THE EFFECTS OF ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) ON FRESHWATER DRUM (*APLODINOTUS GRUNNIENS*) DIET AND GROWTH IN THE SOUTH BASIN OF LAKE WINNIPEG

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ABSTRACT

The freshwater drum (*Aplodinotus grunniens*) is a large-bodied fish found throughout the Lake Winnipeg watershed. Little is known about freshwater drum diet and ecology in Manitoba, as they are not a fish of commercial interest or recreational popularity. However, freshwater drum may be a primary predator of zebra mussels (*Dreissena polymorpha*), which established in Lake Winnipeg in 2013. In this study, the diets and growth of 51 freshwater drum collected in 2019 (6 years post-zebra mussel invasion) were compared to the diets and growth of 64 freshwater drum sampled in 2000 (pre-zebra mussel invasion); all fish were collected from Lake Winnipeg's south basin. Benthic insect larvae were the dominant food item in both sample years. Although mollusks occur in high densities in Lake Winnipeg, they were rarely consumed by freshwater drum in either sample year. Zebra mussels were not a frequent prey item in 2019 as they were only consumed by four of the sampled freshwater drum. Stable isotope analysis of white muscle tissue yielded similar $\delta^{13}C$ and $\delta^{15}N$ values in both sample years, which were consistent with a benthic, insectivorous diet. Length-at-age data derived from otolith aging revealed that the 2019 population had an equal or higher growth rate than the 2000 population. Weight-at-length data suggests that fish condition was greater in 2019 than in 2000, which coincides with increased benthic macroinvertebrate density in Lake Winnipeg. Based on these findings, Lake Winnipeg freshwater drum feed continue to feed preferentially on insect larvae over zebra mussels and were therefore unlikely to have consumed zebra mussels at the onset of invasion.

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INTRODUCTION

1.1 Freshwater Drum Life History

The freshwater drum is North America's only freshwater sciaenid (Boeger *et al.* 2015; Fuller *et al.* 2015), with a latitudinal range spanning from Guatemala to the Nelson River in northern Manitoba (Stewart and Watkinson 2004). In Manitoba, freshwater drum are found in Lake Winnipeg, Lake Winnipegosis, Lake Manitoba, and their connected tributaries (Stewart and Watkinson 2004). Freshwater drum are a silver, deep-bodied fish with a lateral line extending to the caudal peduncle. They have contiguous spiny and soft dorsal fins, an anal fin with two spines, and a rounded caudal fin. Freshwater drum have an inferior, protrusible mouth that facilitates benthic feeding (Scott and Crossman 1973; Becker 1983).

Freshwater drum are reputed molluscivores, due in part to the presence of pharyngeal teeth that are well suited for crushing mussel shells (Moen 1955; French 1997). However, current literature suggests that many freshwater drum populations feed selectively on insect larvae such as chironomids and mayflies, with larger freshwater drum incorporating fish, crayfish, and clams into their diets (Daiber 1952; Moen 1955; Bur 1982; Becker 1983; Jacquemin *et al.* 2014). Ontogenetic dietary shift is brief, with young-of-year freshwater drum progressing from zooplankton to benthic insects at lengths as small as 25-35 mm (Daiber 1952; Becker 1983; Sullivan *et al.* 2012).

Freshwater drum spawn when water temperatures reach 18-22 °C (Holm *et al.* 2009), which occurs from May to June in the Upper Mississippi River (Becker 1983) and late June to early August in Lake Erie (Daiber 1953). In Manitoba, fertile freshwater drum have been caught in the Red River in mid- to late June (Stewart and Watkinson 2004). During courtship, male freshwater drum produce a drumming sound by flexing striated muscle against their swim bladders (Holm *et al.* 2009). Reproduction occurs at night in open water, where females release up to 850,000 pelagic eggs (Shields and Beckman 2015; Eakins 2020). Hatching occurs within two days, and larval fish float head down until their yolk sacs are depleted (Daiber 1953; Davis 1959).

Ages of maturity vary between freshwater drum populations. Male and female freshwater drum mature as early as ages 2-4 in the Upper Mississippi River (Butler and Smith 1950), while females first mature at age 9 in the Red Lakes, Minnesota (Palmer *et al.* 1995). In Lake Erie, Daiber (1953) first observed mature males and females at ages 4-5, while Edsall (1967) identified a maturity window of 2-6 for males and 4-7 for females. Latitude is likely a major contributor to differences in the ages of maturity described in literature (Belk and Houston 2002; Abner and Phelps 2018).

1.2 Zebra Mussels in North America

The zebra mussel, *Dreissena polymorpha*, is a bivalve native to the freshwater and estuarine waterbodies of the Black, Caspian, and Aral Sea drainage basins (Son 2007). Zebra mussels are small (< 50 mm) triangular mollusks with light and dark brown stripes (Benson *et al*. 2020). Unlike other mollusks, which use their foot to burrow into benthic substrate, zebra mussels use hair-like byssal threads to attach to hard underwater structures (Peyer *et al.* 2009). Zebra mussels are believed to have first entered the Great Lakes from the ballast tanks of transatlantic cargo ships in 1986 (O'Neill and Dextrase 1994). Adult mussels were first discovered in Lake St. Clair in June of 1988 (Hebert *et al*. 1989) and in Lake Erie that same year (O'Neill and Dextrase 1994). Zebra mussels have since spread throughout the Great Lakes

Basin, the Mississippi River and Red River drainage basins, and numerous other isolated waterbodies (Benson *et al.* 2020). Rapid spread can be partially attributed to the natural distribution of veliger larvae, which travel with current for up to a month before settling on hard structures as juveniles (Benson *et al.* 2020). Zebra mussels have also been spread by overland boat transport as veligers in undrained water and as adults attached to boats hulls and equipment, where they can withstand desiccation for several days (O'Neill and Dextrase 1994). Zebra mussel presence can have large economic implications as they are known to foul beaches, boats, and other submerged infrastructure (Rosaen *et al.* 2016). Zebra mussels often clog water intake pipes and screens, which is problematic for water treatment and power plants (Rosaen *et al.* 2016). Zebra mussels are projected to cost over $1 billion per year in damages, maintenance, and control efforts in the U.S. alone (Rosaen *et al.* 2016).

The introduction of zebra mussels in a waterbody often leads to dramatic changes in water clarity as well as phytoplankton and invertebrate population structure. Zebra mussels can filter up to 1 L of water a day and retain nearly 100% of suspended particles greater than 1 μm. (Jorgensen *et al.* 1984; Benson *et al*. 2020). In high numbers, populations can decrease suspended solid concentrations and pelagic phytoplankton and zooplankton densities (Higgins and Vander Zanden 2010; Benson *et al.* 2020). Zebra mussels are also associated with proliferated blue-green algae growth, possibly due to selective rejection of cyanobacteria (Bierman *et* al. 2005) or by reducing dissolved nitrate concentrations (Bykova *et al.* 2006). Benthic macroinvertebrate communities generally show short-term increases in biomass, though taxa-specific interactions vary. Collectors and scrapers such as *Hexagenia* mayflies and *Chironomus* midges ('chironomids') may grow in biomass due to increased cover from zebra mussel shells and increased benthic food sources in the form of pseudofeces and biofilm (Young *et al.* 1996; Burlakova *et al.* 2014). By contrast, filter feeders such as clams are regularly outcompeted by zebra mussels, sometimes to the point of extirpation (Schloesser and Nalepa 1994; Higgins and Vander Zanden 2010). In most instances of invasion, zebra mussels become the dominant benthic macroinvertebrate within three years of introduction (Karatayev *et al.* 2011). In Lake Erie, zebra mussels and their counterpart, the quagga mussel (*Dreissena bugensis*), constitute up to 41% of the benthic community in number and 97% of wet biomass (Burlakova *et. al.* 2014). Numerous studies have documented zebra mussels in densities exceeding 4,000/m^2 (MacIsaac 1996; Strayer *et al.* 1999; Haltuch *et al.* 2000) and as high as 750,000/m^2 (Kovalak *et al.* 1993).

1.3 Freshwater Drum Predation on Zebra Mussels

Numerous studies confirm that adult freshwater drum will feed extensively on zebra mussels within years of introduction. Almost half of freshwater drum over 190.5 mm consumed zebra mussels in Lake Dardanelle, Arkansas (Magoulick and Lewis 2002), and zebra mussels accounted for up to 59% of freshwater drum diets by dry weight in Lake Champlain (Watzin *et al.* 2008). In Lake Erie, large freshwater drum (> 375 mm) sampled in 1990 by French and Bur (1993) fed almost entirely on zebra mussels, though a later study by Morrison *et al.* (1997) only found zebra mussels in 40% of large freshwater drum (> 350 mm) stomachs. Both Lake Erie studies found that freshwater drum began feeding on zebra mussels at around 250 mm in length and that zebra mussel consumption increased with fish size (French and Bur 1993; Morrison *et al.* 1997).

The efficacy of freshwater drums as biological controllers of zebra mussels, particularly in reducing zebra mussel spread, is not well documented. Several studies have shown that freshwater drum prefer zebra mussels less than 22 mm in length (French 1995; Morrison *et al.* 1997). French (1995) proposed that this size selection was due to pharyngeal gape limitations, which would prevent freshwater drum from impacting zebra mussel colonies with a large average size. Morrison *et al.* (1997) attributed size selection to the abundance and accessibility of zebra mussels under 11 mm in length, which comprised 90% of the Lake Erie population at the time of study. Even if size limitations exist, several studies have demonstrated that fish can control zebra mussels. A study on Lake Dardanelle, Arkansas found that freshwater drum, blue catfish (*Ictalurus furcatus*) and redear sunfish (*Lepomis microlophus*) significantly reduced the density of zebra mussels colonizing experimental clay tiles (Magoulick and Lewis 2002). A similar predation study in the Mississippi and Ohio rivers determined that fish had the greatest impact on zebra mussel populations when densities were already low (Thorp *et al.* 1998). In this regard, zebra mussel predation by freshwater drum may be most effective at the onset of invasion.

1.4 Freshwater drum and Zebra Mussels in Lake Winnipeg

In 2009, zebra mussels were discovered in Pelican Lake, a Minnesota lake in the Red River basin (Niskanen 2009). Zebra mussels were subsequently found in the North Dakota portion of the Red River in 2010 (Gehring 2010) and Lake Winnipeg in 2013 (DFO 2014). As of 2019, zebra mussels have established throughout Lake Winnipeg, though the extent of encroachment into the north basin is less documented (Enders *et al.* 2019; DFO, unpublished data, 2019). The ecological impacts of zebra mussels in Lake Winnipeg are only beginning to be quantified, but like other waterbodies, the growing population may decrease phytoplankton and zooplankton biomass, alter zoobenthos abundance and diversity (DFO 2017), and concentrate energy production towards the benthic zone of the lake (Mayer *et al*. 2013). If water clarity increases significantly, the biomass and recruitment of light-sensitive fish such as walleye (*Sander vitreus*) may decline (Geisler *et al.* 2016), effectively placing Lake Winnipeg's $100 million-plus commercial and recreational fishery in jeopardy (GNG 2018).

As freshwater drum have consistently consumed zebra mussels in other infested waterbodies, it stands to reason that freshwater drum may be consuming zebra mussels in Lake Winnipeg. Since this possibility has been publicized (CBC News 2015; Forbes 2015; Forbes 2018), freshwater drum–long considered a rough fish and a nuisance in Manitoba (Kives 2013)—are being held in higher regard by commercial and recreational anglers alike (Kevin Casper, personal communication September 18, 2019). The increased recognition of freshwater drum in Manitoba has also been reflected in fisheries regulations. Until recently, there was no recreational possession limit for freshwater drum (Manitoba Sustainable Development 2017). In 2018, a possession limit of 15 freshwater drum was instated (Manitoba Sustainable Development 2018), and in 2020, the possession number was reduced to 10, with a size limit of 60 cm (Manitoba Agriculture and Resource Development 2020). However, the increased attention towards freshwater drum in Manitoba has highlighted how little is known about their life history and feeding behaviour. The interaction between freshwater drum and zebra mussels in Lake Winnipeg also remains undocumented. Currently, the only published research written exclusively on Manitoba freshwater drum is from a growth dynamics study conducted by a past University of Winnipeg student (Hardisty 2007). As such, there remains a considerable knowledge deficit regarding the diet and feeding behaviour of freshwater drum in Manitoba.

