way ANOVA model for seasonality among limnological parameters for Lake Manganese: (spring [April-June], Summer [July-September], Fall [October, December], Winter [January-March]), depth (0.1-7m), and their interactions.

Source	DF	Mean Square	F Ratio	Prob > F
(A) DO (mg/L)				
Season	**2.0**	**27.2**	**17.8**	**<.0001***
Depth (m)	**1.0**	**35.3**	**23.1**	**<.0001***
Season*Depth (m)	**2.0**	**7.5**	**4.9**	**0.0099***
Model Error	70.0	1.5		
(B) Sp Cond (µS/cm)				
Season	**2.0**	**14998.2**	**20.0**	**<.0001***
Depth (m)	**1.0**	**19052.8**	**25.3**	**<.0001***
Season*Depth (m)	2.0	415.8	0.6	0.6
Model Error	77.0	751.7		
(C) TDS (mg/L)				
Season	**2.0**	**5328.9**	**13.9**	**<.0001***
Depth (m)	**1.0**	**10200.0**	**26.6**	**<.0001***
Season*Depth (m)	2.0	704.9	1.8	0.2
Model Error	79.0			
(D) Temperature °C				
Season	**2.0**	**496.4**	**52.0**	**<.0001***
Depth (m)	**1.0**	**337.4**	**35.4**	**<.0001***
Season*Depth (m)	**2.0**	**51.8**	**5.4**	**0.0062***
Model Error	79.0	9.5		

(E) pH				
Season	**2.0**	**3.0**	**16.5**	**<.0001***
Depth (m)	**1.0**	**1.6**	**8.6**	**0.0045***
Season*Depth (m)	2.0	0.1	0.6	0.6
Model Error	79.0	0.2		
(F) ORP (mV)				
Season	2.0	17149.8	3.0	0.1
Depth (m)	1.0	14800.7	2.6	0.1
Season*Depth (m)	2.0	7517.7	1.3	0.3
Model Error	79.0	9.0		

(A) Overall model for DO (mg/L) : N = 97, $R^2 = 0.553$, $P < 0.0001$
(B) Overall model for Sp Cond (µS/cm): N = 83, $R^2 = 0.536$, $P < 0.0001$
(C) Overall model for TDS (mg/L): N = 85, $R^2 = 0.527$, $P < 0.0001$
(D) Overall model for temperature (°C): N = 85, $R^2 = 0.527$, $P < 0.0001$
(E) Overall model for pH: N = 85, $R^2 = 0.362$, $P < 0.0001$
(F) Overall model for ORP (mV): N = 85, $R^2 = 0.527$, $P < 0.0001$

3 Comparison of mercury bioaccumulation by an age class of yellow perch (*Perca flavescens*) over an annual growing season.

3.1 Abstract

Mercury bioaccumulation in fish is associated with a range of biological, ecological and limnological characteristics, many of which exhibit significant seasonal variation. However, few studies have explicitly examined Hg bioaccumulation at such a level of temporal resolution over an annual period. In this study, Hg concentrations were measured monthly over a one-year period in a single age class of the Lake Manganese yellow perch (*Perca flavescens*) population to assess potential seasonal trends in Hg bioaccumulation and relationships with fish proximate composition and carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values. Mercury concentrations demonstrated significant ($P < 0.001$) seasonal variation with monthly averages ranging from 78.5 – 119.3 μg/kg (wet wt.) for spring and fall collections, respectively. Fish protein content and protein mass were significant predictors of Hg bioaccumulation ($P \leq 0.010$) with fish $\delta^{13}C$ values also being significantly ($P = 0.040$) correlated with wet weight Hg concentrations in this population. Mercury concentrations among 8-year old fishes also spanned almost an order of magnitude over one year under both wet (28.4 – 228.5 μg/kg) and dry (114.6 – 948.1 μg/kg) weight concentration measures. Combined, these results demonstrate the high levels of seasonal and individual variability of Hg bioaccumulation in a common and popular recreational and commercial fish species.

3.2 Introduction

Mercury, specifically methylmercury (MeHg) concentrations in fish tissues are of particular concern, as the consumption of contaminated fish is a main source of Hg exposure to humans [94]. MeHg is an acute neurotoxin that binds with high affinity to proteins and is capable of crossing the blood brain barrier [94]. MeHg accounts for > 90 % of total Hg found in freshwater fishes. Factors including fish growth rates [95], water temperature, dissolved oxygen, and acidity [96], fish reproductive cycles [97], trophic position [98], habitat use and resources partitioning [99] have all been shown to contribute to Hg bioaccumulation. However, many of these variables are likely to exhibit spatial and temporal variability within and among ecosystems and populations owing to generally tight coupling with seasonal cycles.

Recreational and commercial fishing activities typically occur throughout the summer months within the Great Lakes basin. Within many inland lakes and northern regions of the Great Lakes, winter fishing (ice fishing) continues far past the close of the commercial season. This suggests for individuals consuming fish captured during this cold water season are consuming fish during conditions when Hg surveys do not typically take place due to difficulty in sampling methods and less in known regarding seasonal variability in Hg bioaccumulation by fishes [1]. Evidence of short-term temporal variability in fish Hg concentrations have been observed during open water seasons or within laboratory settings [96, 100-102]. However, we currently do not know how variable Hg concentrations are among individual fishes on monthly of even on an annual seasonal basis. By quantifying such potential variability, we may better understand

bioaccumulation kinetics in natural populations. This knowledge gap poses a potential human health risk to the people consuming fish caught from ice-covered aquatic ecosystems.

Lab-based and field studies have demonstrated the capacity of fish for the whole body elimination Hg [103, 104] . However, many studies fail to include winter and/or entire growing season datasets when examining temporal changes in Hg concentrations [101, 105, 106] . Being ectotherms, the open water sampling seasons (i.e. spring, summer) are associated with optimal temperatures for yellow perch growth [9, 52, 62] and bioenergetic rates [4]. Faster growth rates also result in a greater potential for growth dilution [102, 107] due to the increased potential for Hg storage in growing muscle tissues. This combination of increased growth rate, growth dilution, and faster bioenergetic rates process all contribute to the capacity for Hg elimination potential being highest during the open water seasons [104, 108] . In contrast, the colder temperatures of fall and winter slow bioenergetics processes and growth rates in fishes [4] resulting in reduced whole-body elimination and limited potential for growth dilution [102, 107] . As a result, Hg elimination rates are thought to decrease in the fall and winter [108]. Field experiments have demonstrated that wild populations of yellow perch to have much slower Hg elimination than predicted by laboratory experiments [104]. This discrepancy between field and laboratory studies could be the result of seasonal changes in ecology and limnology that affect fish physiology but are difficult to replicate in a laboratory setting.

Seasonal changes in reproduction, habitat use, body composition, and prey selection may also affect Hg concentrations of fish. Physiological changes as a result of reproduction represent a pathway for seasonal changes in Hg concentrations as both male and female fish have displayed Hg offloading through gametes [97, 105, 109]. Open water sampling for yellow perch typically occurs after the spawning season [9] and after any potential offloading may have already occurred. In contrast, yellow perch gamete production peaks in winter [10], therefore having the ability to affect seasonal Hg concentrations through Hg offloading as associated with gonad maturation during the winter. Seasonal shifts in fish ecology could also change mercury concentration rates. For example, yellow perch display seasonal shifts in diet as a species [18] and within Lake Manganese (chapter 2). As different prey items have been shown to have differ Hg concentrations [110], this seasonal dietary change may also change mercury concentrations. Furthermore, habitat selection has been shown to affect Hg concentrations [99], and seasonal changes in habitat selection [29] could influence seasonal mercury concentration. For these reasons, a comprehensive full growing season study investigating Hg concentrations in a fish population over one year could address the knowledge gaps regarding the seasonality of Hg bioaccumulation.

Lake Manganese located in Michigan's Keweenaw Peninsula is one of the northernmost yellow perch populations within the contiguous US. This study was designed to investigate temporal changes in Hg bioaccumulation within a single age-class of the yellow perch population in this ecosystem. Specific objectives included; 1) quantifying and contrasting monthly and seasonal Hg bioaccumulation in a single age

class of yellow perch over a one year growing season and 2) evaluating fish proximate composition (moisture, lipid, and protein) and the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to determine how these factors relate with seasonal Hg bioaccumulation patterns in yellow perch. I hypothesize that Hg concentrations will display significant seasonal differences and that protein and nitrogen ($\delta^{15}N$) will be significant predictors of Hg concentration. These results will be valuable for understanding overwintering toxicology of a commercially and recreationally valuable fish species inhabiting north temperate ecosystems.

