Trace element concentrations in riverine fish: relationships with body size, food web dynamics and trace element concentrations in surface water

by

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Abstract

Freshwater fish are an important resource and form an essential component of freshwater ecosystems. However, stressors such as water pollution are negatively impacting freshwater biodiversity. Trace elements can be environmental pollutants and have the potential to negatively impact the health of fish, humans and wildlife. My research builds upon results from a previous study in the Red Deer River watershed in Alberta, Canada, which found trace elements in surface water at concentrations which pose a risk to humans and wildlife. I examined trace element concentrations in fish from the Red Deer River watershed to determine: 1) the concentrations of trace elements in fish tissue and potential risk of these concentrations to humans and wildlife, 2) whether patterns in fish trace element concentrations reflect those in the surface water and 3) if biological characteristics influence trace element concentrations in fish.

To reach these objectives, I examined trace element concentrations in fish muscle tissue from streams and the river mainstem within the Red Deer River watershed. I compared patterns of trace element concentrations in fish to those in the surface water, which vary across four tributary streams, or upstream to downstream in the river mainstem. In both lotic environments, I included physical characteristics (age, body size) as well as food web tracers (stable isotope signatures $\delta^{15}N$ and $\delta^{13}C$) together with trace element analysis. My results show most trace elements were at low concentrations in fish muscle tissue or not detected. However, mercury in many fish exceeded concentration criteria for human consumers, piscivorous wildlife and fish health. Patterns in fish trace element concentrations did not reflect spatial patterns in surface water trace element concentrations and were often species-specific. Correlations between trace element concentrations and fish biological factors varied depending on the fish species and

element considered, but the strongest relationships were with mercury. Mercury was often associated with trophic position and body size, but this relationship was stronger in the mainstem community compared to stream fish. This research indicates that trace element accumulation in fish, particularly mercury, is not limited to areas of high environmental concentrations within this watershed. Therefore, management efforts should be directed to assessing the health of piscivorous wildlife as mercury concentrations in fish pose a potential risk to wildlife health throughout the Red Deer River watershed. Additionally, monitoring mercury concentrations and health of large-bodied fish occupying top trophic level positions should be ongoing as they accumulate the greatest concentrations of mercury.

Preface

This thesis is an original work by Caitlyn R. Donadt.

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were found for mercury, selenium, arsenic and zinc. Positive slopes indicate biomagnification (mercury and selenium), and negative slopes indicate a biodiminishing relationship (arsenic and zinc).

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Chapter 1: General Introduction

Fish are an important resource and an integral part of aquatic ecosystems. Globally, people rely on freshwater fish for food and economic security, cultural significance and recreational services (Lynch et al. 2016). Furthermore, freshwater fish form an essential component of aquatic ecosystem biodiversity and can influence ecosystem functioning through roles such as controlling the flow of biomass across ecosystem boundaries (Wesner 2010), and nutrient cycling (Small et al. 2011). Unfortunately, freshwater environments are experiencing biodiversity loss at a disproportionate rate (WWF 2018, Burkhead 2012). Freshwater biodiversity decline is the result of multiple stressors such as habitat degradation, invasive species and water pollution (Dudgeon et al. 2006). Pollution of aquatic habitats is associated with detrimental effects to fish which reside there, such as disruption to migration (Saunders and Sprague 1967), increasing incidence of disease (Maceda-Veiga et al. 2019), disturbances in behavior (Ward et al. 2008), and negative physiological effects (Holcombe et al. 1976). Therefore, identifying and managing potential pollutants in the environment is an important task for researchers and managers.

Trace elements usually occur in the environment at low concentrations, and include many environmental pollutants. These elements are naturally occurring in the earth's crust, and can be introduced to freshwater ecosystems through processes such as weathering. Non-essential elements, such as mercury, lead, cadmium and arsenic, have no known beneficial aspects (Wood et al. 2012b). Some elements, such as zinc, nickel, selenium, and chromium, are considered essential elements for biota (Wood et al. 2012a). Essential elements play important physiological roles including the formation of proteins (e.g., Selenium; Rayman 2000) or their involvement in aging and immune processes (e.g., Zinc; Mocchegiani et al. 2010). Non-essential elements tend

to exhibit toxicity in biota at lower concentrations than essential elements but both can be hazardous at high concentrations. In the case of selenium, the amount which confers beneficial effects is only marginally lower than a toxic dose (Hamilton 2004). While some trace elements can be found in their elemental form in the environment, many often exist in a variety of oxidation states or in various organic and inorganic compounds. These forms often differ in their toxicity level to biota (Wood et al. 2012a, b) adding to the complexity of understanding the potential risk of trace elements in the environment.

Human activities, such as mining and industrial practices, have increased the export of trace elements into freshwater environments. Negative impacts to wildlife and humans are often associated with an industrial point source. Cases like mercury contamination in Minamata bay, Japan by a chemical plant (Harada 1995), arsenic contamination at giant mine, Canada (Amuno et al. 2018) and chromium contamination from tanneries in India (Shankar 2009) are clear examples of when industrial pollution lead to detrimental effects to human and wildlife health. However, human activities such as fossil fuel combustion can also increase the amount of trace elements emitted to the atmosphere, which can travel great distances and settle upon the landscape far from their original source (Driscoll et al. 2013). Thus, waterbodies can have elevated concentrations of trace elements in the surface water without direct exposure to a point source.