1.5 Objectives

The objective of this study was to compare freshwater drum sampled from Lake Winnipeg in 2000 and 2019 to assess changes in diet, trophic position, and growth following zebra mussel invasion. Freshwater drum collected pre- and post-invasion were used to generate diet indices, ^{13}C and ^{15}N isotope profiles, and weight-at-length and length-at-age relationships. Digestive tract contents were analyzed to characterize the diets of freshwater drum in the south basin. Stable isotope profiles for each sample year were compared to test for differences in long-term diet preferences, and referenced against values in literature to estimate trophic position. Freshwater drum were aged by counting sagittal otolith annuli.

The results from this study will (1) characterize the diet and feeding strategies of freshwater drum in Lake Winnipeg, (2) expand upon past research on freshwater drum growth in Manitoba, (3) identify how freshwater drum diets, feeding strategies and growth rates have changed since zebra mussel introduction, and (4) establish if freshwater drum are integrating zebra mussels into their diets. Study results may also offer some insight into how zebra mussels are impacting the prey of freshwater drum. Findings will provide fisheries managers with a framework to project changes in the freshwater drum population and make informed management decisions as the zebra mussel population continues to expand.

METHODS

2.1 Study Area

With a latitudinal length of 416 km and a surface area of 23,750 km^2, Lake Winnipeg is the world's tenth largest freshwater lake and third largest hydroelectric reservoir (Environment Canada 2011). Lake Winnipeg consists of a north basin with a mean depth of 13.3 m, a smaller south basin averaging 9.0 m, and a narrows separating the two basins (Environment Canada 2011). Lake Winnipeg is highly eutrophic due to heavy nitrogen and phosphorous inputs from its watershed, which spans nearly 1,000,000 km^2 and is populated by over 7 million people (WSRP 2019). In addition to zebra mussels, Lake Winnipeg is experiencing ecological stressors such as blue-green algae blooms, invasive species such as the common carp (*Cyprinus carpio*) and spiny water flea (*Bythotrephes longimanus*) and commercial catch rates that exceed maximum sustainable yield estimates (Environment Canada 2011).

The study area was limited to the south basin of Lake Winnipeg because a) zebra mussels are most prevalent in the south basin; b) between-basin differences in ecological and water quality parameters do not allow for aggregated data analysis (Environment Canada 2011; Hobson *et al.* 2012; Hann *et al.* 2017); and c) time and resource constraints prevented narrows and north basin sampling.

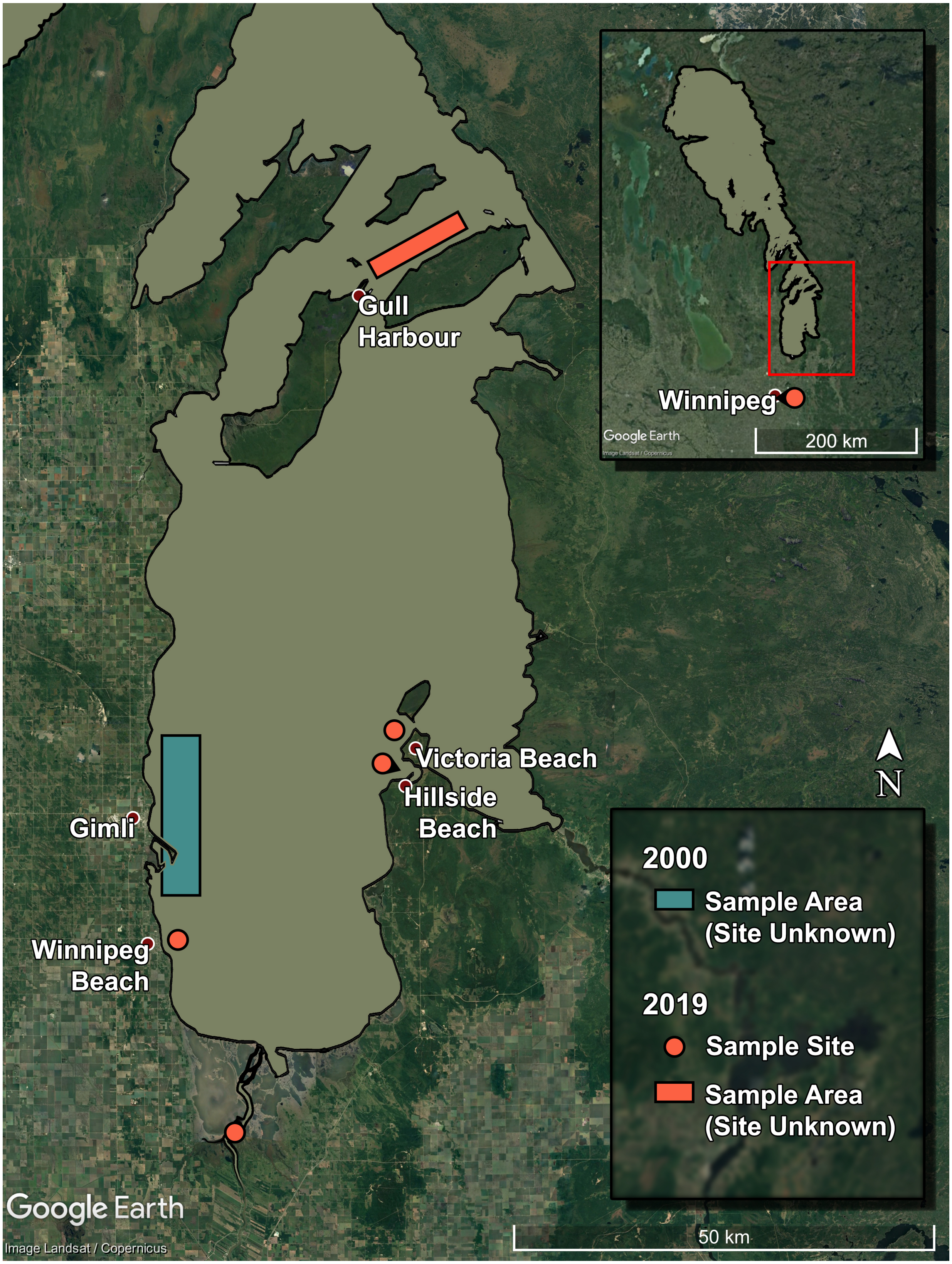

Figure 1. 2000 and 2019 sample sites in the south basin of Lake Winnipeg, MB.

2.2 Lake Winnipeg Freshwater Drum Sampling

Between June and August of 2000, 64 freshwater drum were sampled near Gimli, Manitoba by commercial fishers on behalf of Fisheries and Oceans Canada (DFO). Sample site coordinates and gillnet mesh sizes were not recorded. The fish were thawed in 2018 by DFO personnel, measured for total length and weight, and dissected for study material. Muscle samples and stomachs were sealed in individually marked Nasco bags for freezing, and sagittal otoliths were stored in marked coin envelopes. Fish from the 2000 samples were not sexed.

In 2019, freshwater drum were sampled chronologically from Victoria Beach (n =10, "winter"), the Red River (n = 11), Hillside Beach (n = 15), Winnipeg Beach (n = 4), and Gull Harbour (n = 11). Victoria Beach fish were collected on February 12 by DFO personnel from commercial bycatch found frozen on the ice. Red River, Hillside Beach, and Winnipeg Beach fish were caught using boat electrofishing in 0.5 m to 3 m of water throughout August. The freshwater drum were bagged and frozen at -20 °C and thawed on the day of processing. Fish from Gull Harbour were caught with commercial gillnets in September and supplied by the Gimli Sustainable Development office; these fish were processed on the day of capture. All fish were measured for total length and weight, sexed, and dissected for sagittal otoliths, digestive tracts, and dorsal white muscle tissue.

2.3 Diet Analysis

Digestive tracts from the 2000 and 2019 freshwater drum were processed in the winters of 2018 and 2019, respectively. The digestive tracts of each freshwater drum were excised and partitioned into foregut (esophagus to the pyloric caecae) and hindgut (intestine following the caecae to the anus) sections for diet analysis. Digesta from each section were extruded into conical centrifuge tubes, which were filled with 70% ethanol and stored at -20 °C until further processing. Prey items from each section were viewed under a dissecting microscope and sorted by order; dipterans were sorted by family to account for the relative importance of Chironomidae (midges family; ‘chironomids’) in freshwater drum diets (Daiber 1952; Bur 1982; Becker 1983). Sorted specimens were counted and tabulated for sum, proportion and frequency of occurrence. As bivalves were often crushed, numbers were estimated by counting shell hinges or shell halves and cross-referenced using dry weight comparisons with intact specimens of similar size. Prey-specific abundance was calculated using $P_i = (\Sigma S_i / \Sigma S_{ti})100$, where prey-specific abundance (P) is the proportion that prey i comprises (S_i) of all prey items in the digestive tracts where prey i is present (S_{ti}) (Amundsen *et al.* 1996). Prey items from 2019 were dried in pre-weighed tins for 48 hours at 40 °C to obtain dry weights (Chipps and Garvey 2007); dry weights were not measured for 2000 prey items. Wet weight was not obtained as desiccation of the prey items occurred rapidly during sorting and transport to the analytical balance, which was located in another laboratory room. Because all dominant prey taxa yielded digestion-resistant structures and foregut and hindgut counts were similar, foregut and hindgut diet indices were merged for final analysis.

2.4 Stable Isotope Analysis Preparation

A subsample of fish from each 2019 site (n = 22) and 10 fish from the 2000 samples were selected for stable isotope analysis. Fish were selected to equally represent different size classes (100-275 mm, 276-350 mm, 351-425 mm, 426-500 mm, and > 500 mm). Approximately 2-6 cm^2 of muscle tissue was sampled behind the gill plate and above the lateral line, placed in a Nasco bag, and frozen at -20 °C. The muscle samples were freeze-dried in a Labconco freeze dryer for two days and pulverized with a mortar and pestle. A subsample of each muscle sample was lipid extracted by breaking up the cells in a sonicator bath, then washing the samples with a 2:1 ratio of chloroform and methanol on three consecutive days (Bligh and Dyer 1959). One sample from 2000 was not lipid extracted due to insufficient tissue quantity. Four hundred µg (± 15 µg) of tissue from each sample were weighed into 3.5 x 5 mm tin capsules and run through a continuous-flow isotope-ratio mass spectrometer (IRMS) equipped with a Costech 4010 elemental analyzer to construct ^{13}C and ^{15}N profiles. Untreated tissue was run for ^{15}N profiles, and lipid-extracted tissue was run for ^{13}C profiles (Logan *et al.* 2008). Isotope δ values are expressed as per mil (‰) deviation from international reference materials (δ^{13}C: Vienna Pee Dee Belemnite (VPDB); δ^{15}N: atmospheric N_2) as represented by the equation $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where X is ^{13}C or ^{15}N, R_{sample} is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample, and R_{standard} is the ratio in the standard. Raw IRMS data was normalized to this scale using USGS40 (δ^{15}N = -4.52 ‰, δ13C = -26.39 ‰) and USGS41a (δ^{15}N = +47.55 ‰, δ13C = +36.55 ‰) standards.