3.3 Methods

Yellow perch for this study were collected monthly from Lake Manganese located in Grant Township Michigan. Sampling was completed using hook and line from April 2018 – March 2019. A maximum of 50 fish were collected each month with no fish collections being conducted in November 2018 due to adverse weather and poor ice-conditions on the lake. Fish collections for this research were authorized by the Michigan Department of Natural Resources (MiDNR) Scientific Collection Permits obtained in 2018 and renewed in 2019. All samples were stored in ice filled coolers for transport back to the laboratory where they were subsequently stored frozen (-20°C) until ready for dissection or analysis. Handling and care of the fish was completed under the approval of the Michigan Technological University Institutional Animal Care and Use Committee, project number 1414057-1.

3.3.1 Sample Processing

Biological data collected from each fish included sex, total, fork and standard lengths (mm), whole-body masses (g), lipid and protein content (%), protein mass (g/fish), moisture (%), $\delta^{15}N$, and $\delta^{13}C$ with sagittal otoliths removed for aging purposes. Following dissection, fish were wrapped in solvent rinsed aluminum foil and stored at -20°C until ready for homogenization. Whole-body homogenates were prepared for moisture and lipid content determination and stable isotope analyses using a solvent rinsed stainless steel Waring 700S blender with up to a ~35 g subsample stored in a clean steel tin until ready for analysis. All dissection and homogenization equipment were thoroughly washed and rinsed between each fish.

3.3.2 Fish Aging

Yellow perch ages were estimated from sagittal otoliths removed from each fish during dissection with aging method following the crack and burn procedure outlined in [111]. Otoliths were placed in Scotch™ brand lightweight mounting putty with their concave side facing up before being split in half with a scalpel through the core of the otolith and along the dorso-ventral axis. The cross-sectioned face of the otolith was then held over an open flame for a few seconds until the cut surface turned a light brown in color. The otolith was then inserted into a new piece of mounting putty with the cut surface facing up and then lightly painted with canola oil. Both halves of aged otoliths were imaged using an Olympus SZX9 dissecting microscope equipped with Imagingsource™ camera (Figure 3.1). Images were analyzed in FIJI/ImageJ image processing software. For quality control purposes, a subsample of 16 randomly selected

otoliths were sent for aging by three individuals experienced in aging fish otoliths. Otolith identifications were blind to each reviewer with only fish species information known. In the occurrence of discrepancies between readers, life-history tables and literature based yellow perch length at age ranges were consulted to provide a consensus age estimate for the otolith in question [9, 58, 66]. A frequency distribution histogram indicated 8-year-old yellow perch to be the predominant age class within the Lake Manganese population (Figure 3.2). Subsequently, these fishes were used as a proxy for tracking temporal Hg bioaccumulation among individual fishes in the absence of non-lethal sampling methods for repeated sampling of individual fish Hg concentrations.

3.3.3 Fish Proximate Composition

Sample moisture contents were determined using approximately 1 g (± 0.0001g) of whole-body homogenate. Aluminum weigh boats were pre-dried in an oven for 48 hours at 120℃ and allowed to cool to room temperature inside desiccators prior to sample addition. Weigh boats were pre-weighed empty followed by addition of the sample material and subsequent drying for 48 hours at 60℃. Dried specimens were removed from the oven and allowed to cool to room temperature inside desiccators with dried samples then reweighed (± 0.0001g) and moisture contents determined as per equation (4);

$$\%\, Moisture = \left[1 - \frac{dry\ weight - boat\ weight}{wet\ weight}\right] \times\ 100 \qquad (4)$$

Where dry weight represents the mass of the dried sample and weight boat after 48 hours at 60°C (g); boat weight is the mass of the empty, pre-dried weigh boat (g); and wet weight is the wet mass (g) of sample added to the boat prior to oven drying. Total lipid content for each sample was determined as a component of a paired pollutant extraction procedure outlined in [67]. Approximately 1.0 g of tissue homogenate was mixed with 10 g of pre-combusted sodium sulfate, (Granular 10-60 Mesh) added to a 20 mL glass syringe containing a glass wool plug, 10 mL of a 50:50 (vol:vol) mixture of a hexane and dichloromethane (DCM) extraction solvent attached to a VWR® syringe filter (25 mL 0.45 µm PTFE membrane). The mortar and pestle were then rinsed with an additional 5 mL of the extraction solvent with the 5 mL rinse transferred into the syringe. An additional 5.0g of sodium sulfate was added to the mortar and pestle, mixed in the mortar and quantitatively transferred into the syringe, with a final 5.0 mL rinse of the mortar and pestle with the extraction solvent and transfer into the extraction syringe. Samples were then allowed to stand for one hour with eluents collected in 45 mL glass test-tube with an addition 15 mL of the 50:50 solvent added as a final rinse. Once samples had drained into test tubes, they were transferred into a 125 mL round-bottom flask with each test tubes rinsed with a small volume of hexane to remove any residual lipids in the test tube. The round-bottom flasks were evaporated to a volume < 10.0 mL using Heidolph® Roto Evaporators, and the extract was then transferred to 10 mL volumetric flasks and brought up to volume with hexane. A 1.0 mL subsample was then transferred from the 10 mL flask into a dried, pre-weighed and labelled aluminum weigh boat before being placed in the oven at 110℃ to dry for 1 hour. Following drying, weigh boats containing the lipid volume were removed and allowed to cool to room temperature

in a desiccator before being weighed. Sample lipid contents (%) were calculated using the following equation:

$$\% \, Lipid = \left[\frac{Dried\ weight - Boat\ weight}{Wet\ weight}\right] \times 1000 \qquad (5)$$

where dried weight (g) is the mass of the dried weigh boat containing the lipid sample (± 0.0001g); boat weight (g) is the dried boat mass prior to sample additions; and wet weight represents the wet mass (g) of sample used for the extraction procedure.

Protein contents (%) were estimated using the mass balance approach as outlined in [68]. Briefly, whole-body lipid and moisture masses (g) were determined from the product of sample lipid and moisture contents (% wet weight) and fish total masses. Ash content for fish tissues typically range between approximately 1 - 2 % [69] in [70] and the random number generator function of Excel software was used to estimate an ash content within this range for each individual fish. Fish tissues typically contain very little to no carbohydrates [71], thus protein mass was considered to represent the proportion of whole body tissue remaining following estimation of lipid, moisture and ash contents.

3.3.4 Hg Analysis

Following drying at 60 ℃ for at least 48 hours, samples were ground using a glass mortar and pestle before being quantitatively transferred into scintillation vials that had been washed in 10 % HNO_3 acid bath and then triple rinsed in double distilled water. Samples were then weighed into a pre-cleaned nickel weigh boat on a balance to the nearest ± 0.0001 g before analysis. Samples were analyzed against a certified reference standard material (National Research Council of Canada, DORM-4, 0.410 mg Hg/kg)

with random samples from the field collected samples being run in triplicate. Recovery for the reference standard averaged 96.15 ± 2.53% of the certified concentration. All Hg analyses were completed using a Milestone direct mercury analysis (DMA) DMA-80 instrument following the general methodology outlined by the United States Environmental Protection Agency (USEPA) for the analysis of Hg in biological samples using DMA methodology [112].

3.3.5 Data Analysis

All statistical analyses were completed using the software package JMP Pro version 14.0 for Windows (SAS Institute, Cary, North Carolina, USA) with a criterion for significance of $\alpha = 0.05$ used in all cases. Unless otherwise indicated, all data are reported as means ± 1 standard error. All data were tested for normality using normal probability plots. Dry and wet weight Hg concentrations and lipid, moisture, and protein contents were all determined to be normally distributed. To increase statistical power, monthly sampling data were combined to represent spring (April – June), summer (July – August), fall (September – December) and winter (January – March) seasonal collections. Statistical comparisons of the biological and ecological variables across seasons and between male (n = 51) and female (n = 46) fishes were completed using one way analyses of variance (ANOVA) and corrected for total length or body mass covariates where necessary. Least squares linear regressions were used to evaluate the relationships between dry or wet weight Hg concentrations with fish protein content (%), protein mass, and $\delta^{13}C$, and $\delta^{15}N$ values.

3.4 Results

A summary of biological and Hg concentration data collected from fish analyzed for this study are presented in Table 3.1. Female fishes were larger than males for both total length ($F = 109.2$; $P < 0.001$) and body mass ($F = 53.3$, $P < 0.001$). The heaviest individual was a female (145.5 g) collected in the spring (6/14/19) with the lightest fish (18.1 g) being a male caught in the winter (2/23/19). Individual fish lipid contents ranged from 0.2 - 3.1 % and demonstrated significant seasonal variability (Figure 3.3; $F = 10.5$, $P < 0.001$; Table S3.1). For example, fish lipid contents peaked in winter (2.0 ± 0.1%), which was significantly higher than the lowest lipid average content for spring collected fishes (0.8 ± 0.1%). However, lipid contents did not differ significantly between male and female fish. Fish protein contents (%) also demonstrated significant seasonal differences with highest values determined for summer collected fishes (23.5 ± 0.6%) relative to fall (20.4 ± 0.6%) and winter (20.4 ± 0.4 %) collections ($F = 7.0$, $P < 0.001$; Table S3.1). Fish moisture contents were also seasonally different with spring collected fishes having the highest average moisture contents (77.9 ± 0.7%) and summer collected fishes representing the lower average (73.9 ± 0.8%; $F = 7.6$, $P < 0.001$; Table S3.1).