Once in the aquatic environment, diet can be a main exposure route of trace elements to fish, wildlife and humans. This exposure route is of special concern for trace elements that biomagnify in food chains (e.g., mercury; Lavoie et al. 2013, and selenium; Orr et al. 2006), as hazardous concentrations can accumulate in top predators at relatively low concentrations in the water column. Analysis of stable isotopes, particularly carbon and nitrogen, provide a means of

examining food web dynamics (Post 2002, Vander Zanden et al. 1999). Stable isotope signatures of muscle tissue are advantageous over other common dietary analyses, such as stomach contents, because they provide an integrated signal of consumer diet over weeks to months (Maruyama et al. 2001, Heady and Moore 2013). Recent studies have combined the use of stable nitrogen isotope ratios with trace element analysis to investigate biomagnification in aquatic ecosystems (Reinhart et al. 2018, Griboff et al. 2018). Stable nitrogen isotopes ($\delta^{15}N$) indicate the trophic position of an organism in the food chain, with higher values of $\delta^{15}N$ representing higher positions (Vander Zanden and Rasmussen 2001). While some trace elements consistently biomagnify in food webs (e.g., mercury; Lavoie et al. 2013), conflicting results have been reported regarding the potential biomagnification of others (Ali and Kahn 2018). For example, arsenic has been shown to both biomagnify (Sakata et al. 2015) and biodiminish (decrease in concentration with increasing tropic level; Revenga et al. 2012). As a compliment to $\delta^{15}N$ values, stable carbon isotopes can also be used as a food web tracer in contaminant analysis. Stable carbon isotopes ($\delta^{13}C$) are an indicator of dietary carbon source and can be used to distinguish between terrestrial and aquatic carbon signals in a river system (Vander Zanden and Rasmussen 2001, Riva-Murray et al. 2013a). Studies have found that the source of a fish's diet can influence their contaminant levels (Riva-Murray et al. 2013a, Fletcher et al. 2014). When comparing stream systems, Riva-Murray et al (2013a) found that depleted $\delta^{13}C$ values in consumers were correlated with higher mercury levels, potentially indicating a pathway of elevated mercury transfer from in-stream dietary carbon sources compared to terrestrial sources. Considering biological factors, such as food web dynamics, in addition to investigating trace element concentrations in the environment enhances our understanding of contaminant concentrations in fish.

In this thesis I build upon previous research conducted by Kerr and Cooke (2017) examining trace element concentrations in the Red Deer River watershed in Southern Alberta. Kerr and Cooke (2017) described trace metal concentration patterns in surface water which increased from upstream to downstream in the river mainstem, as well as higher concentrations in the tributaries that were comparable to industrially impacted rivers globally. My study complements these results by examining concentrations of trace elements in fish from this river system. Specifically, I examine mercury concentrations in the surface water and fish from stream habitats (Chapter 2) and a larger community in the river mainstem (Chapter 3). In addition to mercury, I also examine a suite of other trace elements in from fish in the river mainstem. The unifying objective of this research is to determine if fish trace element concentrations reflect patterns of those in surface water, and to what extent the trace elements are accumulated in their tissues. As the accumulation of some trace elements in fish can be linked to biological factors, I included analysis of physical characteristics (fish length and age) as well as food web tracers (stable isotope signatures $\delta^{13}C$ and $\delta^{15}N$) to inform my interpretations of trace element concentrations. The results of this thesis can be incorporated into the larger body of information used to issue fish consumption guidance in Alberta. Additionally, these results will add to our understanding of the extent which contaminants in the environment are transferred to biota.

Chapter 2: Mercury bioaccumulation in stream fish from an agriculturally-dominated watershed

2.1 Executive Summary

Mercury contamination in fish poses a risk to the health of wildlife and humans. Concentrations of mercury in the water column and biological factors may influence the accumulation of mercury in fish. In this study, I assessed concentrations of mercury in surface water and fish from four tributaries to the Red Deer River, Alberta, Canada. I examined relationships between fish mercury concentrations and patterns in unfiltered methyl and total mercury concentrations in surface water among the tributaries as well as fish length, trophic level, and dietary carbon source. Additionally, I compared standardized mercury concentrations in fish from four waterbodies in southern Alberta to determine if mercury concentrations were elevated in fish from the Red Deer River. I found that both fish and surface water total mercury concentrations varied across tributaries, but in differing patterns. In contrast, methylmercury concentrations in surface water did not vary among the tributaries. Mercury concentrations exceeded the tissue residue quality guideline for the protection of wildlife consumers in 99.7% of fish and were correlated fork length and trophic level in some species. When standardized by body size, fish mercury concentrations in the Red Deer River were similar to those from nearby rivers. The results of this study suggest that mercury bioaccumulation in stream fish is not driven by environmental inorganic mercury concentrations. Additionally, the potential risk of mercury to the health of piscivorous wildlife is present in all four tributaries.

2.2 Introduction

Mercury is a widespread contaminant that poses a risk to humans and wildlife. Mercury, specifically methylmercury (MeHg), is a potent neurotoxin which can be transferred to humans

through the consumption of contaminated fish (Harada 1995). In the U.S., the majority (>80%) of fish consumption advisories issued by the government were due to mercury contamination (U.S. EPA 2013). High mercury concentrations in fish can also have negative impacts on fish health by impairing growth and reproduction (Crump and Trudeau 2009, Beckvar et al. 2005, Friedmann et al. 1996). Mercury is emitted into the atmosphere through natural and anthropogenic sources and is deposited on the landscape where it can be exported into freshwater systems (Driscoll et al. 2013). Although global trends in atmospheric emission of mercury from anthropogenic sources have been decreasing since 1990 (Zhang et al. 2016), it has been noted that trends of mercury accumulation in many species are not decreasing along with emissions (Wang et al. 2019, Schartup et al. 2019). Considering the health risks of mercury contamination and the disconnection between trends in emissions and bioaccumulation, investigation of the factors influencing ecosystem mercury dynamics is crucial.

Mercury is commonly found in both inorganic and organic (e.g., MeHg) forms in the environment. While all types of mercury are potentially harmful, organic forms are more toxic to biota than inorganic forms (Boening 2000). Inorganic mercury is converted to MeHg in freshwater predominantly by sulphate- and iron-reducing bacteria (Lin et al. 2011). The primary site of mercury methylation occurs in the upper layers of the sediment (Paranjape and Hall 2017), where mercury is often delivered bound to particulate matter (Xu et al. 2019). MeHg biomagnifies in the aquatic food web and is retained for long periods of time in fish tissue (Kidd et al. 2011). As a result, almost all mercury in fish tissue is MeHg (Bloom 1992), acquired primarily from dietary sources (Hall et al. 1997), and can be greater than concentrations in surface water by orders of magnitude (Scudder et al. 2009). Therefore, investigation of fish

mercury concentrations should be considered when elevated mercury concentrations are detected in surface water.