2.5 Age Determination

Sagittal otoliths were extracted with a dorsal transverse incision at the base of the skull (Secor *et al.* 1992). One otolith from each fish was retained for aging, and otolith nuclei and sulci were marked with a permanent marker. Otoliths were placed sulcus-side up in numbered ice cube trays and immersed in quick-cure epoxy resin. The embedded otoliths were removed the next day and marked for sectioning. Otoliths were bisected along their long-axis with a Buehler IsoMet low speed saw fitted with two 3mm diamond wafering blades and a 0.5mm plastic spacer. Each thin section was placed in a petri dish filled with water and viewed under a dissecting microscope using transmitted light. An age was assigned to each fish by counting the number of annuli in its otolith on three non-consecutive days. Annual increments were defined as the beginning of a translucent zone (representing fast growth in late spring through early fall) to the completion of the subsequent opaque zone (representing slow growth in late fall through winter) (Stewart 2005). Otoliths with incomplete annual increments were assigned an age to the last annulus for analysis, so a fish aged 1+ would be recorded as age 1. Otoliths with conflicting readings were viewed by a more experienced reader to determine a final age. Ten additional otoliths per sample year were verified by the experienced reader. Age-length key plots for both sample years can be found in the Appendix.

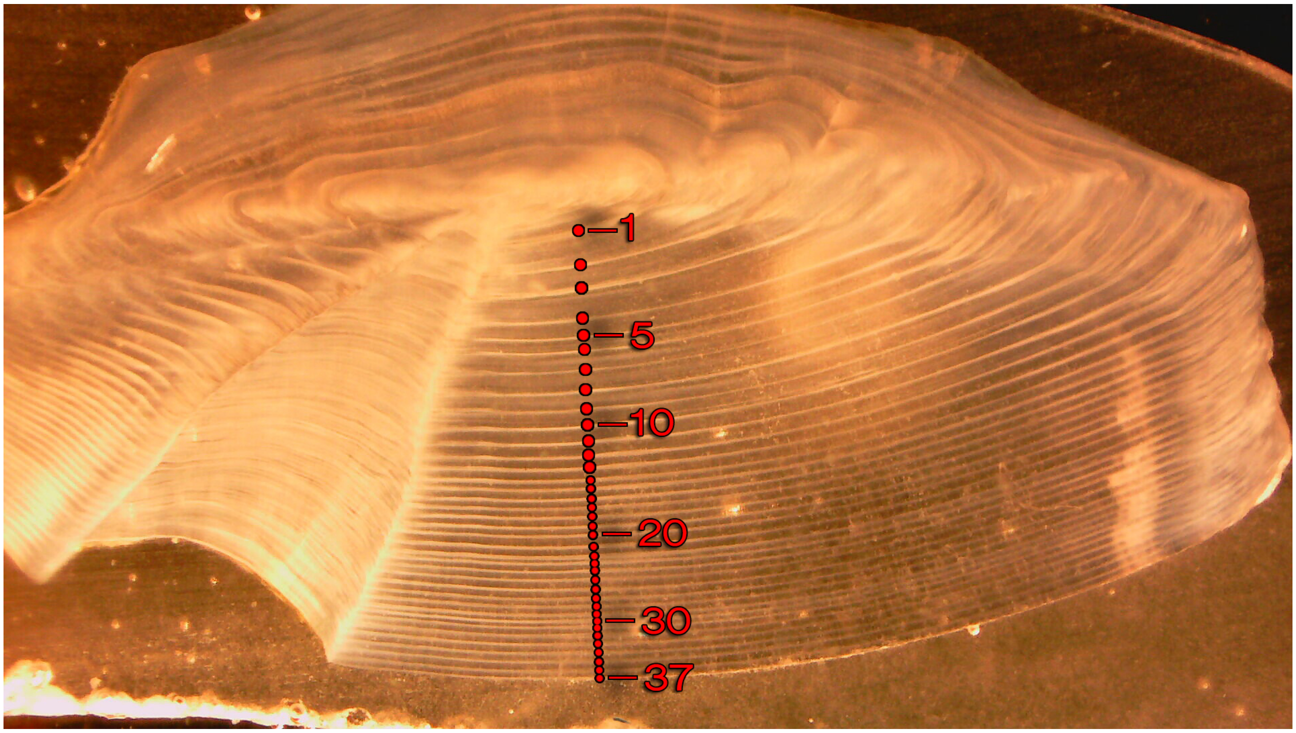

Figure 2. Otolith section of a 37-year-old freshwater drum measuring 460 mm and 1200g. The fish was collected from a gillnet near Gimli on July 21, 2000.

2.6 Data Analysis

Diet indices for the 2000 and 2019 populations were summarized in figures and tables and visually compared to detect possible dietary shift. Prey-specific abundance was plotted against prey frequency of occurrence to evaluate feeding strategy and niche width (Amundsen *et al.* 1996). Samples from 2000 were also grouped by month to identify temporal diet variation, and 2019 digesta were compared by sample site to test for site-specificity. Count and dry weight were both considered while evaluating relative prey importance, as count did not account for prey size and dry weight was heavily biased towards prey with chitinous or calcified structures.

Raw stable isotope data were analyzed with R (R Core Team 2020). Because of the small 2000 sample set (n = 10), a two-tailed Mann-Whitney-Wilcoxon (MWW) test was used to compare the mean rank of 2000 and 2019 δ^{13}C and δ^{15}N values. The Wilcoxon statistic W was calculated with the equation $W = \Sigma\ R_i - (n_i\ (n_i + 1))/2$, where i is the sample year, Σ R is the rank sum of i, and n is the number of values in i; the lowest W of the two sample years was used in the MWW test. The Kruskal-Wallis test was used to test for differences in the δ^{13}C and δ^{15}N values between of the 2019 sample sites (Dinno 2015). The test statistic H is generated with $H = (n - 1)$ $(\Sigma\ n_i (\bar{r}_I - \bar{r})^2 / \Sigma\Sigma\ n_i (\bar{r}_{Ij} - \bar{r}_i)^2)$, where $\Sigma\ n_i (\bar{r}_I - \bar{r})^2$ is the sum of between-sample mean ranks square and $\Sigma\Sigma\ n_i (\bar{r}_{Ij} - \bar{r}_i)^2$ is the sum of within-sample mean ranks square of each sample site. If the Kruskal-Wallis test was significant, a Dunn's test was performed to make pairwise comparisons of the sample sites (Dinno 2015). Dunn's z-test statistic was given by $z_i = y_i / \sigma_i$, where i is one of the pairwise comparisons, y_i is the difference in mean ranks of the sample sites tested, and σ_i is the standard deviation of y_i. To account for familywise type I error, the significance level was reduced with a Bonferroni correction (α / number of comparisons) and expressed inversely as the increase of each p-value (p * number of comparisons).

The mean trophic position of freshwater drum was estimated using $TP_{fish} = 2 + [(\delta^{15}N_{fish} - \delta^{15}N_{zooplankton})/3.4]$, which is the estimated trophic position of zooplankton (2), plus a baseline-corrected $\delta^{15}N$ fish profile, divided by a standardized $\delta^{15}N$ ‰ increase per trophic level (Post 2002; Chipps and Garvey 2007). As zooplankton samples were not collected during this study, a mean zooplankton $\delta^{15}N$ value of 9.6‰ (±2.8‰) was obtained from Ofukany *et al.* (2014). Raw $\delta^{13}C$ and $\delta^{15}N$ data were also referenced against $\delta^{13}C$ and $\delta^{15}N$ profiles of different Lake Winnipeg fish (Ofukany *et al.* 2014) to estimate of trophic position and feeding strategies. Trophic positions were presented as the mean value ± SD.

Age, length, and weight data were analyzed with R (R Core Team 2020) and the *Fisheries Stock Analysis* (FSA) package (Ogle *et al.* 2020). Sex selection bias in the 2019 sample set was tested using a binomial probability distribution. Length-at-age and weight-at-length relationships were tested between sample years (2000, 2019) and sex (2019 only) by conducting analyses of variance (ANOVA) on multiple linear regressions, with sample year or sex as a dummy variable. Age, length, and weight were log(10)-transformed for the regression model to linearize length-at-age and weight-at-length relationships. The test statistic F is calculated with the equation $F = MS_R/MS_E$, where MS_R is the regression mean square and MS_E is the error mean square.

RESULTS

3.1 Diet Composition

In total, 54 digestive tracts from 2000 (85.4%) and 37 digestive tracts from 2019 (73.5%) contained prey items; all 10 digestive tracts from 2019 winter freshwater drum were empty. Only digestive tracts with contents were used for diet analysis. Freshwater drum collected in 2000 fed predominantly on caddisfly larvae and mayfly nymphs (Table 1). Over 80% of 2000 freshwater drum consumed caddisfly larva, which comprised 79% of all food items, and 72% of freshwater drum consumed mayfly nymphs. Chironomid larvae were the third-most common food item but were only found in 26% of freshwater drum and made up only 1.5% of the total prey count. Bivalves—all from the family Sphaeriidae—were consumed by 11% of freshwater drum and were not a primary prey item in any of the digestive tracts. The smallest freshwater drum to consume bivalves was 100 mm, and the largest bivalve consumed was less than 0.5 cm long.

Table 1. Count and frequency occurrence of food items in the digestive tracts of 54 freshwater drum collected in 2000. Freshwater drum were sampled from the south basin of Lake Winnipeg from June through August.

	Count		Frequency of Occurrence	
Food items	Total	%	Number	%F
Trichoptera Larva	**1657**	**78.94**	**44**	**81.48**
Ephemeroptera Nymph	355	16.91	39	72.22
Ephemeroptera Adult	1	0.05	1	1.85
Chironomidae Larva	31	1.48	14	25.93
Hemiptera	3	0.14	3	5.56
Decapoda	4	0.19	2	3.70
Sphaeriida	23	1.10	6	11.11
Odonata Nymph	2	0.09	2	3.70
Ostracoda	18	0.86	1	1.85
Culicidae Larva	4	0.19	2	3.70
Ceratopogonidae Larva	1	0.05	1	1.85

Freshwater drum collected in 2019 primarily fed on chironomid larvae and mayfly nymphs (Table 2). Chironomids were present in 62% of freshwater drum digestive tracts, but comprised 82% of all food items. About half of the freshwater drum consumed mayfly larvae, which accounted for 13% of prey. No other identified taxa where found frequently or in large numbers in the grouped 2019 samples. Zebra mussels were only present in the digestive tracts of two freshwater drum caught in the Red River and two freshwater drum caught at Hillside Beach; 90% of the mussels were found in a single individual from Hillside Beach. The smallest fish that consumed zebra mussels was 433 mm. Crayfish accounted for the largest proportion of dry weight, followed by fish, chironomids, and mayflies. The smallest freshwater drum to consume crayfish and fish was 352 mm and 396 mm, respectively.

Table 2. Count, dry weight, and frequency of occurrence of food items in the digestive tracts of 37 freshwater drum collected in August of 2019. Freshwater drum were sampled from the Red River and three sites in the south basin of Lake Winnipeg.

	Number		Dry Weight		Frequency of Occurrence	
Food items	Total	%	g	%	Number	%F
Trichoptera Larva	8	0.33	0.0268	0.20	3	8.11
Ephemeroptera Nymph	311	12.86	1.6107	11.95	18	48.65
Chironomidae Larva	**1977**	**81.73**	2.3371	17.35	**23**	**62.16**
Hemiptera	34	1.41	0.0289	0.21	3	8.11
Amphipoda	1	0.04	0.0018	0.01	1	2.70
Hirudinea	4	0.17	0.1242	0.92	2	5.41
Decapoda	16	0.66	**4.6878**	**34.79**	8	21.62
Sphaeriida	1	0.04	0.0031	0.02	1	2.70
Gastropoda	2	0.08	0.0029	0.02	2	5.41
Fish	~16	0.66	2.5132	18.65	8	21.62
Plant Material[1]	N/A	N/A	1.5459	11.47	3	8.11
D. polymorpha	~49	2.03	0.5908	4.39	4	10.81

[1] Plant material could not be traced to individual plants, so counts were not possible.