Monthly and seasonal comparisons of Hg bioaccumulation in Lake Manganese yellow perch are presented in Figures 4 and 5. Dry weight Hg concentrations for individual fishes ranged from 114.6 – 948.1 µg/kg in comparison to 28.4 - 228.5 µg/kg for wet weight concentrations. The highest individual wet weight concentration (228.5 µg/kg) was for a male caught during the winter (1/21/19). This individual also represented the highest dry weight Hg concentration. Significant seasonality was evident

for wet weight Hg concentrations (F = 3.3, P = 0.025) but not for dry weight concentrations (F = 2.4, P = 0.077). Fall collected fishes were determined to have the highest average dry (484.6 ± 58.2 μg/kg) and wet (119.3 ± 12.1 μg/kg) weight Hg concentrations. Wet weight Hg concentrations for spring collected fishes were approximately 65.8 % lower relative to values quantified in fall collected fishes. Of the 10 highest Hg concentrations among individual fishes (both wet and dry weight), the 9 highest concentrations were males. On average, males had higher Hg concentrations under both wet (109.3 ± 7.9 μg/kg) and dry weight (452.8 ± 35.1 μg/kg) relative to females (82.8 ± 5.2 μg/kg wet wt.; 352.0 ± 23.2 μg/kg dry wt.; Table 3.2). There were no significant differences in Hg bioaccumulation between males and females for both wet (F = 2.1, P = 0.157) and dry (F = 1.1, P = 0.293) weight Hg concentration metrics.

Fish $\delta^{15}N$ values ranged from 5.7 – 8.8 ‰ and were not significantly correlated with either dry (P = 0.882) or wet weight (P = 0.803) Hg concentration data (Figure 3.6; Table 3.2). In contrast, fish $\delta^{13}C$ values were significantly correlated with wet weight Hg concentrations (P = 0.040) but not for dry weight Hg concentrations (P = 0.064). Mercury concentration data (wet and dry wt.) were negatively correlated with fish protein content (%) and protein mass (g) metrics. Protein mass was significantly correlated with both wet (P = 0.023) and dry (P = 0.006) Hg concentration data. For protein content, no significant relationship was observed with wet weight Hg concentrations (P = 0.365). However, protein content was significantly correlated with dry weight Hg concentrations (P = 0.010). Coefficients of determination for all linear regressions were generally low ($R^2 \leq 0.13$) and explained minimal variance in each relationship.

3.5 Discussion

Mercury concentrations measured in a single age class of yellow perch over a one-year period demonstrated significant seasonal variability with highest concentrations occurring during the fall and winter seasons. These seasonal differences emphasize the importance of collecting such data in order to generate a better understanding of the factors that can influence the bioaccumulation of environmental pollutants such as Hg. For example, fish biology and ecology are highly regulated by limnological conditions, such as water temperature, which can also dictate the temporal structure and functioning of aquatic food-webs. Thus, quantifying Hg concentrations in fish species over multiple time points may help capture the various processes that contribute to an individual's exposure to Hg and their capacity to assimilate and bioaccumulate such pollutants.

Mercury concentrations in fishes are typically positively correlated with animal length, mass and age [95, 108]. However, in this study, no significant relationships were evident for yellow perch Hg concentrations and these predictor variables. By investigating Hg bioaccumulation in a single age class of fish, the design of this study eliminated the role of age as a cofactor in Hg bioaccumulation and thus likely also minimized the possible effects of age and growth related increases in fish length and mass. Mercury is a unique environmental pollutant in that it is hydrophobic but does not partition into lipid tissues rather tending to bioaccumulate in tissues having higher protein content such as dorsal muscle [93]. Mercury bioaccumulation by Lake Manganese yellow perch was negatively correlated with fish protein mass with smaller individuals generally exhibiting a higher Hg concentrations relative to larger individuals within the

same age class. The smaller size at age individuals represent more slowly growing fishes that have reduced capacity for the growth dilution of environmental pollutants such as Hg [113]. Specifically, growth dilution represents the bioaccumulation mechanism in which animal's growth rate exceeds that for the rate of pollutant assimilation into somatic tissue and the pollutant mass becomes diluted by a rapid increase in tissue mass [113]. Such contrasts in fast or slow growth have been demonstrated to be important for contrasting patterns of Hg bioaccumulation among walleye and bluegill sunfish at the population scale but not at the individual level [95, 102]. The results of the current study demonstrate this effect at the individual level and also suggest that periods of the year during which slow growth predominates (i.e. winter) are more likely to increase Hg bioaccumulation relative to the warm open water season when temperatures are more amenable to faster growth in poikilothermic species [114]. For example, fish collected during the summer tended to have proportionally higher protein content and lower wet weight Hg concentrations, and faster growing individuals with higher protein content also tended to have lower Hg concentrations independent of season. These results demonstrate how both seasonal changes and individual differences in proximate composition and growth rates can significantly affect Hg bioaccumulation.

As poikilotherms, yellow perch growth and bioenergetics are highly coupled with ambient temperature [4]. In addition to very slow growth predicted for the overwintering period, the rates of bioenergetic processes such as respiration, excretion, egestion are also predicted to be highly reduced in the cold water season. These biological functions also represent the general pathways for the whole-body elimination of pollutants such as Hg

by fish species [114]. Van Walleghem et al. [103] demonstrated very slow rates of Hg elimination in wild yellow perch under natural temperature conditions relative to elimination rates determined from controlled laboratory studies during which fish are often held at a constant temperature. Such slow Hg elimination patterns in the wild complements the results observed in this study for the Lake Manganese yellow perch population. Specifically, Hg concentrations were highest during the fall and winter when slow growth predominates and reduced bioenergetics functioning is also predicted for the metabolic pathways such as excretion, egestion and respiration (ie. elimination across the gill surface) that could contribute to the loss of Hg by fishes [4, 114] .

In addition to the contributions of excretion, egestion and respiration to Hg elimination, fish reproductive biology can also play a significant role in seasonal Hg changes as associated with the offloading of Hg into gonads and gametes. For example, Johnson et al. [115] demonstrated that MeHg concentrations in walleye eggs can range from 1 – 12% of the concentrations measured in muscle tissues. Also, differences in the extent of Hg bioaccumulation between male and female fishes have been attributed to contrasting levels of Hg that are transferred to gametes and released during spawning [116, 117] . For yellow perch, gonad maturation peaks in the late winter and, for females, gonadosomatic indices can exceed 25% but rapidly decline to < 5% following spawning in early spring [26]. This agrees well with the results of the current study where yellow perch Hg concentrations declined following the spring spawning period. This decline in Hg concentration from winter to spring also occurred in the absence of any significant changes in protein growth that could contribute to the dilution of the Hg

body burden pre- to post-spawning activities. These patterns suggest that reproductive tissue growth and the release of gametes could represent an important Hg offloading mechanism for yellow perch [105]. However, because Hg was measured in whole body homogenates for this study, rather than in specific tissues, additional studies comparing the temporal transfer of Hg into gonad tissue and gametes during reproductive development and spawning are needed to confirm that this seasonal change is the result of reproductive offloading.

A unique observation of this study was the relationship between yellow perch Hg concentrations and $\delta^{13}C$ and also the relatively wide range of $\delta^{13}C$ values (-27.4 to -35.4 ‰) determined for individual fishes over the course of this study. For example, Happel et al. [81] demonstrated $\delta^{13}C$ values ranging from approximately -18 to -24 ‰ for yellow perch collected from across multiple locations and habitats within Lake Michigan. Such a wide range of $\delta^{13}C$ values for yellow perch from within Lake Manganese is surprising given the small size (21.2 ha) of this ecosystem relative to a location such as Lake Michigan (5.8×10^6 ha) for which residential, industrial and agricultural inputs can contribute to both stable isotope signatures and Hg loadings [50, 76, 77]. Much of the inflow into Lake Manganese from French Annie and Aetna Creeks transitions through wetland habitat with evidence of beaver lodge construction (B. Duxbury *pers. obs.*). Greenfield et al. [97] demonstrated for small aquatic ecosystems such as Lake Manganese that even the presence of relatively small wetlands within the watershed can influence Hg bioaccumulation in fishes. Beaver ponds can also increase the rate of MeHg production in affected areas due to the development of anoxia [118]. The

deciduous trees preferred by beavers for dam construction can contribute to lower $\delta^{13}C$ values relative to when dams are absent [119]. Also, bacterial respiration in low oxygen environments such as beaver ponds can further reduce $\delta^{13}C$ values to the more negative of values observed in the current study [85]. Future research would be valuable to quantify the extent to which the surrounding watershed of Lake Manganese contributes to patterns of both $\delta^{13}C$ signatures and Hg concentrations in this ecosystem.