Mercury concentrations in fish are not solely determined by exposure in the water column. Biological factors can mediate mercury concentrations in fish resulting in high variability between sites, even from stream systems receiving similar inputs of atmospheric mercury (Ward et al. 2010a). Moreover, mercury concentrations in fish occupying top trophic levels can be altered by food web characteristics such as exposure to different concentrations of mercury at the base of the food web, changes in food chain length and differences in community taxa present (Kidd et al. 2011). Within taxa, mercury acquisition and storage in fish tissue can be influenced by body size (Eagles-Smith et al. 2016a, Roxanna Razavi et al. 2019), age (Redmayne et al. 2000, Donald et al. 2015), trophic level (Donald et al. 2015, Pandey et al. 2017) and dietary carbon source (Riva-Murray et al. 2013a). Analysis of stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope ratios can be used to determine fish trophic level and sources of dietary carbon, respectively (Vander Zanden and Rasmussen 2001). In fish, $\delta^{15}N$ values typically increase with higher positions in the food chain (Vander Zanden and Rasmussen 2001) and more depleted $\delta^{13}C$ values are interpreted as higher use of in-stream dietary carbon sources (Hershey et al. 2007, Broadley et al. 2019). Examination of fish body size, age and stable isotope signatures in combination with mercury analysis can provide an effective way to investigate the influence of biological factors on fish mercury concentrations.

Streams provide important habitat and resources which support both aquatic and terrestrial ecosystems. Much of our knowledge about mercury accumulation in freshwater fish comes from studies in lakes, but more emphasis on understanding mercury dynamics in riverine environments has occurred in the last decade (Chasar et al. 2009, Ward et al. 2010b).

Understanding mercury dynamics in riverine systems is important in western North America, where spatial patterns indicate fish mercury concentrations are elevated in some riverine environments compared to lakes (Eagles-Smith et al. 2016a). Streams with forest dominated land cover may be at risk of mercury contamination as trees, particularly conifers, scavenge mercury from the atmosphere potentially increasing the introduction of mercury into the aquatic environment compared to open ecosystems (Witt et al. 2009). Much of the research on mercury bioaccumulation in streams has been conducted in forested environments (e.g., Ward et al. 2010a, Jardine et al. 2013, Riva-Murray et al. 2013a, de Wit et al. 2014). Although forested streams effectively scavenge atmospheric mercury, mercury deposited upon the landscape can be easily mobilized in agriculturally dominated watersheds (Balogh et al. 1998). More arid regions have also been noted to accumulate disproportionately high concentrations of mercury in biota compared to waterbodies in forested regions, which may receive higher mercury deposition (Eagles-Smith et al. 2016a). Investigation of agriculturally dominated watersheds is needed to provide insight into mercury dynamics in these important stream systems.

The Red Deer River is an agriculturally dominated watershed in Southern Alberta, Canada. High total mercury (THg; measurement including both inorganic and organic forms) concentrations in surface water from this river have been reported in association with high levels of suspended sediment in the water column, especially in certain tributaries (i.e. Michichi Creek; Kerr and Cooke 2017). In this study, I sought to evaluate mercury accumulation in fish from this watershed. My objectives were to 1) determine mercury concentrations in surface water and fish among four tributaries to the Red Deer River, 2) examine the relationship between fish mercury concentrations and biological factors, and 3) determine if mercury concentrations in fish from the Red Deer River are elevated compared to other waterbodies in Southern Alberta. I predicted that

fish mercury concentrations would generally reflect patterns in aqueous THg concentrations among the tributaries, but would also be positively correlated to fish body size, trophic level and in-stream dietary carbon signatures. Additionally, due to high peak concentrations of THg previously recorded in the surface water, I predicted that mercury concentrations in fish would be elevated compared to other waterbodies in southern Alberta.

2.3 Methods

2.3.1 Study area

The Red Deer River flows from its headwaters in the Rocky Mountains of Alberta to Saskatchewan, where it joins the South Saskatchewan River (Campbell 1977a; Figure 2.1). The south-eastern corner of Alberta is a semi-arid region and the river is flanked by badlands (Campbell 1977a). Upstream of the badlands, the bedrock geology is formed from Quaternary clay-rich alluvium (Allan 1922). Within the badlands, it transitions to outcropping bedrock cretaceous in age formed of clays and bentonite, with ironsone and coal bands (Allan 1922). Four tributaries – Kneehills Creek, Threehills Creek, Michichi Creek and Rosebud River – drain the central region of the Red Deer River watershed, and confluence near the Town of Drumheller. The subwaterhseds of the four tributaries range in size from 2735 km^2 (Kneehills Creek) to 6204 km^2 (Michichi Creek) (Aquality Environmental Consulting Ltd, 2009). Land use around the tributaries is predominantly agricultural (proportional area: 64 – 77%; Kerr and Cooke 2017), with minimal wetland cover (0.67 – 5.53%; Aquality Environmental Consulting Ltd 2009). Further information about these four subwatersheds can be found in Aquality Environmental Consulting Ltd (2009).

2.3.2 Field and data collections

Fish and benthic macroinvertebrates were collected to compare mercury bioaccumulation among four tributaries to the Red Deer River. Fish were collected from 19 sites on Kneehills Creek, Michichi Creek, Rosebud River and Threehills Creek during June to August 2017 (Figure 2.1). At each site, fish were collected by electrofishing (backpack electrofisher; Smith Root LR24) 150 m sections of wadeable stream area. Fish were identified to species and measured to fork length. Common species that were targeted for this study included native lake chub (*Couesius plumbeus*), white sucker (*Catostomus commersonii*) and fathead minnow (*Pimephales promelas*) as well as invasive Prussian carp (*Carassius gibelio*). Prussian carp was first introduced to North America in the Red Deer River watershed (Elgin et al. 2014). Benthic macroinvertebrates were collected by a two-minute kick-net sample at each site. Fish and macroinvertebrates were frozen until processing. Fish from this dataset were aged and tissue was analyzed for stable isotope ratios and THg concentrations.