Plots of prey-specific abundance against occurrence present both 2000 and 2019 freshwater drum as dietary specialists, with most freshwater drum from 2000 feeding heavily on caddisfly larvae and mayfly nymphs (Figure 3) and freshwater drum from 2019 relying on either mayflies or chironomids (Figure 4). In both years, each non-dominant prey item was found in less than 30% of digestive tracts. Although crayfish and ostracods were primary prey when present in 2000 freshwater drum digesta, they were rarely consumed by sampled freshwater drum (Figure 3).

The relative balance of chironomid and mayfly frequencies in 2019 suggests alternate prey selection within the freshwater drum population. Chironomids and mayflies comprised over half the prey items in the digestive tracts in which they were found, but were only found in 62.2% and 48.7% of digestive tracts, respectively (Figure 4). This may be evidence of alternative feeding strategies within the population, or that site-specific autocorrelation is occurring on account of prey availability.

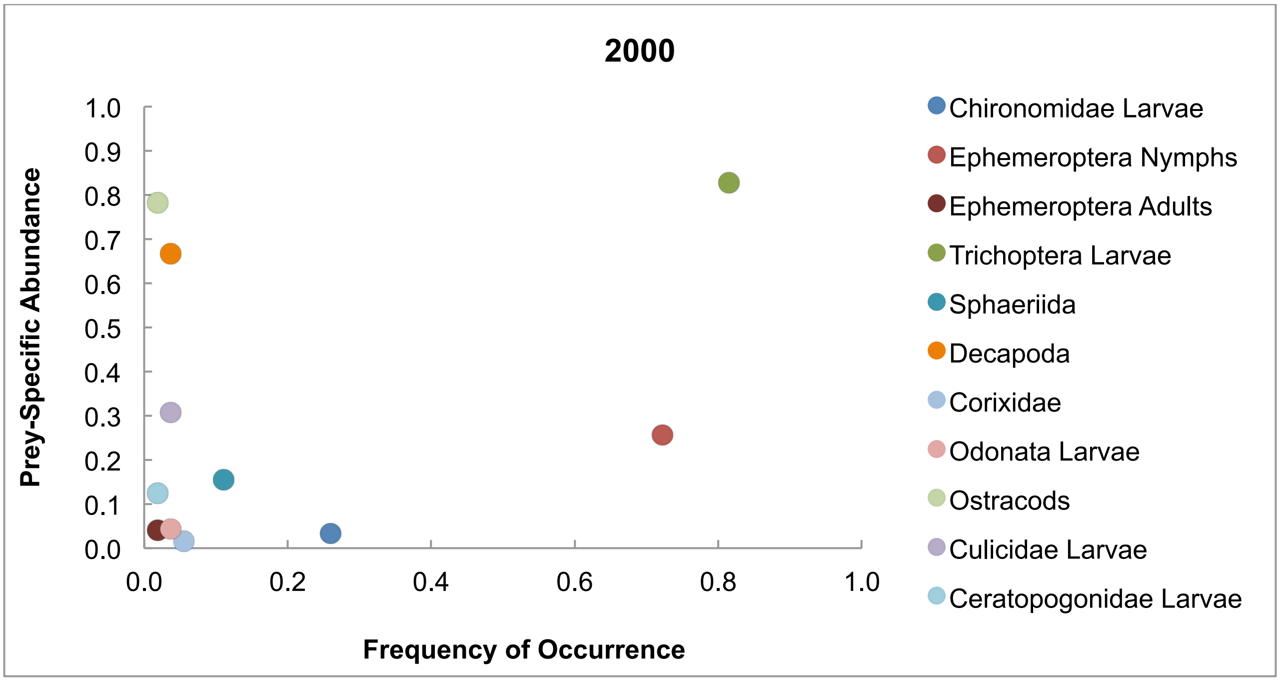

Figure 3. Prey-specific abundance (P_i; by count) plotted against frequency of occurrence (% F) of prey items found in the digestive tracts of freshwater drum (n = 54) collected in June through August of 2000.

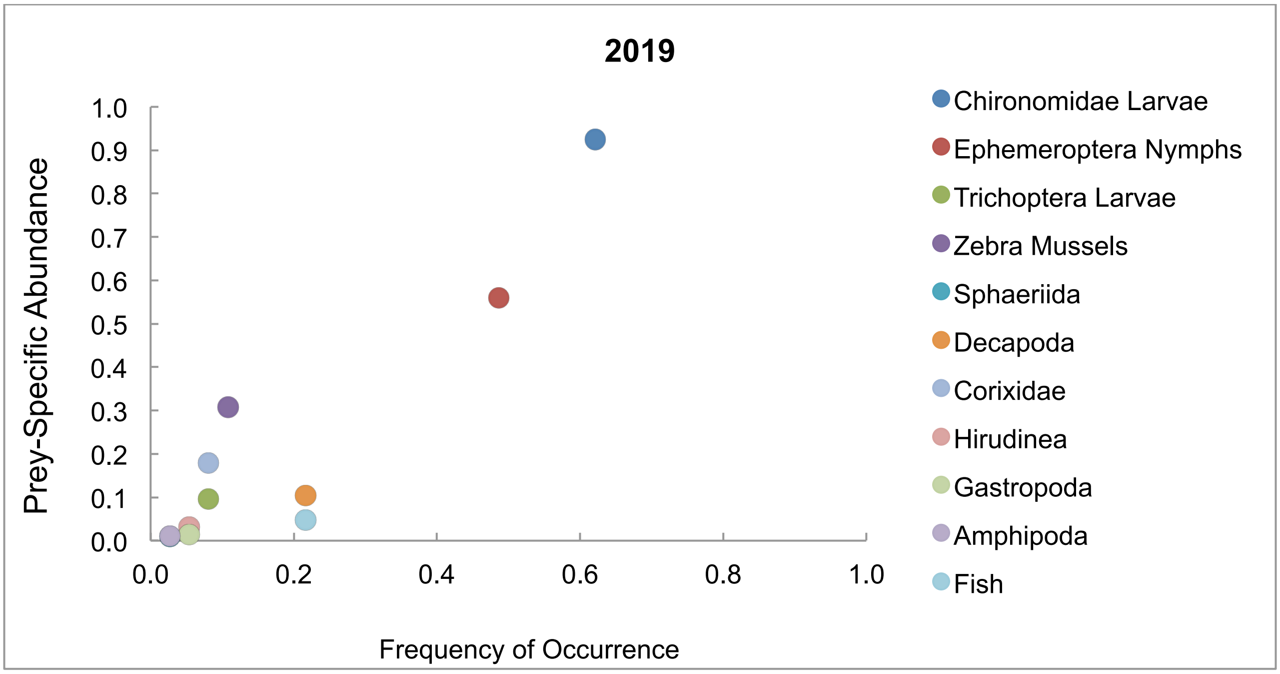

Figure 4. Prey-specific abundance (P_i; by count) plotted against frequency of occurrence (% F) of prey items found in the digestive tracts of freshwater drum (n = 37) collected in August of 2019.

Diet indices were also evaluated by month (2000) and sample site (2019) to test temporal and spatial autocorrelation. Dietary trends in 2000 were largely consistent across sample months (Table 3). Caddisflies were the dominant prey, and taxa other than caddisflies or mayflies comprised less than 10% of the cumulative count in each month. Mayflies were the second-most important prey item in July and August but unimportant in June.

Table 3. Percent count and frequency of occurrence of freshwater drum digestive contents collected from the south basin of Lake Winnipeg in 2000. Freshwater drum were sampled by gillnet in June (n = 4), July (n = 27), and August (n = 23).

	June		July		August	
Food items	% Count	%F	% Count	%F	% Count	%F
Trichoptera Larva	**95.74**	**100.00**	**77.89**	**77.78**	**73.51**	**82.61**
Ephemeroptera Nymph	0.66	25.00	19.54	70.37	19.9	**82.61**
Ephemeroptera Adult	---	---	---	---	0.14	4.35
Chironomidae Larva	3.28	75.00	1.42	22.22	0.81	21.74
Hemiptera	---	---	0.09	3.70	0.27	8.70
Decapoda	---	---	0.38	7.41	---	---
Sphaeriida	0.33	25.00	0.19	3.70	2.70	17.39
Odonata Nymph	---	---	0.09	3.70	0.14	4.35
Ostracoda	---	---	---	---	2.43	4.35
Culicidae Larva	---	---	0.28	3.70	0.14	4.35
Ceratopogonidae Larva	---	---	0.09	3.70	---	---

There was distinct site-specific prey selection in the 2019 samples. Chironomids were the dominant prey item by count in the Red River and Hillside Beach sites, while mayflies were the primary prey item at the Winnipeg Beach and Gull Harbour sites (Table 4). Prey counts were significantly lower in Red River fish as a large proportion of the digestive tracts were filled with large crayfish (79.6% dry weight, 62.5% occurrence). Similarly, fish only accounted for 17.2% of prey items in Winnipeg beach, but were found in all 4 fish and made up 74.5% of dry weight. Freshwater drum feeding habits from the 2019 sample can be best characterized by sample site: Red River freshwater drum preyed on crayfish and various insects, Hillside Beach freshwater drum fed predominantly on chironomid larvae, Winnipeg Beach freshwater drum fed on fish and mayflies, and Gull Harbour freshwater drum consumed mostly mayflies.

Table 4. Count and dry weight percentages and frequency of occurrence of food items in the digestive tracts of freshwater drum collected in the Red River (n = 8), Hillside Beach (n = 15), Winnipeg Beach (n = 4), and Gull Harbour (n = 10). All sites were sampled in August of 2019.

	Red River			Hillside Beach			Winnipeg Beach			Gull Harbour		
Food items	% Count	% Dry Weight	%F	% Count	% Dry Weight	%F	% Count	% Dry Weight	%F	% Count	% Dry Weight	%F
Trichoptera L.	3.40	0.30	25.00	---	---	---	5.17	0.40	25.00	---	---	---
Ephemeroptera N.	23.13	6.27	50.00	0.20	0.16	20.00	**67.24**	24.84	50.00	**92.13**	**69.80**	**90.00**
Chironomidae L.	**40.14**	0.21	25.00	**96.63**	28.37	**100.00**	6.90	0.08	25.00	7.87	30.20	20.00
Hemiptera	22.45	0.51	25.00	0.05	0.02	13.33	---	---	---	---	---	---
Amphipoda	---	---	---	0.05	0.02	6.66	---	---	---	---	---	---
Hirudinea	---	---	---	0.20	1.71	13.33	---	---	---	---	---	---
Decapoda	6.80	**79.61**	**62.50**	0.26	6.07	13.33	1.72	0.09	25.00	---	---	---
Sphaeriida	---	---	---	0.05	0.04	6.66	---	---	---	---	---	---
Gastropoda	---	---	---	0.05	0.02	6.66	1.72	0.05	25.00	---	---	---
Fish	0.68	3.04	12.50	0.26	4.95	20.00	17.24	**74.53**	**100.00**	---	---	---
Plant Material	---	---	---	N/A	21.25	13.33	---	---	---	---	---	---
D. polymorpha	3.40	10.06	25.00	2.25	**37.39**	13.33	---	---	---	---	---	---

3.2 Trophic Position

Isotope medians and distributions of 2000 and 2019 freshwater drum tissue were nearly identical (Figure 5, 6), which suggests that the trophic position and feeding strategy of Lake Winnipeg freshwater drum has not changed. The mean ranks of $\delta^{13}C$ values did not significantly differ between the 2000 samples (median -27.41‰) and 2019 samples (median -27.08‰) (Mann-Whitney-Wilcoxon test: $W = 65$, $p = 0.15$). The mean ranks of $\delta^{15}N$ values also did not significantly differ between 2000 (median 13.70‰) and 2019 (median 13.90‰) (Mann-Whitney-Wilcoxon test: $W = 106$, $p = 0.88$).