Seasonal changes in Lake Manganese yellow perch diets may also have influenced observed the relationships between Hg bioaccumulation and fish $\delta^{13}C$ values. Freshwater snails (*Physa spp.*) are abundant in Lake Manganese yellow perch diets (Chapter 2) and this taxa tends to have more negative $\delta^{13}C$ values relative to other prey species identified in the diet of population [83]. *Physa* spp. are also small (< 10 mm) bodied and declining growth efficiencies have been demonstrated as a consequence for larger consumers feeding on such small prey [120, 121] . This is associated with the increased number of small prey that must be consumed to meet daily energy requirements and also the increased amount of time spent foraging to consume numerous small prey [121]. Mayflies (*Hexagenia limbata*) were also documented in Lake Manganese gut contents and this benthic invertebrate can also have more negative $\delta^{13}C$ relative to other aquatic invertebrate prey observed. Mayflies are a burrowing invertebrate and during field collections, yellow perch were visually observed diving into Lake Manganese sediments with mayflies contributing to fish diets in this ecosystem (B. Duxbury *pers. obs.*). Sediments typically represent the sink for environmental pollutants such as Hg in aquatic ecosystems [51]. These unique aspects of yellow perch foraging ecology in Lake

Manganese likely contribute to the observed relationships between Hg concentrations and $\delta^{13}C$ in this population.

3.5.1 Conclusion

This research demonstrates that Hg concentrations in fish species may be much more dynamic over relatively short periods in an individual's life history than previously considered. These results complement the conclusions of Kolka et al. [96] and Braaten et al. [100] which also demonstrated the potential for seasonal change in Hg concentrations. However, the study of Kolka et al. [96] sampled young-of-the-year yellow perch (YOY) once a year but across multiple years. Mercury concentration data generated under such a field design are likely more representative of changes in bioavailable Hg as YOY perch tend to feed primarily on zooplankton for which Hg bioaccumulation is primarily driven by dissolved Hg concentrations [114]. The study of Braaten et al. [100] provides valuable insight into temporal Hg bioaccumulation dynamics in perch populations but not at the resolution of an individual age class scale as completed in the current study. Greenfield et al. [98] showed that a variety of biological, ecological, and limnological factors can predict Hg concentrations in yellow perch. The results of my study also suggest that seasonal variability in such factors help regulate short term fluctuations in Hg bioaccumulation in fishes. This research further builds on perspectives of Block et al. [1] who proposed the need for more winter biological, limnological, and ecological datasets. This research is also important from a human risk management perspective as the results indicate potentially elevated Hg exposure for population sub-groups such as ice-fishermen. Fish consumption guidelines are generally established based on Hg

concentrations generated from specimens that are frequently collected during the spring and summer open water seasons. However, the results of this study and those of Braaten et al. [100] generally demonstrate, lower seasonal Hg concentrations for summer collected fishes may underestimate the exposure risks to human consumers at other times of the year.

3.6 Tables and Figures

Table 3.1 Summary biological data including length (mm), mass (g), lipid, moisture and protein contents (%), Hg concentrations (µg/kg dry and wet wt.), and $\delta^{13}C$ and $\delta^{15}N$ (‰) values for 8-year-old yellow perch collected from Lake Manganese, Grant Township, Michigan. Collections occurred April 11, 2018 through March 2, 2019. Values represent mean with ± 1 standard error in parentheses.

Variable	Female (n = 46)	Male (n = 51)
Length	173.7 (2.5)	140.1 (2.0)
Mass	51.4 (2.7)	25.7 (2.2)
Lipid	1.0 (0.1)	1.5 (0.1)
Moisture	76.2 (0.7)	75.5 (0.4)
Protein	22.0 (0.6)	21.6 (0.4)
Hg (dry wt.)	352.0 (23.2)	452.8 (35.1)
Hg (wet wt.)	82.8 (5.2)	109.3 (7.9)
$\delta^{15}N$	7.6 (0.1)	7.2 (0.1)
$\delta^{13}C$	-31.5 (0.3)	-32.8 (0.3)

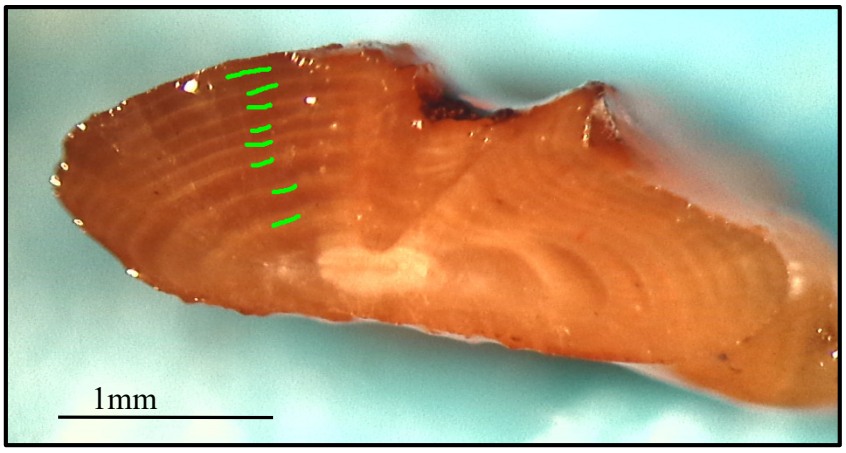

Figure 3.1. 8-year-old otolith picture taken from a Lake Manganese yellow perch. Overwintering annuli are indicated by the green lines in green with a scale bare representing 1mm.

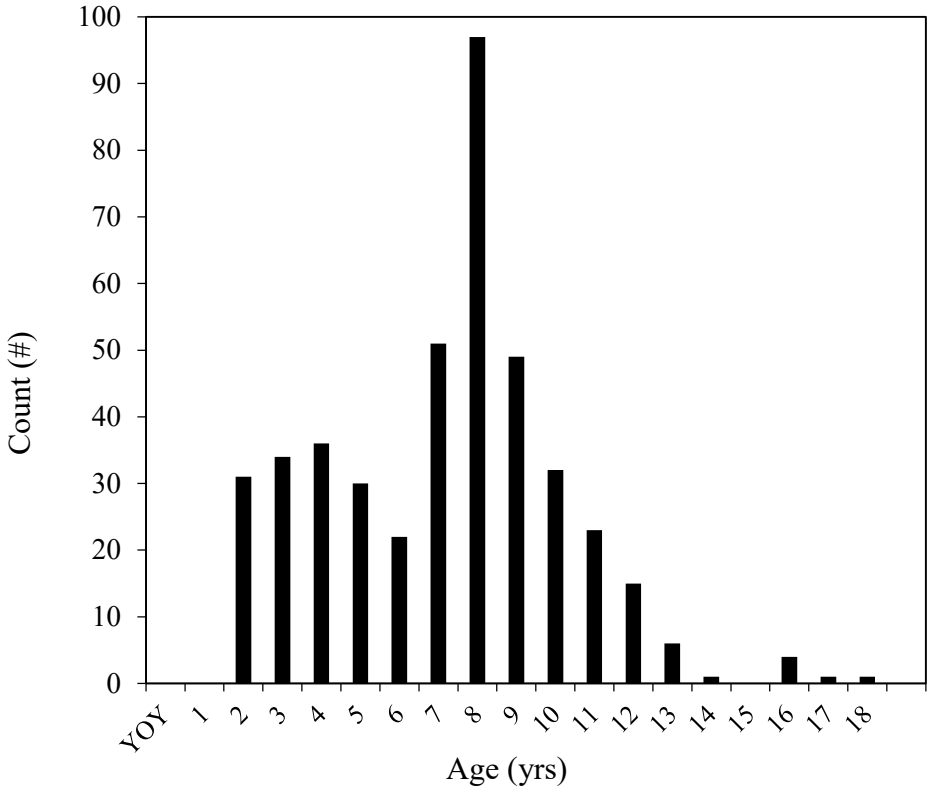

Figure 3.2. Frequency distribution histogram of yellow perch age classes (n = 433) collected from Lake Manganese in Keweenaw County, MI between April 11, 2018 and March 17, 2019.