A secondary dataset of fish mercury concentrations was compiled to compare fish from the Red Deer River to other waterbodies in Alberta as a part of a larger monitoring program (Alberta Health 2018). In addition to mercury concentrations in fish from the tributaries, data was added from two sites on the Red Deer River mainstem (samples collected in September 2017) and from other waterbodies compiled in an open government dataset (sampled between 1997 and 2008, Table A2.1). The species sampled from the most waterbodies in the dataset, white sucker, was selected for the comparison. Fish from this dataset were weighed and THg concentrations were determined from fish tissues.

Surface water samples were collected from the tributaries as part of a larger monitoring program by Alberta Environment and Parks (AEP) (Kerr and Cooke 2019). Samples collected monthly

between April 2016 and August 2017 were selected to be included in this study. Conditions often prevented sampling in December, January, February and March, so these months were excluded from analysis. Samples also could not be collected from Michichi Creek in May 2016, and July 2017. Surface water was collected at one site per tributary located downstream of biota collection sites. Samples were taken just below the surface, approximately at the midpoint between the banks of each tributary following a "clean hands – dirty hands" sampling protocol (U.S. EPA 1996). From this dataset, unfiltered THg and MeHg concentrations were selected for analysis. Unless otherwise noted, all THg and MeHg concentration in water data presented below are all from unfiltered water samples. Additional water quality information (pH, dissolved organic carbon, turbidity, dissolved oxygen and dissolved sulphate) as well as concentrations of THg and MeHg in filtered samples can be found in Table A2.2.

2.3.3 Laboratory processing

Fish were thawed, rinsed, blotted and weighed in the laboratory. Skinless, boneless muscle tissue samples were placed in clean glass vials for processing. Lapillus otoliths were collected to estimate age (n = 232). When lapillus otoliths could not be found, sagittal otoliths were used instead (n = 5). Macroinvertebrate samples were thawed, and invertebrates were removed from debris and rinsed. Macroinvertebrates were sorted to family and classified into functional feeding groups based on the literature (Clifford 1991, Resh and Carde 2009, Thorp and Covich 2010, Voshell 2002). Invertebrates of the same family were pooled to form one sample for each taxon per site. Fish and invertebrate samples were placed in clean glass vials, freeze-dried and homogenized with a ceramic mortar and pestle or stainless steel pulverizing instrument for analysis.

2.3.4 Otolith age estimation

Otoliths were embedded in epoxy resin, and then either sectioned with a low speed dual-blade saw through the nucleus (Prussian carp and white sucker) or aged whole (fathead minnow and lake chub). A subset of otoliths were read by a second independent reader for validation (n = 115).

2.3.5 Stable isotope analysis

Homogenized fish and invertebrate samples were analyzed for stable isotopes. Samples were placed in tin capsules and analyzed with a Vario Pyrocube elemental analyzer and an Elementar IsoPrime visIon continuous-flow isotope ratio mass spectrometer for $\delta^{15}N$ and $\delta^{13}C$ ratios. Isotope ratios were determined as follows:

$$\delta R‰ = ((R_{sample}/R_{standard})-1) \times 1000$$

Where $\delta R‰$ is the heavy isotope, R_{sample} indicates the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample, and $R_{standard}$ is a reference value determined in air ($\delta^{15}N$) or Vienna Pee Dee Belemite ($\delta^{13}C$). An additional QA/QC check was used (NIST 8415 whole egg powder SRM) every 20 samples for $\delta^{15}N$ (6.89‰) and $\delta^{13}C$ (-23.99‰) with a precision of $\delta^{15}N$ ±0.2‰ and $\delta^{13}C$ ±0.01‰, respectively.

2.3.6 Mercury analysis

In fish, THg concentrations can be used to approximate MeHg concentrations because almost all mercury accumulated in the tissues is MeHg (Bloom 1992, Mason et al. 2000, Jardine et al. 2013). For fish sampled in 2017, THg analysis was conducted on dry fish muscle tissue using atomic absorption spectrophotometry in a Milestone Direct Mercury Analyzer (DMA-80) following EPA method 7473 (U.S. EPA 1998). Quality control was conducted using SRM

controls (DORM-3 and DORM-4 dogfish muscle, NRC, Ottawa, Canada). SRM results were within ±10% of certified values, and duplicates were within ±10% value of each other. The first value was reported for duplicate samples and the method detection limit was 0.003 mg/kg. For fish sampled between 1997 and 2008, THg concentrations were determined in wet tissue through a flow injection Hg system and cold-vapour atomic absorption detection. The method detection limit (MDL) was 0.003 mg/kg.

THg in water was analyzed by cold vapour atomic fluorescence spectroscopy (CVAFS) and MeHg was analyzed with isotope dilution, purge and trap, and inductively coupled plasma mass spectrometry (ICP- MS). The MDLs for THg and MeHg were 0.05 ng/L and 0.016 ng/L, respectively.

2.3.7 Data analysis

Data was examined for outliers by computing z-scores, normality and homogeneity using QQ-plots and residual analysis. Statistical significance for all tests was at a level of $p < 0.05$. Data analysis was conducted in R (version 3.5.3; R Core Team 2019). Data manipulation and visualization was done using dplyr (Wickham et al. 2019), ggplot2 (Wickham 2019), ggpubr (Kassambara 2019), ggforce (Pedersen 2019), ggthemes (Arnold 2019) and rcompanion (Mangiafico 2019) packages.