$\delta^{13}C$ values were fairly consistent between 2019 sample sites, but there is evidence of site-specificity for $\delta^{15}N$ values (Figure 6). The mean ranks of $\delta^{13}C$ values did not significantly differ between fish muscle sampled from the Red River (median -27.06‰), Hillside Beach (median -25.69‰), Winnipeg Beach (mean -27.08‰), Gull Harbour (median -27.12‰), and in Victoria Beach winter fish (median -27.47‰) (Kruskal-Wallis test, $H = 2.54$, $p = 0.64$, $df = 4$). By contrast, there was a significant difference in $\delta^{15}N$ mean ranks between two or more of the Red River (median 15.70‰), Hillside Beach (median 12.63‰), Winnipeg Beach (mean 17.23‰), Gull Harbour (median 13.26‰) and winter fish (median 13.43‰) sample sites (Kruskal-Wallis test, $H = 13.478$, $p = 0.0092$, $df = 4$). The Dunn's test with Bonferroni correction did not identify any statistically significant differences in pairwise comparisons, but comparisons with Red River and Winnipeg Beach yielded low to moderate probabilities ($0.07 < p < 0.42$) except when compared to each other. See the Appendix for all adjusted and unadjusted Dunn's test results.

Interestingly, tissue from three of the four freshwater drum that had recently consumed zebra mussels yielded the highest $\delta^{13}C$ values from their sampling location (Red River: -27.28‰,

-26.08‰; Hillside Beach: -24.70‰, -23.67‰), and all freshwater drum had the highest $\delta^{15}N$ values among their respective sites (Red River: 17.09‰, 17.54‰; Hillside Beach: 13.61‰, 14.16‰).

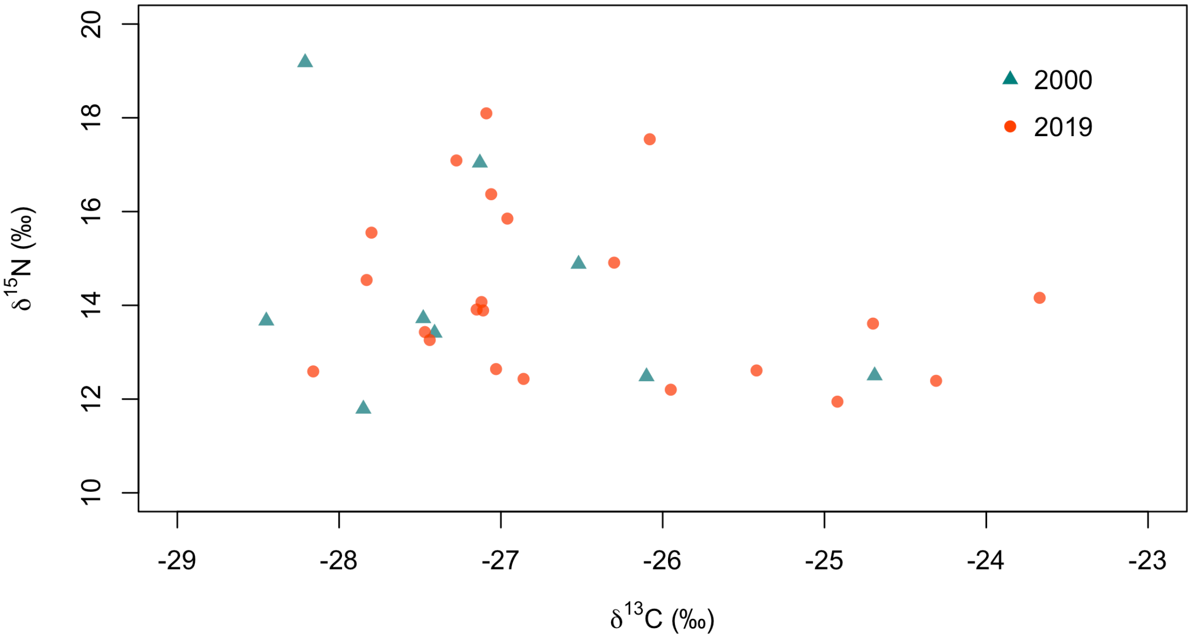

Figure 5. $\delta^{13}C$ and $\delta^{15}N$ values (‰) of freshwater drum muscle sampled from Lake Winnipeg in 2000 (n = 9) and 2019 (n = 22). Values are expressed as per mil (‰) deviation from international reference materials ($\delta^{13}C$: Vienna Pee Dee Belemnite (VPDB); $\delta^{15}N$: atmospheric N_2).

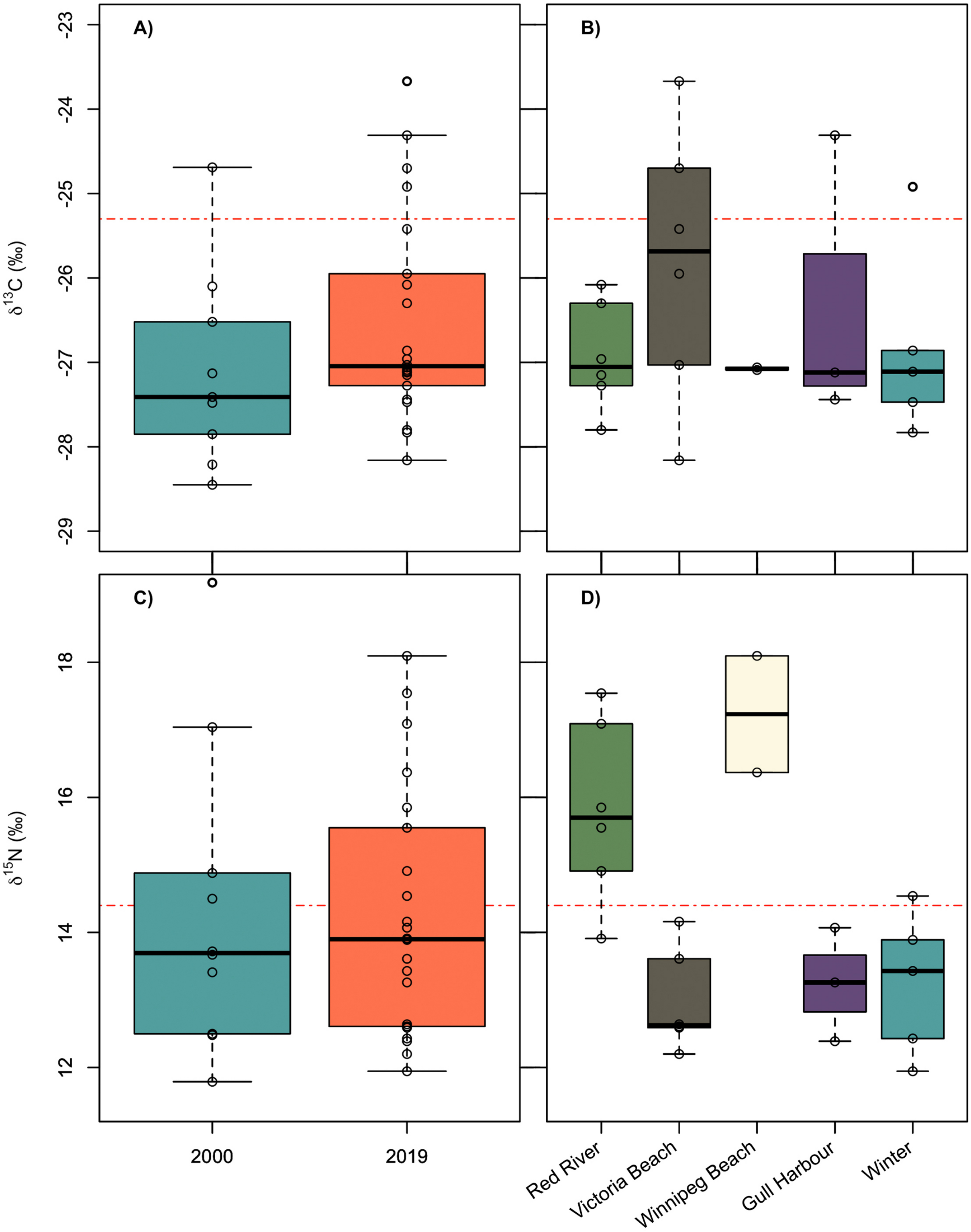

Figure 6. Boxplots of $\delta^{13}C$ and $\delta^{15}N$ data of freshwater drum muscle sampled from Lake Winnipeg in 2000 (A, C; n = 10, 9) 2019 (A, C; n = 22) and divided by 2019 sample site (B, D; from left to right: n = 6; 6; 2; 3; and 5). The red points indicate freshwater drum that had zebra mussels in their digestive tract and the red lines represent the mean isotope values of Lake Winnipeg drum tissue sampled by Ofukany *et al.* in 2014 (n = 64 for $\delta^{13}C$, n = 60 for $\delta^{15}N$).

Trophic position calculations revealed that freshwater drum from both the 2000 and 2019 samples were a combination of secondary and tertiary consumers. The mean trophic position of freshwater drum was nearly identical between years, with 2000 fish sitting at 3.39 (± 0.67) and 2019 fish occupying a trophic position of 3.36 (± 0.54). The estimated trophic position of freshwater drum varied by 2019 sample site and was directly correlated with the $\delta^{15}N$ values of fish from each site. Freshwater drum from Hillside Beach (2.99 ± 0.22, n = 6), Gull Harbour (3.07 ± 0.25, n = 3), and the Victoria Beach winter site (3.07 ± 0.31, n = 5) were secondary consumers, whereas fish from the Red River (3.83 ± 0.40, n = 6) and Winnipeg Beach (4.24 ± 0.36, n = 2) fed mostly at the tertiary level. The freshwater drum that had consumed zebra mussels at the Red River site (4.20, 4.34) and Hillside Beach site (3.18, 3.34) had the highest calculated trophic positions of their respective sites.

3.3 Age and Growth

Freshwater drum collected in 2000 ranged from 1 to 38 years of age, with a median age of 4 years (29 of 60 aged fish). The smallest 2000 freshwater drum (age 1) was 96 mm and 11 g, and the largest was 500 mm and 2000 g. Young freshwater drum were disproportionately represented in the 2000 sample, with 51 of 60 freshwater drum being under 5 years of age. Four sets of otoliths were missing from the 2000 sample set, including those of the largest freshwater drum. Ages of freshwater drum collected in 2019 ranged from 2 to 57 years, with a median age of 8 years (8 of 51 freshwater drum). The smallest 2019 fish (age 2) was 187 mm and 76 g, and the largest (age 57) was 555 mm and 2800 g. Only 4 freshwater drum under the age of 3 were caught, but year classes were otherwise evenly distributed. The oldest female freshwater drum caught was 57, and the oldest male was 56.

Of the 51 2019 fish, 25 were females, 16 were males, and 10 were undetermined due to ambiguous or premature gonads. Assuming an equal chance of catching a male or female fish, there was a 10.6% probability of catching fewer than 16 males and a 5.9% probability of catching more than 25 females. Sex did not significantly affect length-at-age or weight-at-length relationships (Figure 7, 8). An ANOVA of the length-age regression (Adjusted $r^2 = 0.82$, $F_{3,37} = 63.29$, $p < 0.001$) determined that the interaction between $\log_{10}$ age and log sex did not predict the slope of the regression ($F_1 = 2.24$, $p = 0.14$), and sex did not significantly influence the length of a fish at a given age ($F_1 = 0.12$, $p = 0.73$). Likewise, the slope and intercept of the $\log_{10}$ weight and $\log_{10}$ length regression model (Adjusted $r^2 = 0.96$, $F_{3,37} = 288.80$, $p < 0.001$) did not differ between the sexes ($F_1 = 0.00$, $p = 0.77$; $F_1 = 0.00$, $p = 0.92$). These regressions provide a sufficient basis to compare the ungrouped 2019 dataset against the unsexed 2000 dataset for differences in length-at-age and weight-at-length. Sex-specific regression lines for length-at-age and weight-at-length can be seen in Figures 7 and 8.