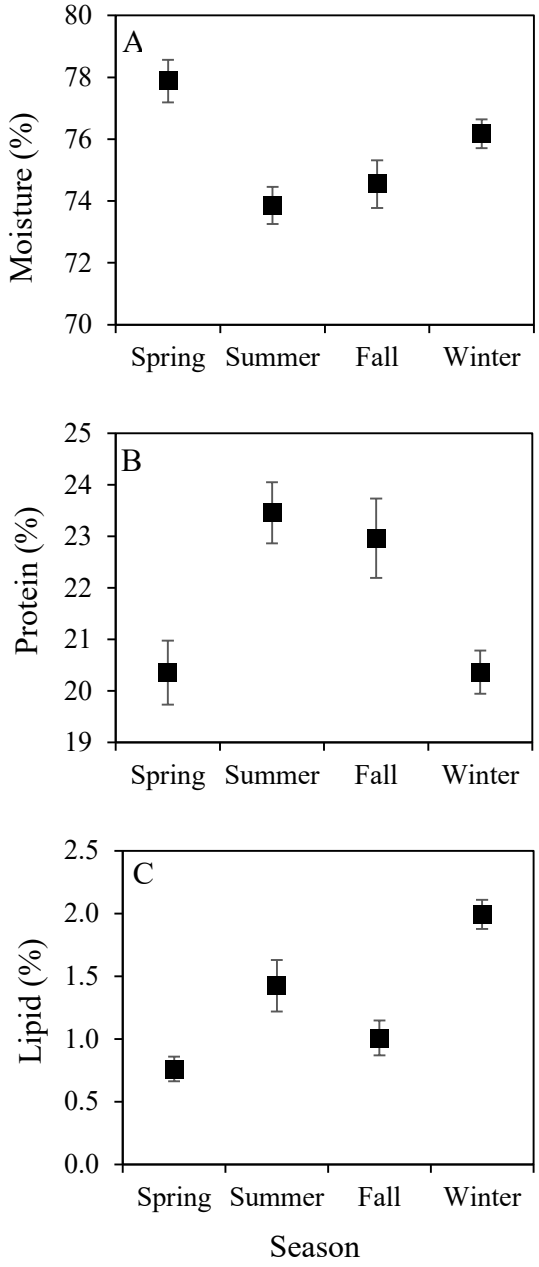

Figure 3.3. Average seasonal proximate (%) composition A) moisture (B) protein and (C) lipid contents for 8-year-old yellow perch including sampled from Lake Manganese in Keweenaw County, MI between April 11, 2018 and March 17, 2019. Symbols indicate mean with error bars indicating ± 1 standard error.

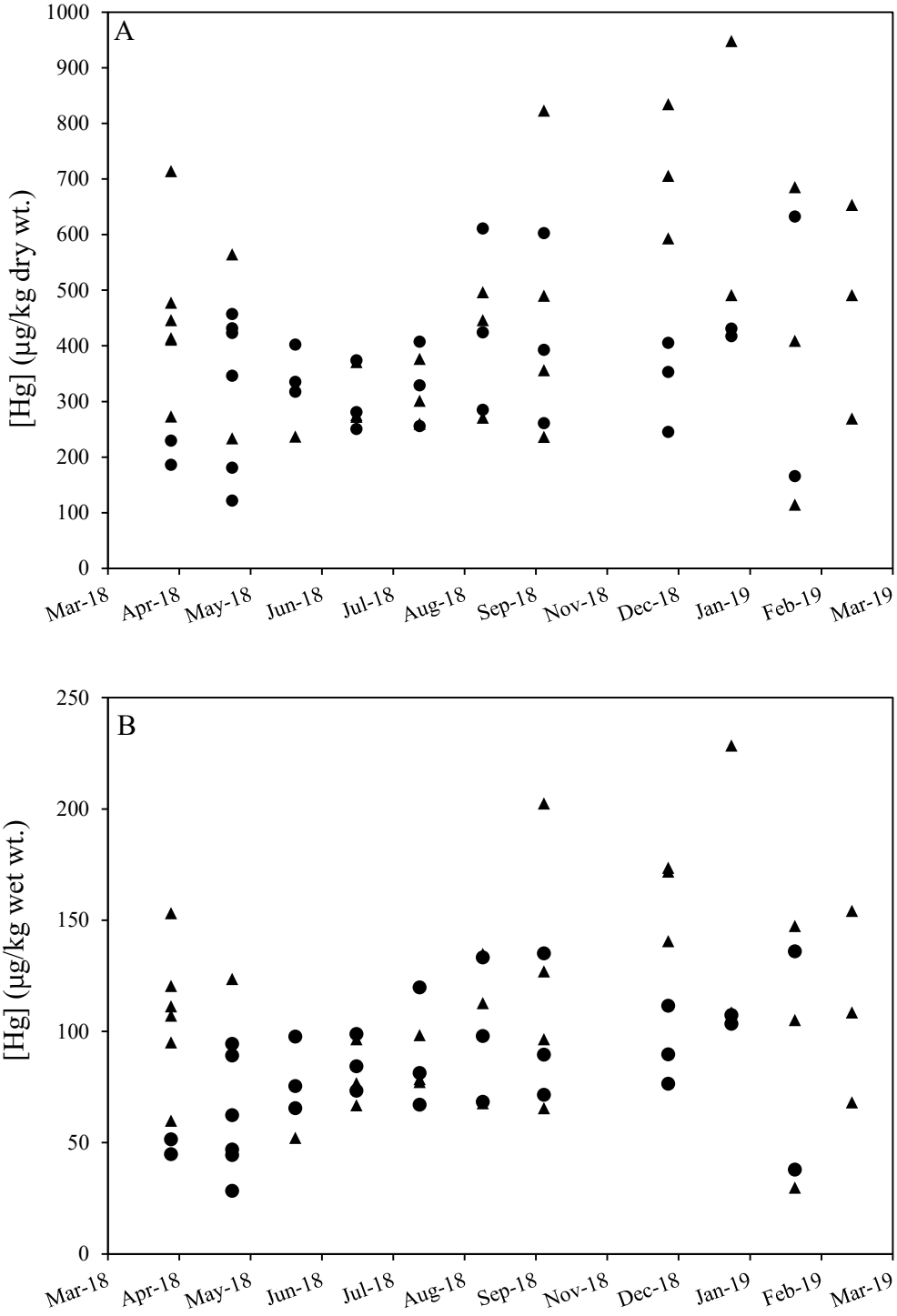

Figure 3.4 Monthly dry (A) and wet (B) weight Hg concentrations (µg/kg) for 8-year-old male (▲) and female (●) yellow perch collected from Lake Manganese in Keweenaw County, MI from April 11, 2018 and March 17, 2019.