Comparisons of mercury concentrations in fish and surface water among the tributaries were made to identify patterns between environmental mercury concentrations and fish. Kruskal-Wallis tests were used to compare between group differences of THg, MeHg and %MeHg in surface water. Pairwise comparisons among tributaries were conducted using Dunn's post hoc test using the FSA package (Ogle et al. 2019). In fish, between-group differences were compared

among the tributaries using ANOVA with type III sums of squares for unequal sample sizes and pairwise comparisons among tributaries were conducted using Tukey HSD post hoc test. Lake chub sampled from Kneehills Creek were not included in the statistical comparison due to low sample size collected from that tributary (n = 3). Fish THg concentrations were also compared among species using ANOVA with type III sums of squares for unequal sample sizes and Tukey HSD post hoc test. For this comparison, fish THg concentrations were log10 transformed to meet assumptions of normality and homogeneity. ANOVA comparisons were done using the car package in R (Fox and Weisberg 2019).

Mercury concentrations in all fish (no outliers removed) were also compared to a tissue residue quality guideline for the protection of piscivorous wildlife (0.033 mg/kg, CCME 2000), a criterion at which issued fish consumption advice suggests limiting consumption for subsistence consumers (0.2 mg/kg, Government of Alberta 2019a), and an estimated threshold associated with potentially diminished fish health (0.2 mg/kg; Beckvar et al. 2005). Mercury concentrations in fish from this study are reported in muscle tissue on a dry-weight basis, therefore some modifications were made for comparison to the above values. The threshold suggested by Beckvar et al. (2005) was originally reported as a whole body concentration but was converted to a muscle concentration by dividing by 0.74 following Eagles-Smith et al. (2016a). All above criteria were originally reported in wet weight concentrations, but were converted to dry weight concentrations for comparison in this study following the approach by Magalhães et al. (2007):

$C_D = C_W/((100-\%H)/100)$

Where C_D is the concentration of mercury in the dry tissue, C_W is the concentration of mercury in wet tissue, and %H is the moisture percentage in the muscle tissue (estimated at 80% based on literature values; Scudder Eikenberry et al. 2015).

Calculating fish trophic level using baseline primary consumers is suggested to correct for environmental variation in $\delta^{15}N$ among sites (Post 2002). Biota collected from the Rosebud River were elevated in $\delta^{15}N$, likely reflecting inputs from an external nitrogen source (Figure A2.1; Brinkmann and Rasmussen 2012). Therefore, trophic positions were calculated for all fish to correct for differences in fish $\delta^{15}N$ related to potential external nitrogen sources. Collectors (Caenidae, Chironomidae, Corixidae) were the most widespread macroinvertebrate taxa in the tributaries, so they were used as baseline primary consumers for calculation of fish trophic level (Table A2.3). Trophic level was calculated within each tributary following the approach of Post (2002):

$$TP = ((\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/ 3.4) + \lambda$$

Where $\delta^{15}N_{consumer}$ was the fish value, $\delta^{15}N_{baseline}$ was the average value of baseline primary consumers within a tributary, 3.4 is the fractionation value of one trophic level (Post 2002) and λ is the trophic level of the primary consumers, assumed to be 2 (Cabana and Rasmussen 1996).

Linear multiple regression analysis was used to examine whether biological factors were correlated with fish mercury concentrations. Otoliths could not be recovered from all fish, therefore age-length keys and a multinomial logistic model were used to predict ages according to fork length for each species following Ogle (2016) using the nnet package (Venables and Ripley 2002). A model was constructed to predict fish mercury concentrations for each species with tributary, age, fork length, $\delta^{13}C$ signature, trophic level and a length – tributary interaction

as explanatory variables. If no significant interaction was detected, the interaction term was removed from the model. Fish mercury concentrations were log transformed to meet assumptions of normality and homogeneity. Multicollinearity was examined though VIF values using the car package (Fox and Weisberg 2019), with those higher than 5 considered problematic (Gareth et al. 2013). Two separate models were created for Prussian carp due to multicollinearity between age and fork length. Akaike's information criteria bias-corrected for small samples (AICc) was used to evaluate model support between the two models using the MuIn package (Barton 2019). The model with the lowest AICc was selected as best if the difference between both AICc values was less than two (Burnham and Anderson 2002).

Mercury concentrations in white sucker from three other waterbodies in Alberta were compared to those in the Red Deer River to assess if mercury concentrations in fish from the Red Deer River were elevated in relation to surrounding waterbodies (Table A2.1). All mercury concentrations were converted to wet weight values assuming 80% tissue moisture content (Magalhães et al. 2007). Fish mercury concentrations are often correlated with body size (Eagles-Smith et al. 2016a, Roxanna Razavi et al. 2019), and the size of white sucker varied widely among waterbodies (Table A2.1). Therefore, fish mercury concentrations needed to be standardized by body size for comparison among waterbodies. Fork length is a common body size metric chosen for mercury standardization because it is often highly correlated to mercury concentrations (Scudder Eikenberry et al. 2015), but weight can also be used (Åkerblom et al. 2014). In the present study fish mercury concentrations were standardized by weight as it was the most commonly recorded metric of body size across waterbodies. A linear mixed effects model was constructed using the lme4 package (Bates et al. 2015) with weight as a fixed covariate, and waterbody and sampling year as random effects to predict fish mercury concentrations at a

median body weight. Fish mercury concentrations were log10 transformed to meet assumptions of normality and homogeneity. The residuals of the model were added to predicted mercury concentrations and back-calculated to create weight-standardized mercury concentrations. Sample sizes varied widely among waterbodies (n=10 to n = 187), so only those with greater than 100 white sucker sampled were included in statistical comparisons (Little Bow River, Red Deer River and Oldman River; Table A2.1). Standardized mercury concentrations in fish were compared using ANOVA with type III sums of squares for unequal sample sizes and a Tukey HSD post hoc test was used to determine between waterbody differences.