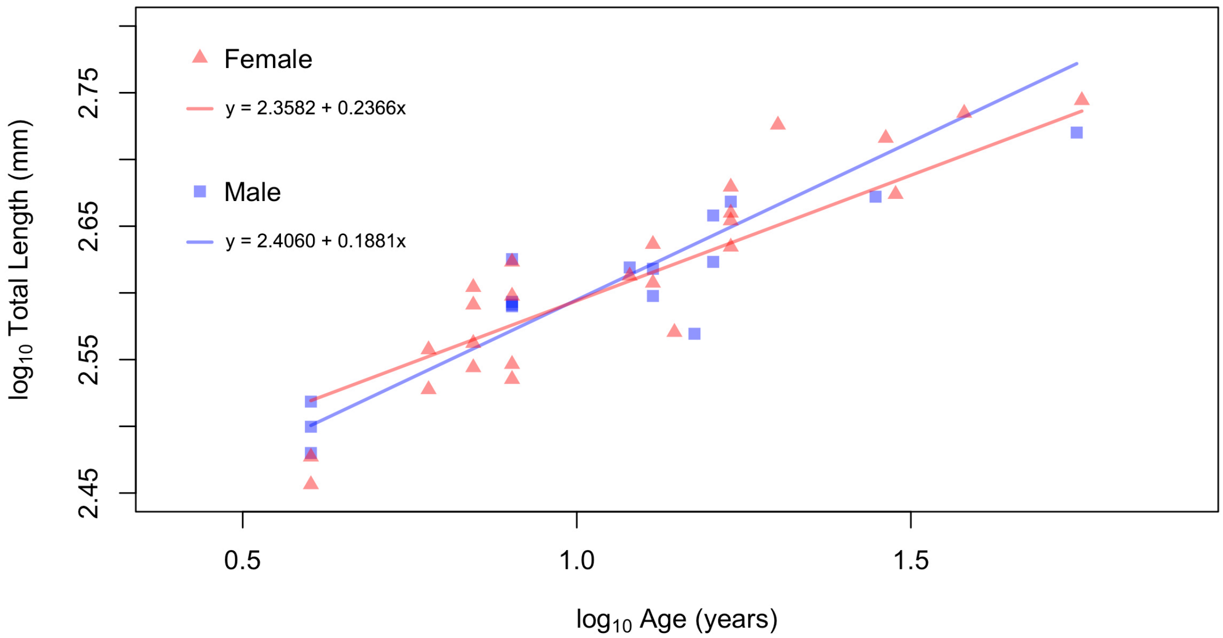

Figure 7. Log_{10} length at $\log_{10}$ age of female ($r^2 = 0.83$, n = 25) and male ($r^2 = 0.81$, n = 16) freshwater drum collected in August of 2019. Freshwater drum were sampled from two sites in the Red River and three sites in the south basin of Lake Winnipeg.

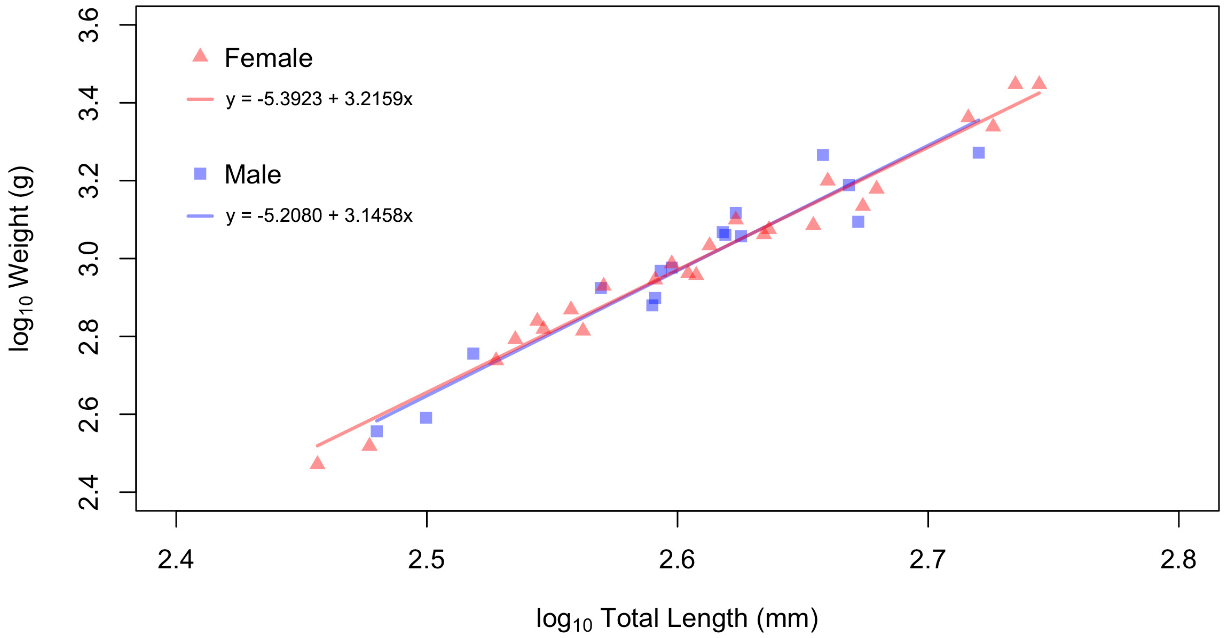

Figure 8. Log_{10} weight at $\log_{10}$ length of female ($r^2 = 0.97$, n = 25) and male ($r^2 = 0.91$, n = 16) freshwater drum collected in August of 2019. Freshwater drum were sampled from two sites in the Red River and three sites in the south basin of Lake Winnipeg.

Freshwater drum sampled in 2019 were longer at a given age than freshwater drum sampled in 2000. An analysis of variance of the length-at-age regression (Adjusted $r^2 = 0.92$, $F_{3,107} = 428.50$, $p < 0.001$) yielded significantly different slopes and intercepts between years ($F_1 = 21.07$, $p < 0.001$; $F_1 = 82.63$, $p < 0.001$). The difference in regression coefficients suggests that the 2000 freshwater drum have a faster growth rate, but the regression lines show that freshwater drum from 2019 were overall larger at a given age than freshwater drum sampled in 2000 (Figure 9).

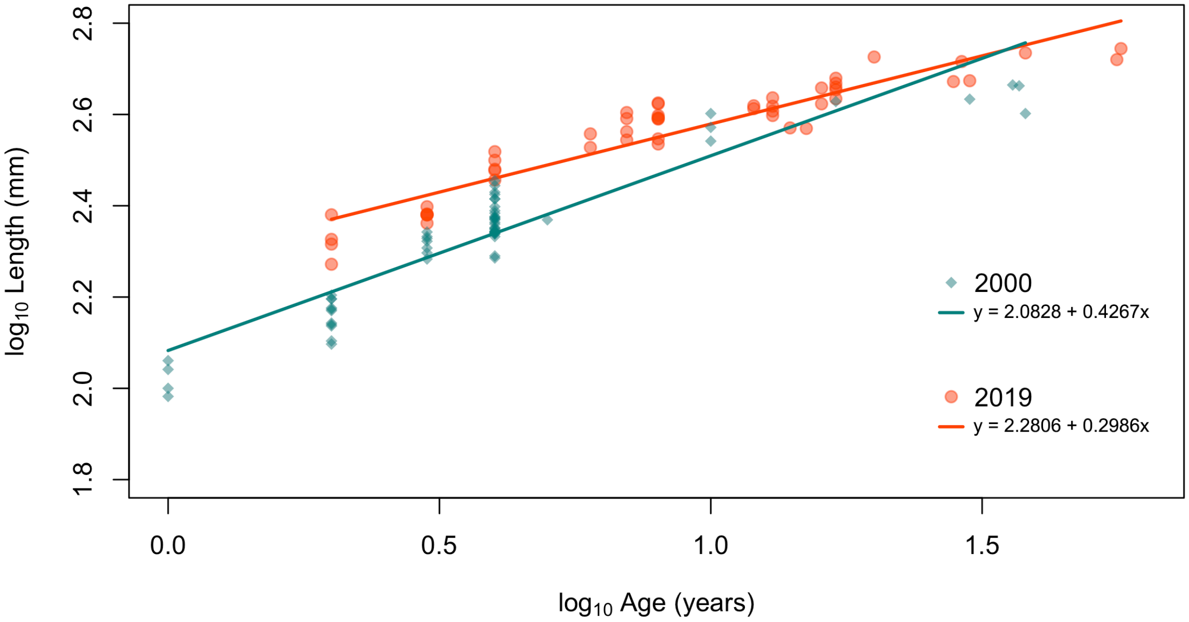

Figure 9. Log_{10} length at $\log_{10}$ age of freshwater drum collected from the south basin of Lake Winnipeg in 2000 ($r^2 = 0.86$, $n = 60$) and 2019 ($r^2 = 0.87$, $n = 51$).

Freshwater drum sampled in 2019 were also heavier at a given length than freshwater drum sampled in 2000. An analysis of variance of the weight-at-length regression (Adjusted r^2 = 0.96, $F_{3,111}$ = 959.90, $p < 0.001$) determined that the 2000 and 2019 samples had the same slope but a different intercept (F_1 = 0.03, p = 0.86; F_1 = 32.24, $p < 0.001$) (Figure 10). The result suggests that the freshwater drum from both population put on weight at the same rate relative to length, but the regression lines show that 2019 freshwater drum were heavier at a given length than 2000 freshwater drum (Figure 10).

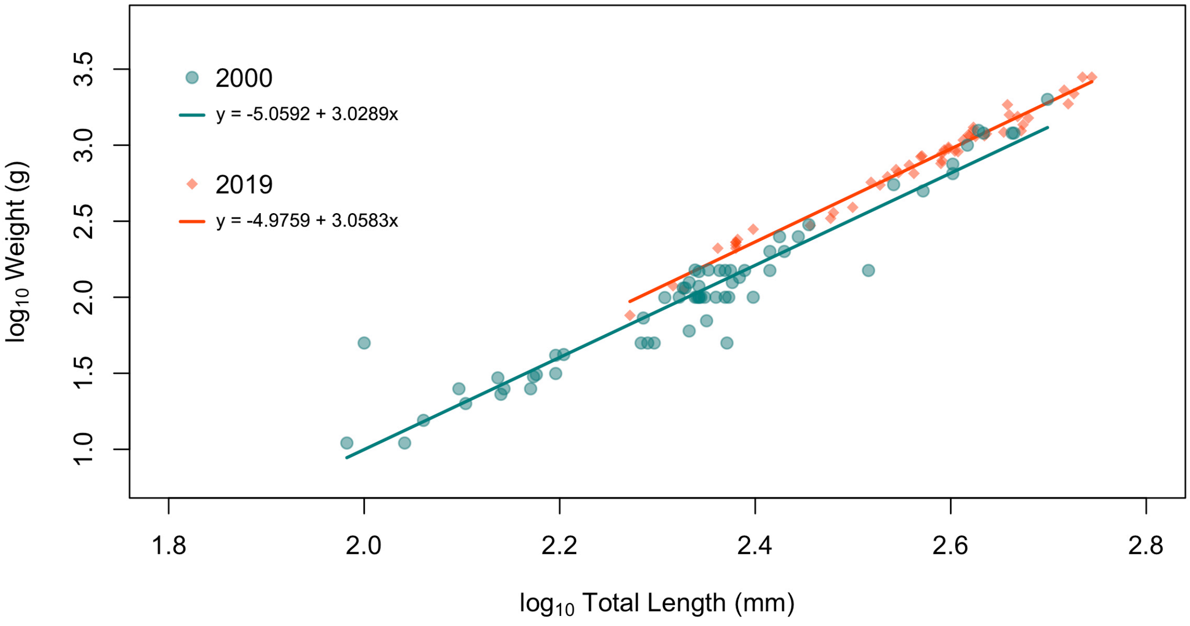

Figure 10. Log_{10} weight at $\log_{10}$ length of freshwater drum collected from the south basin of Lake Winnipeg in 2000 (r^2 = 0.92, n = 64) and 2019 (r^2 = 0.98, n = 51).