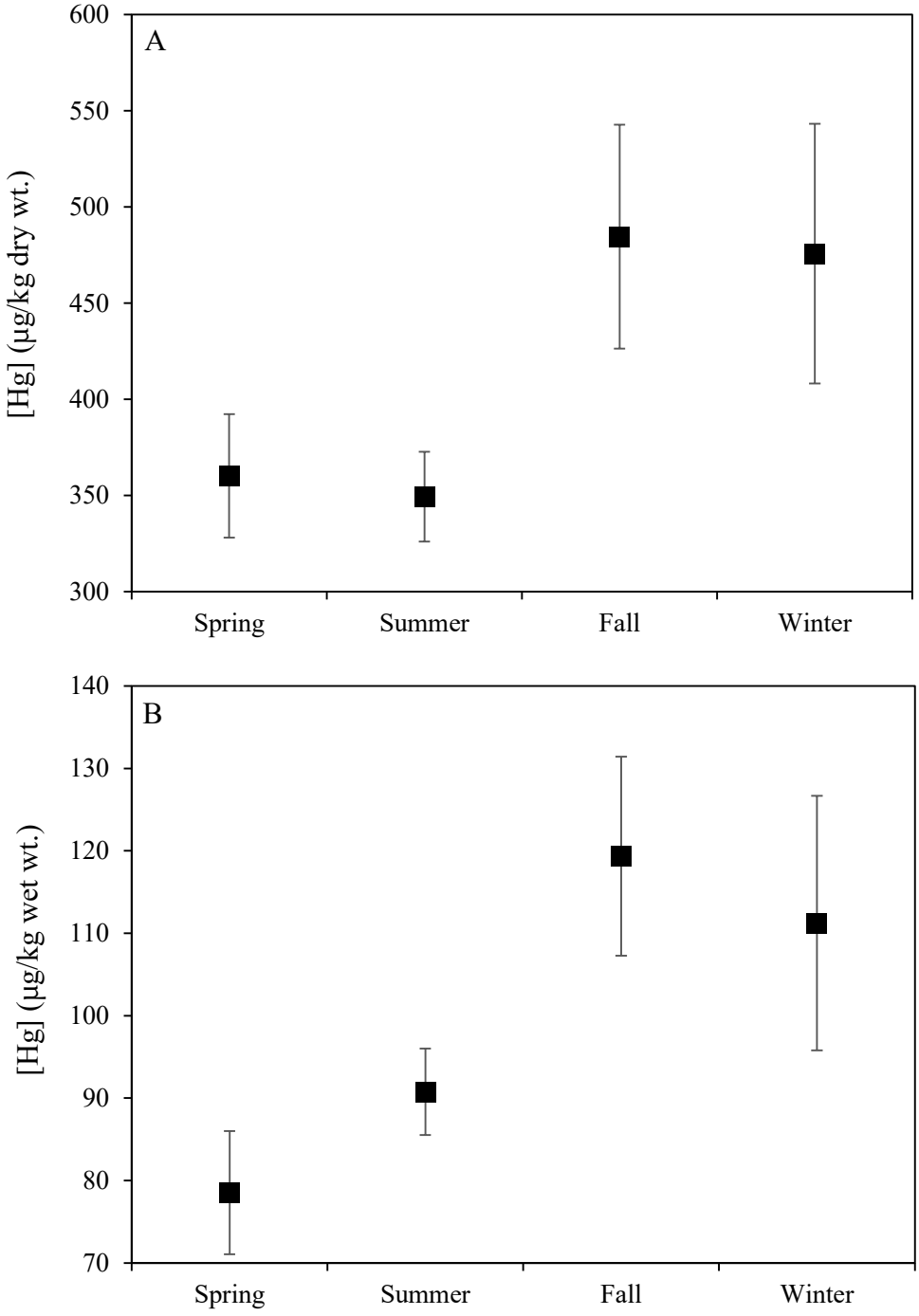

Figure 3.5. Average dry (A) and wet (B) weight Hg concentrations for 8-year-old yellow perch collected from Lake Manganese in Keweenaw County, MI during the spring, summer, fall, and winter between April 11, 2018 and March 17, 2019. Note the difference in y-axis scales between panels A and B. Symbols indicate mean with error bars indicating ± 1 standard error.

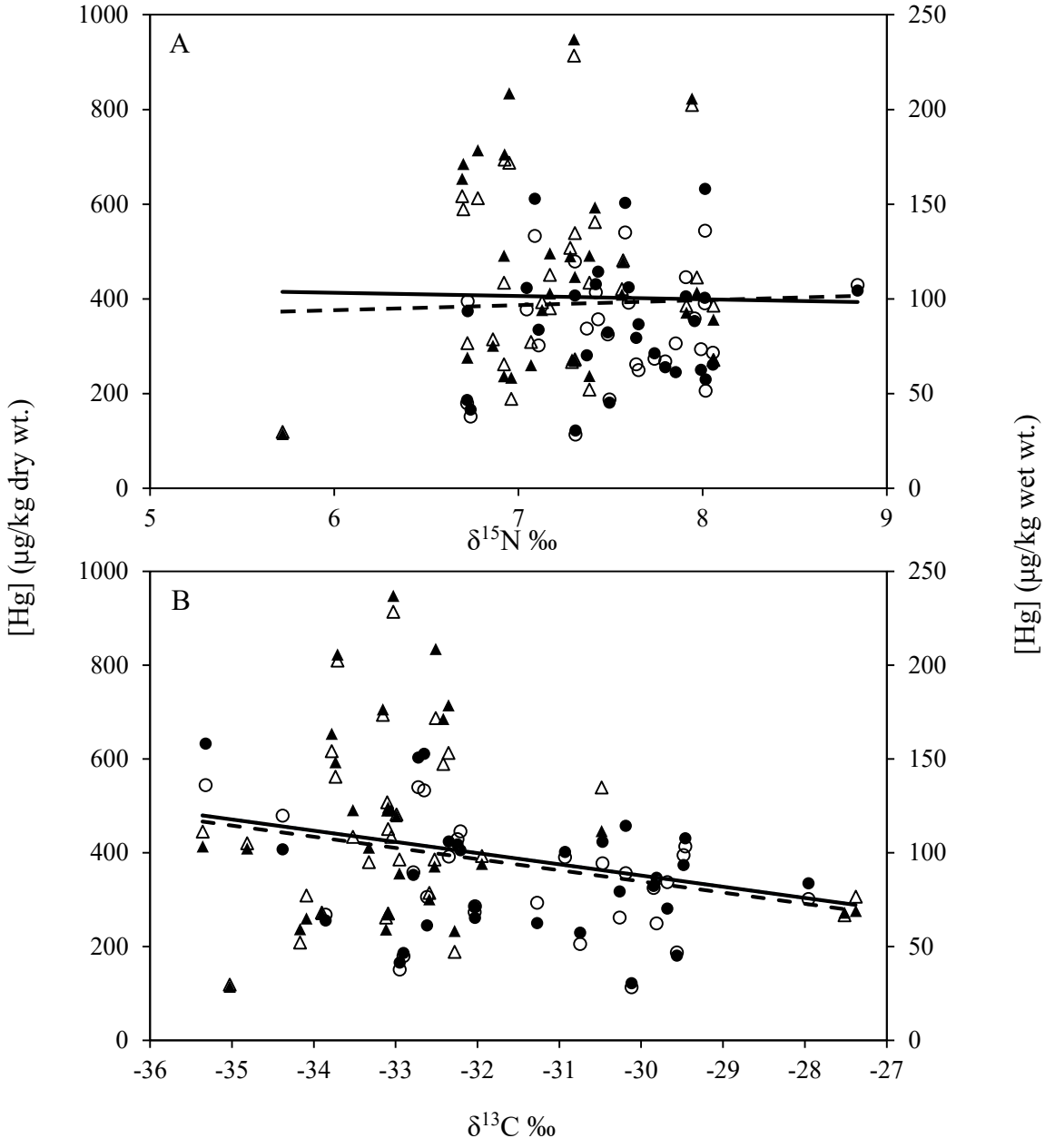

Figure 3.6. Dry and wet weight Hg concentrations (µg/kg) relative to stable isotopes of nitrogen $\delta^{15}N$ (A) and carbon $\delta^{13}C$ (B) for male (▲) and female (●) yellow perch. Solid and open symbols represent dry and wet weight Hg concentrations, respectively, with dry weight concentrations plotted on the primary y-axis and wet weight concentrations on the secondary y-axis. Solid and dashed lines in each panel represent the least squares regression lines between dry or wet weight Hg concentrations, respectively, and the independent variables. Summary regression statistics are provided in Table S3.2.

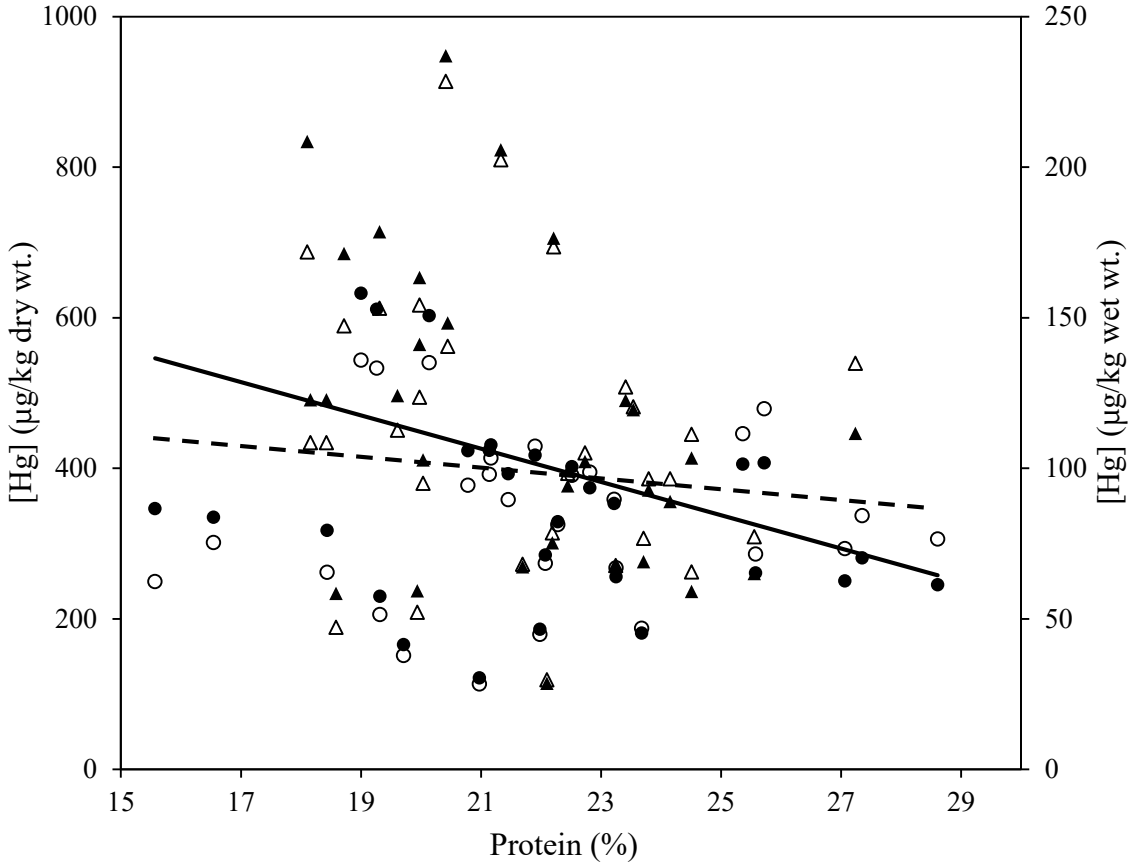

Figure 3.7 Dry and wet weight Hg concentrations (µg/kg) relative to protein content (%) (▲) and female (●) yellow perch. Solid and open symbols represent dry and wet weight Hg concentrations, respectively, with dry weight concentrations plotted on the primary y-axis and wet weight concentrations on the secondary y-axis. Solid and dashed lines in each panel represent the least squares regression lines describing the relationships between dry or wet weight Hg concentrations, respectively, with the independent variables. Summary regression statistics are provided in Table S3.2.