2.4 Results

2.4.1 Mercury in the water

THg concentrations in water were highly variable (range: 1.14 – 615.33 ng/L; Figure 2.2), and there were significant differences among the tributaries (Kruskal-Wallis test, $\chi2 = 20.2$, df = 3, $p < 0.001$). THg concentrations in Michichi Creek were significantly elevated compared to other creeks (Kneehills: $p < 0.001$, Rosebud: $p = 0.003$, Threehills: $p = 0.004$). Although no significant differences were found in MeHg concentrations among creeks, mean MeHg concentrations were almost double in the tributary with the highest mean (Kneehills Creek: 0.70 ng/L) compared to the lowest (Rosebud River: 0.39 ng/L). %MeHg was significantly different among tributaries (Kruskal-Wallis test, $\chi2 = 29.4$, df = 3, $p < 0.001$). Specifically, Kneehills Creek had significantly higher %MeHg compared to Michichi Creek, Threehills Creek and Rosebud River (Dunn's test, $p < 0.001$, $p = 0.036$ and $p = 0.024$, respectively).

2.4.2 Mercury in the fish

Mercury concentrations differed significantly among species (ANOVA test, $F = 15.2$, df = 3, $p < 0.001$), where Prussian carp and fathead minnow had lower mercury concentrations than lake

chub and white sucker but were not different from each other. Fish mercury concentrations were significantly different among tributaries for lake chub (ANOVA test, $F = 11.3$, $df = 2$, $p < 0.001$), Prussian carp (ANOVA test, $F = 17.5$, $df = 3$, $p < 0.001$) and white sucker (ANOVA test, $F = 6.1$, $df = 3$, $p < 0.001$) but not fathead minnow (Figure 2.3). However, patterns of fish mercury concentrations between the tributaries varied depending on species. For example, mercury concentrations in lake chub sampled from Rosebud River were significantly higher than those from Michichi Creek (Tukey test, $p < 0.001$) and Threehills Creek (Tukey test, $p < 0.001$), whereas mercury concentrations in white sucker from Rosebud River were significantly lower than those from all other tributaries (Tukey test, Kneehills Creek: $p = 0.002$, Michichi Creek: $p = 0.046$, Threehills Creek: $p = 0.006$). Few fish exceeded the criterion for issuing fish consumption advice for subsistence consumers (4.0%), or the estimated threshold for the protection of fish health (0.8%), but almost all exceeded tissue residue quality guidelines for the protection of wildlife consumers (99.7%; Figure 2.3).

2.4.3 Fish biological characteristics and mercury accumulation

The largest fish sampled were white sucker (maximum fork length = 240 mm), followed by Prussian carp (max = 201 mm), with maximum ages of 5 years old for both (Figure 2.4). Fathead minnow were generally smaller (<75 mm) and younger (≤ 2 years) than other fish species (Table 2.1, Figure 2.4). All fish species had similar $\delta^{13}C$ signatures (~ 29‰) and trophic positons (~3 – 4) indicating these species occupy similar positions in the food web as tertiary consumers (Cabana and Rasmussen 1996). Multiple regression models were significant for white sucker, Prussian carp and lake chub (all: $p<0.001$) but not fathead minnow. Of the two models created for Prussian carp, including only fork length was better supported than age (AICc = -119 and -108 respectively), therefore the length based model was selected for comparison. Sampling

location (tributary) was a significant explanatory variable in multiple regression models for Prussian carp, white sucker and lake chub (Table 2.2). Fish length was a significant factor for white sucker, and also Prussian carp. A significant length – tributary interaction was found for Prussian carp, indicating that the accumulation of mercury with fish size varies depending on the environment. Trophic level was only significant in the Prussian carp model, and $\delta^{13}C$ was not a significant factor for any species. Significant fish characteristics and sampling locations explained a moderate amount of variation in fish mercury concentrations (R^2 = 0.33 to 0.49), but a considerable amount of variation was not explained by these characteristics (Figure 2.4).

2.4.4 Comparison of mercury concentrations in white sucker

Standardized white sucker mercury concentrations were significantly different between the Red Deer River, Oldman River and Little Bow River (ANOVA test, $F = 39.556$, $df = 2$, $p < 0.001$). Fish from the Red Deer River were significantly higher in mercury than those from the Little Bow River (Tukey test, $p < 0.001$), but not the Oldman River (Figure 2.5).

2.5 Discussion

Understanding the extent which mercury in the water column is accumulated by fish is necessary to identify the potential risk to human and wildlife consumers. Mercury concentrations in surface water of the Red Deer River have been shown to exceed surface water guidelines for the protection of freshwater biota (Kerr and Cooke 2017). In the present study, THg concentrations in surface water were highly variable and elevated in Michichi Creek. In contrast, MeHg concentrations were no different in Michichi compared to the other tributaries. MeHg concentrations in the tributaries were comparable to streams with historical mining activity and naturally high geologic deposits (Domagalski 2001), contaminated liquid effluents (Xu et al. 2019), as well as industrial spills and discharges (Mathews et al. 2013). Wetland cover is often

associated with dissolved MeHg in surface water (Hurley et al. 1995, Brigham et al. 2009), whereas agricultural landscapes are more associated with mercury particulate complexes (Hurley et al. 1995, Balogh et al. 2003). Indeed, aqueous THg concentrations are associated with suspended sediment supply in the Red Deer River (Kerr and Cooke 2017), but MeHg in this system is comparable to those with "high" wetland cover (e.g., St. Louis et al. 1994, Hurley et al. 1995) despite a paucity of wetlands in the subwatersheds (Aquality Environmental Consulting Ltd 2009). Although MeHg concentrations were not variable among the tributaries, the percent MeHg was the highest in Kneehills Creek, intermediate in Rosebud River and Threehills Creek, and the lowest in Michichi Creek, suggesting Michichi Creek might be a site of decreased methylation efficiency (Gilmour et al. 1998). Due to the vast differences in patterns of THg and MeHg concentrations among these tributaries, THg measurements should not be considered a reliable indicator of MeHg present in the water column.