DISCUSSION

4.1 Diet

As of 2019, zebra mussels are not a significant prey item of freshwater drum in the south basin of Lake Winnipeg. Zebra mussels were only found in four digestive tracts (10.8%), and over 80% of these mussels were consumed by a single fish. Given that zebra mussels were present at or near all sample sites (Enders *et al*. 2019), freshwater drum do not appear to be regularly incorporating zebra mussels into their diets in the south basin of Lake Winnipeg. Similarly, the freshwater drum sampled in 2000 rarely consumed mollusks and did not feed on them in high quantities, even though they likely constituted over 20% of the benthic invertebrate community (Hann *et al.* 2017). Due to the low number of large freshwater drum (> 350 mm [Morrison *et al.* 1997], n = 9) sampled in 2000, it is still possible that bivalves were a primary prey of freshwater drum prior to zebra mussel invasion. If freshwater drum rarely consumed mollusks as the data suggests, and the current population is not regularly feeding on zebra mussels, it is unlikely that freshwater drum fed on zebra mussels upon introduction or slowed their spread to any meaningful extent. By contrast, the Lake Erie freshwater drum population fed heavily on zebra mussels within two years of their discovery (French and Bur 1993, Morrison *et al.* 1997). This juxtaposition may be explained by the different rates of zebra mussel spread in Lake Winnipeg and Lake Erie. While zebra mussels reached peak densities in Lake Erie within three years of first detection (Karatayev *et al.* 2011), the zebra mussel population is still expanding in Lake Winnipeg and remains low throughout much of the profundal zone (Enders, unpublished data, 2020). Furthermore, zebra mussels have readily colonized the soft substrates of Lake Erie's main basins (Dermott and Munawar 1993; Berkman *et al.* 1998), whereas mussels were rarely sampled on fine sediments in Lake Winnipeg (Enders *et al*. 2019); This may be due to differences in lake depth, as the central (mean depth 18.3 m) and eastern (mean depth 24.4 m) basins of Lake Erie remain largely undisturbed by wind action (Dermott and Munawar 1993), while much of the substrate in Lake Winnipeg's south basin (mean depth 9.0 m) can be disrupted by wind-induced currents (Wassenaar 2012). Because zebra mussels have not spread as quickly in Lake Winnipeg as in Lake Erie, it is possible that preferred prey items are still in high abundance and easily accessible.

Insect larvae were the dominant prey item of both 2000 and 2019 freshwater drum by count and frequency of occurrence, which is consistent with many past freshwater drum diet studies (Daiber 1952; Moen 1955; Bur 1982; Jacquemin *et al.* 2014). Although larger freshwater drum (> 350 mm) also consumed fish and crayfish, benthic insect larvae remained an integral part of their diets. Caddisfly larvae comprised 79% the prey items consumed by freshwater drum in 2000, and mayfly nymphs made up another 17%. Caddisflies were preyed upon selectively, as they constituted just over 1% of benthic invertebrates in the south basin near the time of sampling (Hann *et al.* 2017). While it is possible that freshwater drum were caught in a location or depth where caddisflies were found in higher densities, it is difficult to verify without knowing the exact location that the freshwater drum were sampled. The high frequency of mayfly consumption in July and August is expected, as this period coincides with mass emergence of Lake Winnipeg mayflies (Neave 1932). Molting nymphs and subimagos (sub-adults) were rarely among freshwater drum digesta, suggesting that freshwater drum did not actively feed on the lake's surface. Only four fish with gut contents were sampled in June, so the dietary importance of mayflies in June cannot be characterized.

Freshwater drum sampled in 2019 fed heavily on chironomid larvae (81.7% count) and to a lesser extent, mayfly nymphs (12.9% count). The dominant prey taxa differed by sample site, with freshwater drum feeding on crayfish and insects in the Red River, chironomid larvae in Hillside Beach, fish and mayflies in Winnipeg Beach, and mayflies in Gull Harbour. This spatial variation may be due to differences in habitat, and consequently prey item composition. For example, the Red River site was muddy and rocky with stretches of submerged lumber, which is optimal crayfish habitat (Bergman and Moore 2003). Past studies on Lake Winnipeg have also highlighted spatial variation in the benthic invertebrate community that roughly parallel the site-specific diets of freshwater drum. One historic survey sampled chironomids in high densities near the Hillside Beach site, though mayflies were also sampled in high densities (Saether 1979; Chang *et al.* 1993). Mayflies are also found in high densities near the Winnipeg Beach and Gull Harbour sites (Chang *et al.* 1993), while chironomids were found in low to moderate densities near Winnipeg Beach (Chang *et al.* 1993) and not sampled near the Gull Harbour site (Saether 1979).

It may be worth noting that freshwater drum sampled in 2019 fed on chironomids over mayflies at a 6:3:1 ratio, which is the exact fraction of chironomid to mayfly densities documented in the south basin by Hann *et al.* (2017). Superficially, the preservation of this ratio suggests that freshwater drum feed on benthic insect larvae indiscriminately, though this interpretation may not hold when taking sampling times and locations into account. The 2019 freshwater drum were collected at nearshore sites, whereas Hann *et al.* (2017) collected benthic samples at many depths throughout the south basin. For the ratio to be significant, sample sites would have to collectively represent the south basin. Adding to the uncertainty are the diets of freshwater drum sampled in 2000, which appeared to selectively feed on the less abundant caddisfly. The 6.3:1 ratio may therefore be coincidental, through the strong correlation between chironomid and mayfly densities in the benthic community and in the 2019 freshwater drum diets merits further consideration.

4.2 Trophic Position

The consistency of stable isotope values between the 2000 and 2019 samples suggests that the feeding habits of Lake Winnipeg freshwater drum have remained unchanged in 19 years, even after zebra mussel introduction. Freshwater drum from both years were found to occupy a trophic position (TP) of about 3.4, which suggests predation on primary consumers (TP of 2) and some predation on secondary consumers (TP of 3). These values are consistent with prey items found in the digestive tracts of freshwater drum: chironomids and mayflies are primary consumers and detritivores (Burlakova *et al.* 2014), and crayfish and baitfish such as emerald shiners are secondary consumers (Bergman and Moore 2003; Ofukany *et al.* 2014). Ofukany *et al.* (2014) also determined that freshwater drum occupied a mean trophic position of 3.4 over a range of depths in the south basin, which suggests that the 2000 and 2019 are accurate representations of freshwater drum diets in the south basin of Lake Winnipeg. Although it was suboptimal to derive a δ^{15}N baseline from literature for trophic position calculations, Ofukany's zooplankton values were specific to the south basin of Lake Winnipeg in the summer of 2010 (Ofukany 2012). Therefore, any changes in south basin ^{15}N concentrations between 2000 and 2019 were likely averaged through Ofukany's mean δ^{15}N baseline value.

Though not statistically significant with a Bonferroni-corrected Dunn's test, δ^{15}N values (and therefore, trophic position), exhibited site-specificity. The spatial variation of these values were remarkably consistent with the spatial variation in digestive tract contents. Hillside Beach and Gull Harbour freshwater drum fed mostly on chironomids and mayflies, and also had the lowest $\delta^{15}N$ values of the 2019 fish. Freshwater drum sampled in the Red River and Winnipeg Beach sites ate crayfish and fish, respectively, and their tissue had the highest $\delta^{15}N$ values. This result is surprising, as it can take several months for stable isotope concentrations in muscle to quantifiably shift in response to dietary change (Thomas and Crowther 2015; Vander Zanden *et al.* 2015; Busst and Britton 2018). Therefore, the site-specific gut contents of freshwater drum are a direct reflection of long-term, site-specific feeding habits. The correlation between short-term and long-term dietary trends has several possible interpretations. If freshwater drum are exhibiting site affiliation, it may be due to the presence of preferred prey, which would suggest different feeding strategies within the freshwater drum population. Alternatively, the results may reflect opportunist feeding behaviour in freshwater drum, with non-motility being a product of high freshwater drum densities or uniform fish distribution. This proposition is supported by the diet data, which shows that benthic insects remained a primary prey of freshwater drum at all sample sites and size classes. Telemetry data from Fisheries and Oceans Canada's fish tagging study on Lake Winnipeg also shows that freshwater drum are widely distributed throughout the south basin and are not constrained to certain depths or substrates (DFO, unpublished data, 2018-2019). At present, there are not enough published data to validate either theory.

$\delta^{13}C$ values did not significantly differ between the 2000 and 2019 freshwater drum samples, which suggests that freshwater drum fed in the same lake zone (Post 2002). However, the $\delta^{13}C$ values of freshwater drum tissue were significantly lower than the $\delta^{13}C$ values of freshwater drum sampled by Ofukany *et al*. (2014). Freshwater drum sampled by Ofukany (2014) had the second-highest $\delta^{13}C$ values within the fish community isoscape, which is consistent with littoral or benthic feeding strategies (Peterson and Fry 1987; Post 2002). By contrast, the lower $\delta^{13}C$ values of 2000 and 2019 freshwater drum suggested pelagic feeding habits when fitted to the same isoscape. This result is counterintuitive, as Ofukany caught freshwater drum at a range of depths, and the fish from this study were sampled exclusively from the littoral zone. Given that the $\delta^{13}C$ values from 2000 and 2019 were nearly identical, it seems more likely that differences between these values and Ofukany *et al.*'s values were due to differences in the calibration reference materials and software used to normalize the data to international standards (Ofukany *et al.* 2014).

4.3 Growth

The $\log_{10}$ length-age multiple linear regression results show that that freshwater drum sampled in 2019 were longer at a given age than freshwater drum sampled in 2000, especially in the first few years of life. These results indicate that the current growth rate of freshwater drum exceeds the growth rate of freshwater drum from 2000. This change in growth rate may be attributed to an increase in preferred prey availability: benthic macroinvertebrate densities have grown steadily in Lake Winnipeg since the 1920s due to elevated nutrient inputs (Hann *et al.* 2017). This explanation is also consistent with the proportional increase in body weight of 2019 freshwater drum from 2000, as greater weight at a given length is generally associated with increased fish condition and therefore prey availability (Jones *et al*. 1999).