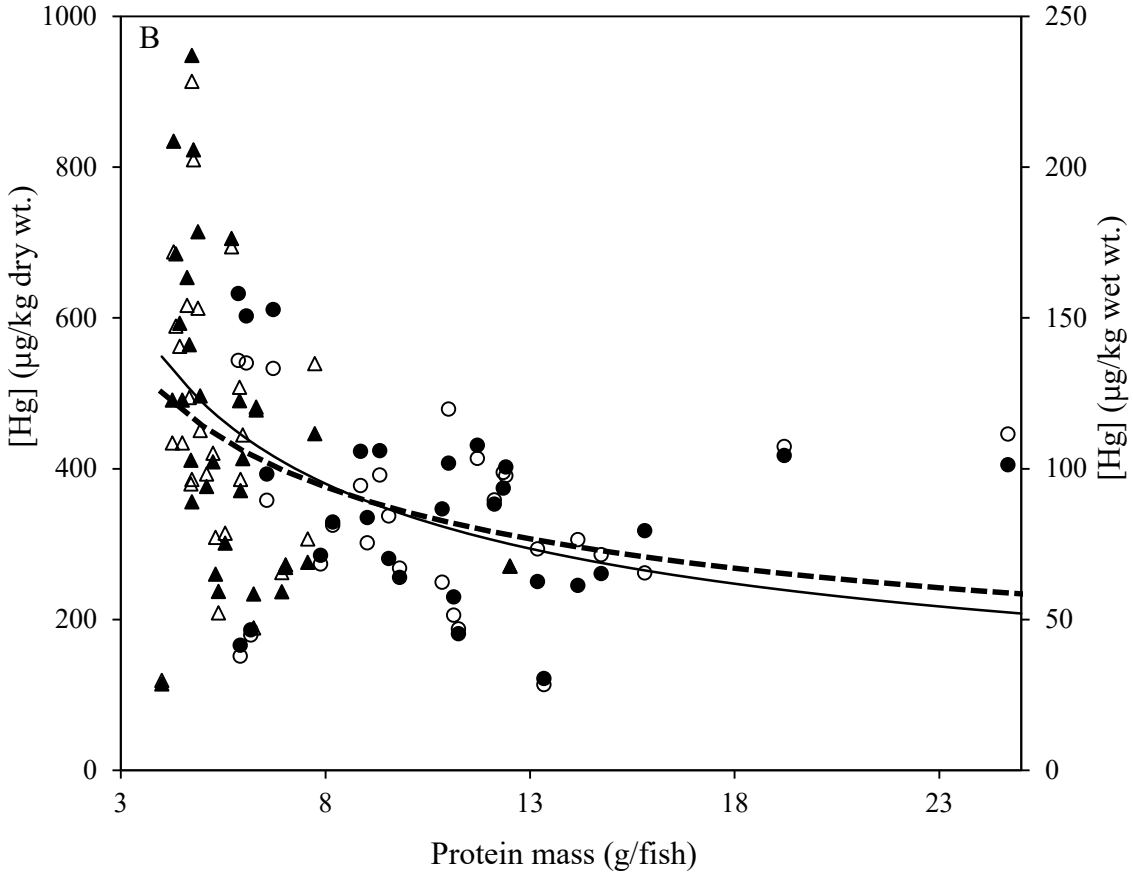

Figure 3.7 Dry and wet weight Hg concentrations (µg/kg) relative to protein mass (g/fish) for male (▲) and female (●) yellow perch. Solid and open symbols represent dry and wet weight Hg concentrations, respectively, with dry weight concentrations plotted on the primary y-axis and wet weight concentrations on the secondary y-axis. Solid and dashed lines in each panel represent the least squares regression lines describing the relationships between dry or wet weight Hg concentrations, respectively, with the independent variables. Summary regression statistics are provided in Table S3.2.

3.7 Supplemental Information

Table S3.1. Results of the one-way ANOVA model for the effects of Season (Spring [April-June], Summer [July-September], Fall [October, December], Winter [January-March]), and the covariates: Sex (Male, Female), length (mm), and mass (g) on the lipid content (%), moisture content (%), protein content (%), dry mass Hg concentration (µg/kg), wet mass Hg concentration (µg/kg) on Lake Manganese yellow perch *Perca flavescens*. All factors were treated as fixed effects, and bold values indicate a significant effect at $\alpha = 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
(A) Moisture (%)				
Season	**3.0**	**59.8**	**7.6**	**<0.001**
Sex	1.0	8.3	1.1	0.308
Total length (mm)	1.0	7.3	0.9	0.340
Body mass (g)	1.0	7.0	0.9	0.348
Model Error	61.0	7.8		
(B) [Hg] (µg/kg dry wt.)				
Season	3.0	66590.5	2.4	0.077
Sex	1.0	31253.1	1.1	0.293
Total (mm)	1.0	3784.0	0.1	0.713
Total (g)	1.0	9317.3	0.3	0.564
Model Error	56.0	277725.9		
(C) [Hg] (µg/kg wet wt.)				
Season	**3.0**	**4443.2**	**3.3**	**0.025**
Sex	1.0	2732.3	2.1	0.157
Total (mm)	1.0	523.1	0.4	0.533
Total (g)	1.0	930.1	0.7	0.406
Model Error	56.0	1328.2		
(D) Lipid (%)				
Season	**3.0**	**3.2**	**10.5**	**<0.001**
Sex	1.0	0.5	1.7	0.202
Total (mm)	1.0	0.5	1.5	0.226
Total (g)	1.0	0.6	2.0	0.163
Model Error	51.0	0.3		
(E) Protein (%)				
Season	**3.0**	**40.8**	**7.0**	**<0.001**
Sex	1.0	2.2	0.4	0.542
Total (mm)	1.0	4.2	0.7	0.400
Total (g)	1.0	1.8	0.3	0.579
Model Error	52.0	5.8		

(A) Overall model for Moisture (%): N =67, $R^2 = 0.302$, $P < 0.0009$
(B) Overall model for dry mass Hg concentration (μg/kg): N = 63, $R^2 = 0.194$, $P < 0.0518$
(C) Overall model for wet mass Hg concentration (μg/kg): N = 63, $R^2 = 0.259$, $P < 0.0081$
(D) Overall model for lipid (%): N = 58, $R^2 = 0.482$, $P < 0.0001$
(E) Overall model for Protein (%): N = 59, $R^2 = 0.304$, $P < 0.0033$

Table S3.2 Results of the one-way ANOVA model for the effects of season (Spring [April-June], Summer [July-September], Fall [October, December], Winter [January-March]) and sex (Male, Female) on total length (mm) and mass (g) on Lake Manganese yellow perch *Perca flavescens*. All factors were treated as fixed effects, and bold values indicate a significant effect at $\alpha = 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
(A) Length (mm)				
Season	3	275.6	1.5	0.2333
Sex	**1**	**20755.6**	**109.2**	**<0.001**
Model Error	92	190.0	36.9	
(B) Mass (g)				
Season	3	1532.8	2.3	0.088
Sex	**1**	**12085.3**	**53.3**	**<0.001**
Model Error	92	226.9	19.3	

(A) Overall model for total length (mm): N =97, $R^2 = 0.616$, $P < 0.001$
(B) Overall model for dry mass Hg concentration (µg/kg): N = 97, $R^2 = 0.456$, $P < 0.001$

Table S3.3 Summary least squares regression statistics describing the relationships between wet and dry wet Hg concentrations with biological predictor variables including protein content (%), protein mass (g), $\delta^{13}C$ (‰), $\delta^{15}N$ (‰) for 8-year-old yellow perch collected from Lake Manganese, Grant Township, Michigan. Collections occurred April 11, 2018 through March 2, 2019. Values indicated in bold represent statistically significant ($P < 0.05$) relationships.