While mercury concentrations in most fish species differed among the tributaries, they did not reflect the same pattern as aqueous THg concentrations. Mercury in the aquatic environment is largely composed of inorganic compounds. Larger spatial patterns of inorganic mercury concentrations are often decoupled with bioaccumulation in biota, such that the areas of greatest bioaccumulation do not coincide with those with the greatest mercury deposition (Eagles-Smith et al. 2016b). Other studies suggest that aqueous MeHg concentration is a predictor of mercury concentrations in fish (Chasar et al. 2009, Riva-Murray et al. 2013b). Yet, fish mercury concentrations in this study did not follow the same pattern as aqueous MeHg concentrations among the tributaries either. Nonetheless, the dissimilarity between patterns of fish mercury and aqueous MeHg concentrations among the tributaries is likely due to the absence of variation in aqueous MeHg concentrations captured in the study and the influence of fish biological factors.

In other studies where aqueous MeHg concentrations had a much greater range among sampling locations, fish mercury concentrations did reflect the patterns of aqueous MeHg in their environment (Souza-Araujo et al. 2016, Matthews et al. 2013, Riva-Murray et al. 2013b). Biological factors such as body size (Eagles-Smith et al. 2016a), age (Donald et al. 2015), dietary carbon source (Riva-Murray et al. 2013a) and trophic level (Donald et al. 2015, Pandey et al. 2017) can have a strong influence on fish mercury concentrations; however, the extent to which these factors influence fish mercury concentrations can vary greatly. In the present study, the influence of biological characteristics was species-specific, apart from dietary carbon source which was not significant for any species. Fathead minnow mercury concentrations did not vary significantly among the tributaries and therefore seem to be determined entirely by environmental MeHg concentrations. However, for other species length and trophic level were correlated with fish mercury levels. Previous research indicates other biological factors not accounted for in this study, such as growth efficiency (Ward et al. 2010a, Sandheinrich and Drevnick 2016) and sex (Madenjian et al. 2014, 2015), can also influence fish mercury concentrations. Therefore, it is likely that fish mercury concentrations in the Red Deer River are largely influenced by water column MeHg, not THg, concentrations the base of the food web are exposed to in combination with the species specific influence of biological factors.

Small fishes, like the ones targeted in this study, form a key component of the ecosystem. Studies of mercury bioaccumulation in fish often target top predator fish species, as these are usually the preferred game fish that may be consumed by humans. However, elevated mercury in small fishes, like cyprinids, can impact the larger ecosystem as they are important food sources for a variety of predators. For example, piscivorous birds which feed on contaminated fish may accumulate high body burdens of mercury, resulting in negative impacts on behavior, physiology

and reproduction (Evers et al. 2008). Almost all fish collected in this study had mercury concentrations exceeding the tissue residue guideline for the protection of wildlife consumers, raising concerns for the health of wildlife in the Red Deer River watershed. Additionally, while the mercury concentrations in these small fishes do not regularly exceed the criterion for issuing fish consumption advice for subsistence consumers, they can be incorporated into the diet of piscivorous fish species targeted by anglers, and through biomagnification, lead to potentially hazardous levels of mercury consumption. Furthermore, changes in the feeding dynamics of primary and secondary consumers such as small fish species can have implications for mercury concentrations at higher levels of the food chain. Invasive species have been shown to shift food web structure in aquatic systems where they are introduced (Baxter et al. 2004), which can alter mercury dynamics through competition with other species causing a shift in their diet (Eagles-Smith et al. 2008), or through extension of the aquatic food chain length (Kidd et al. 2011). In the Red Deer River, an invasive species, Prussian carp, was introduced in the early 2000s and is spreading rapidly (Docherty et al. 2017), with negative impacts on native biota including alterations to community structure (Ruppert et al 2017). Here, I demonstrated that Prussian carp occupy a very similar dietary niche to native small fish species and are likely competing for food resources (Figure 2.4). Given the generalist diet of Prussian carp (Özdilek and Jones 2014) and ability to successfully establish in large numbers, they could impact larger food webs in the Red Deer River system. Considering the potentially hazardous concentrations of mercury in top predator fish from the Red Deer River and the strong influence food web dynamics exert on mercury biomagnification, closer investigation of how invasive Prussian carp may alter mercury dynamics in larger food webs in the Red Deer River is warranted.

Mercury concentrations in white sucker from the Red Deer River were similar to those from surrounding waterbodies in southern Alberta. When compared to the literature, median mercury concentrations of white sucker from the Red Deer River (0.12 mg/kg, range = 0.05 – 0.38, n = 122) also resembled median mercury concentrations from Canada (0.12 mg/kg, range = <DL – 4.39 mg/kg, n = 12 717; Depew et al. 2013) and western North America (0.12 mg/kg, range = 0.001 to 5.70, n = 1764; Eagles-Smith et al. 2016a). However, white sucker from the Red Deer River were considerably smaller (median = 142 mm), than those from across Canada (median = 413 mm; Depew et al. 2013) and western North America (median = 361 mm; Eagles-Smith et al. 2016a). Body size is a significant factor effecting mercury accumulation in this species, therefore, in comparison to widely sampled white sucker from Canada and western North America, white sucker from the Red Deer River may have elevated mercury concentrations for their size. Taken together, this suggests that although mercury concentrations were not unique for southern Alberta, this region may be elevated compared to other regions. However, an analysis of multiple species aggregated by watershed by Eagles-Smith et al. (2016a) does not indicate a mercury bioaccumulation hotspot in Alberta. Mercury bioaccumulation hotspots have been identified on the eastern side of Canada (Evers et al. 2007). These hotspots possess stream chemistry and land cover characteristics associated with high mercury in biota including acidic water conditions with a high amount of dissolved organic carbon and high percentage of wetlands in the watershed (Ward et al. 2010b, Evers et al. 2007, Scudder et al. 2009). Although the Red Deer River may not be a mercury bioaccumulation hotspot, fish mercury concentrations are high despite the watershed not possessing many qualities which have been associated with high mercury concentrations in biota.