However, the result of the length-at-age regression is confounded by potential differences in sex distribution and by temporal variation of the 2000 data. While the ratio of female to male fish sampled in 2019 was not outside the realm of chance ($p > 0.05$), female freshwater drum made up a disproportionate number of fish in the 2019 sample set. Several papers have described sexual dimorphism within freshwater drum populations, with females being slightly larger than males (Bur 1984; Rypel 2007). Although the sex-specific length-at-age regressions did not significantly differ, the regression coefficient representing $\log_{10}$ growth was greater in female fish than male fish (Figure 6). Thus, it is possible that sexual dimorphism of freshwater drum exists in Lake Winnipeg but that the 2019 sample size was too small to detect it. If sexual dimorphism does exist in the Lake Winnipeg freshwater drum population and the 2000 fish were equally represented or male-dominant, then the differences in the 2000 and 2019 regressions may be exaggerated. Length-at-age values may also be incomparable due to differences in collection date. Fish species in Manitoba generally grow fastest from June through September (Rick Wastle, personal communication September 20, 2019), and Edsall (1967) determined that freshwater drum in Lake Erie grow fastest in August and continue to grow into October. Because freshwater drum were grouped by annuli count and fish from both samples were collected over a range of time, the relative length-at-age of fish would be higher at the end of sampling than at the beginning. As 58% of freshwater drum sampled in 2000 were collected earlier in the summer than the first freshwater drum sampled in 2019 (August 1), many of the 2000 fish would not have exhibited the same increase in length as the 2019 fish. This reduced growth period is particularly significant for the 2000 regression, as Lake Winnipeg freshwater drum have the highest growth rates from ages 1-5 (Hardisty 2007). Since the majority of the fish in the 2000 sample set were less than 5 years of age, the intercept of the regression was skewed low, and the slope was skewed high to account for older fish. With these factors taken into account, freshwater drum sampled in 2019 were as long or longer at a given age as 2000 freshwater drum.

4.4 Final Considerations

After six years of zebra mussel presence in Lake Winnipeg, the diet, feeding behaviour, and growth of freshwater drum have gone largely unchanged. This delayed response to zebra mussel invasion differs from other drum populations, in which zebra mussels became a dominant prey item within a few years of introduction (French and Bur 1993; Watzin *et al.* 2008; Davis-Foust 2012). While the results of this study do not directly explain the unresponsiveness of freshwater drum to zebra mussels, they allow for a degree of inference. Drawing from optimal foraging theory, freshwater drum would be expected to forage on prey that offer maximum energy content relative to search and capture effort (Pyke 1984). The diet data revealed that freshwater drum feed heavily on benthic insects such as chironomids and mayflies, which have whole organism energy contents that are as high or higher than the flesh of zebra mussels (Salonen *et al.* 1976; Magoulick and Lewis 2002). Chironomids and mayflies are also found in high densities in Lake Winnipeg (Hann *et al.* 2017), which minimizes search time. Because the most abundant chironomid and mayfly taxa in Lake Winnipeg are soft-bodied and shallow burrowers (Neave 1932; Flannigan 1979), freshwater drum would spend minimal energy capturing and processing them. By contrast, freshwater drum have been observed actively searching for removable 'clumps' of zebra mussels and crushing each clump with their pharyngeal teeth, which would expend considerable energy (Watzin 2008). Therefore, it is likely that benthic insect larvae are a more energy-efficient prey item than zebra mussels. This theory is consistent with the findings of French and Bur (1996), who determined that the growth rates of freshwater drum slowed with zebra mussel consumption in Lake Erie. It is also possible that zebra mussels can become the most energy-efficient prey item by dominating the benthic invertebrate community and obscuring insect-rich substrates (Berkman *et al.* 1998). Currently, these hypotheses are little more than conjecture, though they may serve as a subject for a future literature review.

The presence of zebra mussels in a few digestive tracts suggests that dietary shift may still be occurring, albeit slowly. As the zebra mussel population continues to grow, several scenarios may arise. If zebra mussels dominate the littoral and sub-littoral benthic invertebrate community, freshwater drum may shift to a zebra mussel-dominant diet. In this scenario, zebra mussels would be an expected prey item of fish as small as 250 mm (age 3-4) and the dominant prey item of fish over 350 mm (age 6-8) (French and Bur 1993; Morrison *et al.* 1997). Conversely, freshwater drum may congregate in deeper water in search of more accessible benthic insects, thereby decreasing freshwater drum numbers in the littoral zone and increasing predation pressure on the profundal benthic invertebrate community. As such, the slow growth of the Lake Winnipeg zebra mussel population presents a unique opportunity to monitor for changes in freshwater drum feeding behaviour. If the foraging strategies of Lake Winnipeg freshwater drum continue to be monitored, findings may be used to predict the response of freshwater drum to early zebra mussel invasion in other waterbodies.

CONCLUSIONS

1. Lake Winnipeg freshwater drum feed mostly on benthic insect larvae, but larger freshwater drum will also consume fish and crayfish.

2. The mean $\delta^{13}C$ and $\delta^{15}N$ values of freshwater drum tissue were consistent with an insectivorous diet and remain unchanged after almost 20 years.

3. Growth (length-at-age) and body condition (weight-at-length) of freshwater drum in 2019 were equal to or greater than the growth and body condition of 2000 freshwater drum.

4. As of 2019, zebra mussels are not a significant prey item of freshwater drum.

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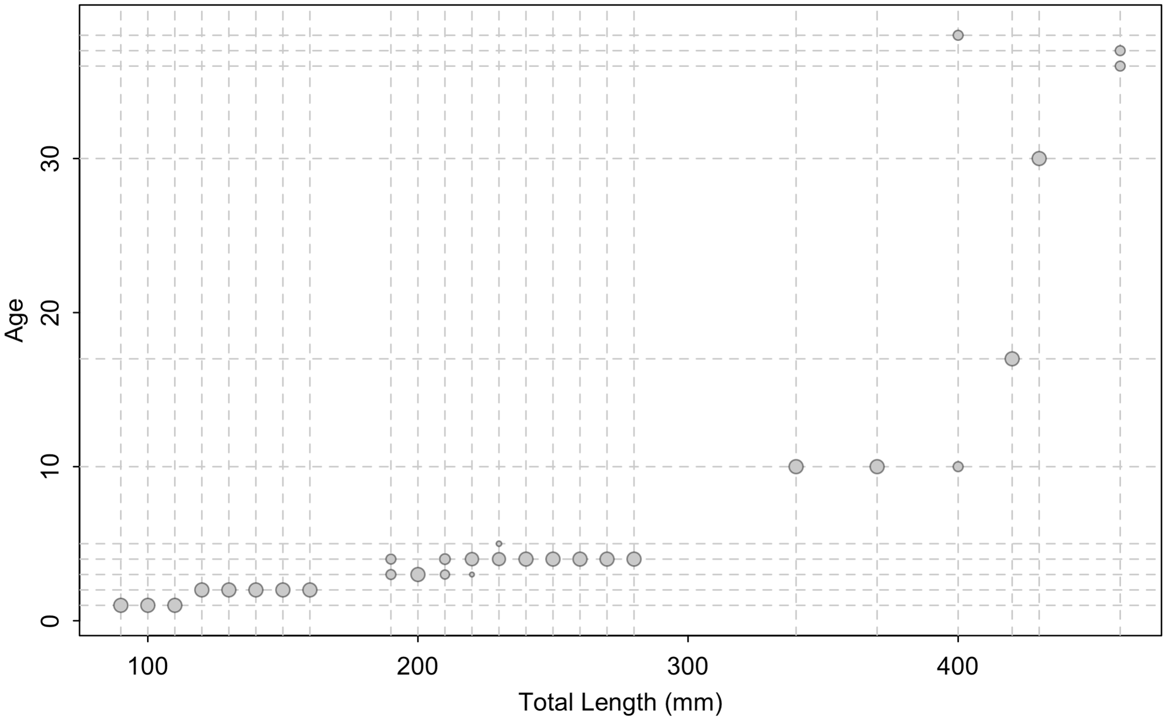
Appendices

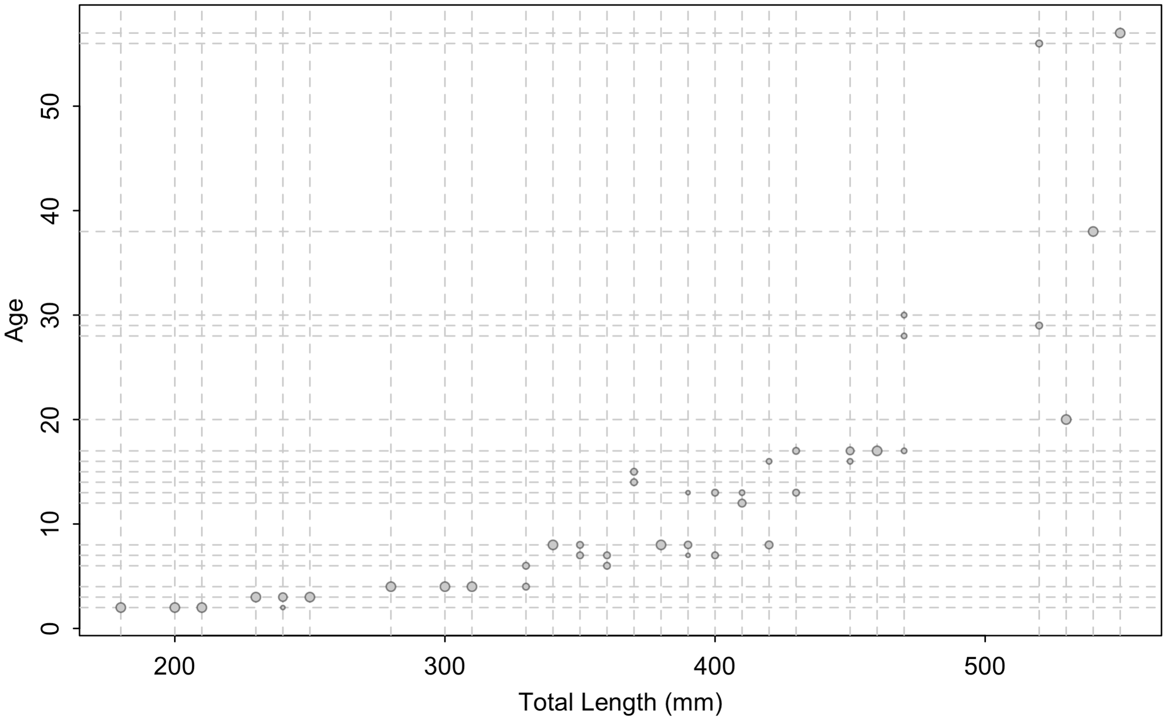
Appendix I: Results of a pairwise Dunn's test comparing $\delta^{15}N$ mean ranks of freshwater drum tissue sampled from Red River (median 15.70 ‰, n = 6), Hillside Beach (median 12.63 ‰, n = 6), Winnipeg Beach (mean 17.23 ‰, n = 2), Gull Harbour (median 13.26 ‰, n = 3) and Victoria Beach ("Winter"; median 13.43 ‰, n = 5) sites.

Comparison	Z	P (unadjusted)	P (Bonferroni adjustment)
Gull Harbour – Red River	-2.0327	0.0421	0.4209
Gull Harbour – Hillside Beach	0.1452	0.8846	1.0000
Red River – Hillside Beach	2.6673	0.0076	0.0765
Gull Harbour – Winter	0.0000	1.0000	1.0000
Red River – Winter	2.3736	0.0176	0.1761
Hillside Beach – Winter	-0.1695	0.8654	1.0000
Gull Harbour – Winnipeg Beach	-2.1087	0.0350	0.3497
Red River – Winnipeg Beach	-0.5973	0.5503	1.0000
Hillside Beach – Winnipeg Beach	-2.4833	0.0130	0.1302
Winter – Winnipeg Beach	-2.3008	0.0214	0.2140

Appendix II: Age-length key plot of freshwater drum collected in 2000 (n = 60). Freshwater drum were sampled from the south basin of Lake Winnipeg from June through August.



Appendix III: Age-length key plot of freshwater drum collected in August of 2019 (n = 51). Freshwater drum were sampled from the Red River and the south basin of Lake Winnipeg.



Appendix IV: Freshwater drum length-age data fitted with a back-transformed best-fit curve. Fish were collected from the south basin of Lake Winnipeg in 2000 (n = 60) and 2019 (n = 51).