Predictor	[Hg]	R^2	F	p	SE	Y-intercept	Slope
Protein content	Wet	0.12	0.8	0.365	2.0	137.9	-1.8
(n = 58)	**Dry**	**0.11**	**7.1**	**0.010**	**8.3**	**890.4**	**-22.1**
Protein mass*	**Wet**	**0.09**	**5.5**	**0.023**	**1.3**	**122.8**	**-2.9**
(n = 58)	**Dry**	**0.13**	**8.1**	**0.006**	**5.5**	**534.9**	**-15.1**
$\delta^{13}C$	**Wet**	**0.06**	**4.4**	**0.040**	**2.8**	**-93.5**	**-5.9**
(n = 58)	Dry	0.07	3.6	0.064	12.7	-366.4	-23.9
$\delta^{15}N$	Wet	<0.01	0.1	0.803	10.6	78.0	2.7
(n = 58)	Dry	<0.01	<0.1	0.882	47.2	455.1	-7.0

4 General Conclusion

Seasonality is a ubiquitous component of aquatic ecosystems, yet focused studies investigating fish population responses to this characteristic are generally limited. The overall objective of this thesis was to examine the importance of seasonality on the biology and ecology of the Lake Manganese yellow perch population and their association with limnological characteristics over one year. It is important to understand how yellow perch respond to seasonal changes owing to their broad latitudinal range across North America, ecological role in aquatic ecosystems, and their contributions to recreational and commercial fisheries [9, 10] . During an annual growing season, the cold water overwintering seasons generally represents an understudied period for aquatic ecology [1]. Furthermore, the bioaccumulation and biomagnification of pollutants, such as mercury (Hg), are tightly coupled with animal ecology and the role of seasonality in these processes has received relatively limited attention [122]. This research greatly contributes to our understanding of the role that seasonality and winter conditions have on aquatic ecology, limnology, and ecotoxicology.

Seasonal changes in lacustrine limnological factors have been generally well studied [5]. However, under-ice limnology is relatively understudied outside of polar environments [1]. In this study, we quantified daily average water temperatures at various depths throughout Lake Manganese from August 2018 through June 2019. For a cool-warm water fish species such as yellow perch, the availability of water temperatures from 20 – 23 °C define the optimal habitat for growth [4]. During the summer months, only the top 4 m of Lake Manganese approached this temperature range with the month

of August 2018 demonstrating the highest average water column temperature (16.7 °C). In contrast, under ice-cover, the 'warm' water temperature was largely restricted to 4 °C and only encompassed the bottom 1m of Lake Manganese. These patterns indicate substantial differences in the availability of thermal habitat for the growth of species such as yellow perch that prefer water temperatures ≥ 20 °C. Additional fishes captured from Lake Manganese during the course of this research included bluegill sunfish (*Lepomis macrochirus*), golden shiner (*Notemigonus crysoleucas*), largemouth bass (*Micropterus salmoides*) and white sucker (*Catostomus commersonii*). Each of these species also have preferred water temperatures ≥ 20 °C [123] and the annual thermal stratification of Lake Manganese is likely limiting for their growth. Specifically, each of these species have only a relatively short duration of optimal growth habitat available which likely creates a high degree of competition among these fish species for habitat and prey resources in Lake Manganese. Investigating how such potential competition contributes to the stunted growth of yellow perch in Lake Manganese could contribute to our knowledge of resource partitioning in aquatic ecosystems.

The remote location of Lake Manganese and the sparse population density of Michigan's Keweenaw Peninsula creates a great opportunity for long term limnological monitoring programs. Due to its high northern latitude, I believe that a long-term study with various data loggers could greatly benefit our knowledge of seasonal limnology and would be an excellent tool to examine the responses of limnological variables under the perspectives of climate change. By using temperature profiles to accurately map out habitat volumes of various species, data sets such as mine could be used to predict

species at risk of extirpation due to climate change while simultaneously characterizing habitat for species likely to thrive in these new conditions. For example, based on continued oxygenation of the water column and availability of cool-cold water habitat over one year, Lake Manganese may represent a candidate for brook trout (*Salvelinus fontinalis*) restoration/reintroduction. Lake Manganese also receives almost 2 m of snow and ice-cover during winter (B. Duxbury, *pers. obs.*), and a more in-depth study as to how pH, dissolved oxygen, and zooplankton communities change in such prolonged and extreme winter environments would improve our understanding of under-ice limnology.

Yellow perch were sampled on a monthly basis to observe seasonal changes in their biology and ecology. Significant seasonal variation in lipid contents, and gonadosomatic and hepatosomatic indices were expected and observed in Lake Manganese yellow perch [26]. The seasonal changes observed in energy density was surprising as it did not follow the same seasonal pattern as lipids. Another surprising observation was the significant seasonal variability in nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope values. The stable isotope of carbon can be used to describe habitat and prey resource exploitation activities [77, 83, 124]. In Lake Manganese, yellow perch $\delta^{13}C$ values were generally quite negative for a small inland lake population [81] and encompassed a wide range of values (-35.3 to -28.0 ‰) and were overall very negative relative to, for example, those reported for Lake Michigan yellow perch by Happel et al. [81] across a much ecosystem scale. This wide range in $\delta^{13}C$ values for Lake Manganese yellow perch suggests that this population is exploiting a diversity of habitat and prey resources rather than maintaining a high degree of fidelity to any one resource. From this

consideration, a stable isotope study of the entire Lake Manganese food web could provide novel insight regarding the trophic ecology of this ecosystem. For example, owing to the general lack of top predator species and the generally smaller sizes of fishes collected during this study, I predict that the trophic structure of the fish population as indicated by $\delta^{15}N$ should not differ among the fish species within Lake Manganese. Such an in-depth investigation of stable isotopes especially within the Lake Manganese fish community could be a key component for examining the role of competition in structuring this food web and possible contributors to stunted yellow perch growth. The occupancy of all fish species at the same tropic position would indicate that resource limitation is occurring within Lake Manganese. I believe that the limiting resource would be identified as benthic invertebrates due to a large abundance of small fish and in the absence of any large piscivores in the lake.

To our knowledge, this study is among the first to examine Hg bioaccumulation in a single age class of fish over an annual temperature cycle. Among 8-year old yellow perch, Hg concentrations varied almost ten-fold among individuals and across the seasonal sampling. These results complement those of Braaten et al. [100] who quantified Hg concentrations in populations of Eurasian perch (*Perca fluviatilis*) over an annual spring, summer, fall and winter seasonal cycle. However, our results demonstrate the variable nature of Hg bioaccumulation among individual fish and emphasize the importance of fish growth at this scale [125]. Our results also emphasize the need for pollutant bioaccumulation data during the cold water and ice-covered periods. Specifically, Hg concentrations quantified yellow perch were highest during the fall and

winter seasons when the contributions of mechanisms, such as growth dilution and whole-body elimination, are likely to be limited for reducing the extent of Hg bioaccumulation by individuals [4, 103] . Additional investigations of pollutant bioaccumulation kinetics, including Hg and hydrophobic polychlorinated biphenyls (PCBs), would prove a valuable contribution for our understanding of the ecotoxicology of these pollutants in aquatic ecosystems, especially under the auspices of climate change. For example, future investigation into Hg concentrations throughout the entire food web may help explain the significant seasonal variation that was observed within Lake Manganese. The use of passive integrated transponder (PIT) tags and mussel plugs in Lake Manganese sampling methods could be expanded to include non-lethal seasonal sampling while also specifically tracking temporal Hg bioaccumulation patterns among specific individual fish. These results could expand upon our knowledge of seasonal ecotoxicology data and the role winter and seasonal changes in growth play on Hg bioaccumulation.

4.1 Summary

The extent of seasonality for the range of limnological characteristics and ecological and ecotoxicological variables demonstrated for Lake Manganese and the yellow perch population in this lake underscore the importance of seasonal and overwintering datasets. The most important expansion of this dataset would be to further increase the intensity of winter and entire growing seasonal sampling across multiple areas of aquatic ecology. Efforts to standardize and demonstrate the importance of winter sampling as emphasized by Block et al. [1] and the methodologies used in the current

study provide examples for generating novel and additional data for this emerging field of ecology [126]. Lake Manganese represents an excellent site for a long-term study on the effects of seasonality owing to its smaller size and scale for sampling, and relatively remote location and accessibility. Specifically, high frequency sampling of various ecological and toxicological metrics in this ecosystem could further our understanding of the role of winter on aquatic ecology and pollutant bioaccumulation.

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