2.6 Conclusion

Stream systems in southern Alberta support aquatic and terrestrial wildlife but contain high concentrations of mercury in surface water. This study showed that THg concentrations were elevated in Michichi Creek, but MeHg concentrations were not different among the tributaries. Additionally, patterns of mercury concentrations in fish among the tributaries were species-specific and not reflective of variation in aqueous THg concentrations. Few fish sampled exceeded the criterion for issuing fish consumption advice for subsistence consumers or the estimated threshold associated with potentially diminished fish health, but almost all exceeded guidelines for the protection of wildlife consumers. Although some biological characteristics were correlated to fish mercury concentration, such as body size and trophic level, the relationship was not consistent among all species. Fish mercury concentrations in the tributaries are likely influenced by a combination of environmental MeHg concentrations and biological factors. When mercury concentrations in white sucker were compared across waterbodies in southern Alberta, fish from the Red Deer River were not elevated. Yet comparisons of mercury concentrations and body size of white sucker from the Red Deer River to median values reported in the literature across Canada and western North America indicates fish from southern Alberta may be elevated compared to the broader region. The results from this study highlight management concerns regarding the risk of mercury accumulation in fish eating wildlife and potential impacts on mercury dynamics in the aquatic food web by invasive Prussian carp.

2.7 Acknowledgements

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2.8 Figures and Tables

Table 2.1. Summary of fish biological characteristics (age, length, $\delta^{13}C$, $\delta^{15}N$, trophic position), and total mercury concentrations (THg). Data is presented as mean values ± standard deviation. Fish species are fathead minnow (FTMN), lake chub (LKCH), Prussian carp (PRCR) and white sucker (WHSC).

Tributary	Species	Count	Length (mm)	Age (yrs)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Trophic Position	THg (mg/kg)
Kneehills	FTMN	20	58 ± 6	1 ± 0.56	-29.99 ± 1.5	10.8 ± 0.8	3.5 ± 0.2	0.47 ± 0.13
	LKCH	3	88 ± 22	2 ± 1.73	-29.17 ± 1.22	11.8 ± 0.5	3.8 ± 0.1	1.23 ± 0.49
	PRCR	25	115 ± 21	1.8 ± 0.6	-29.59 ± 0.86	9.6 ± 0.6	3.2 ± 0.2	0.44 ± 0.15
	WHSC	16	130 ± 31	3.6 ± 1.7	-30.02 ± 0.31	10.3 ± 0.6	3.4 ± 0.2	0.71 ± 0.21
Michichi	FTMN	13	58 ± 6	0.8 ± 0.4	-31.34 ± 1.02	11.1 ± 0.4	3.2 ± 0.1	0.46 ± 0.21
	LKCH	24	73 ± 12	0.8 ± 0.5	-29.76 ± 0.78	11.6 ± 0.9	3.4 ± 0.3	0.48 ± 0.09
	PRCR	35	134 ± 24	2.1 ± 0.6	-30.29 ± 0.97	11.2 ± 1.1	3.3 ± 0.3	0.6 ± 0.2
	WHSC	28	154 ± 35	3.3 ± 1.2	-30.68 ± 0.73	11.8 ± 0.9	3.4 ± 0.3	0.6 ± 0.17
Rosebud	FTMN	25	60 ± 6	1.3 ± 0.7	-28.86 ± 1.00	15.1 ± 1.3	3.2 ± 0.4	0.46 ± 0.22
	LKCH	12	87 ± 14	1.8 ± 1	-27.9 ± 0.80	16.8 ± 1.3	3.7 ± 0.4	0.83 ± 0.31
	PRCR	31	131 ± 35	2.1 ± 1	-28.05 ± 1.08	15 ± 1.3	3.2 ± 0.4	0.36 ± 0.11
	WHSC	32	146 ± 25	3.3 ± 1.1	-28.39 ± 0.67	15.5 ± 1.1	3.4 ± 0.3	0.46 ± 0.15
Threehills	FTMN	7	59 ± 9	1.4 ± 0.8	-29.3 ± 1.36	12.8 ± 1	3.3 ± 0.3	0.42 ± 0.16
	LKCH	24	97 ± 19	2.3 ± 1.3	-27.69 ± 1.34	12.8 ± 0.3	3.3 ± 0.1	0.62 ± 0.24
	PRCR	11	98 ± 28	1.6 ± 1.2	-28.57 ± 2.3	11.8 ± 0.7	3 ± 0.2	0.34 ± 0.13
	WHSC	18	140 ± 25	3.7 ± 1.3	-28.75 ± 1.2	13.1 ± 0.6	3.4 ± 0.2	0.68 ± 0.26

Table 2.2. Multiple regression results modelling mercury concentrations in fathead minnow (FTMN), lake chub (LKCH), Prussian carp (PRCR) and white sucker (WHSC). Mercury concentrations were modelled as a response variable with fish age, fork length, $\delta^{13}C$, trophic position (TP) and the tributary they were sampled from as explanatory variables. The tributaries are Kneehills Creek (KC), Michichi Creek (MC), Rosebud River (RR) and Threehills Creek (TC). Mercury concentrations were log10 transformed.

	Biological Characteristics				Tributary						
Species	**Age (yrs)**	**Length (mm)**	**$\delta^{13}C$ (‰)**	**TP**	**KC[a]**	**MC**	**RR**	**TC**	**Adj. r^2**	**p**	**n**
WHSC	0.015	0.002*	-0.033	0.110	-1.868*	0.078*	-0.051	-0.086**	0.33	<0.001	94
LKCH	-0.007	0.004	-0.026	0.087	-1.505*	0.174	-0.142**	0.045**	0.39	<0.001	63
FTMN	-0.003	0.002	0.035	0.149	0.064	0.013	0.067	-0.029	0.04	0.231	65
PRCR[b]	—	-0.003*	-0.023	0.134**	0.194	-0.629**	-0.534**	-0.754**	0.49	<0.001	102

*p<0.05
**p<0.01
[a]intercept
[b]singificant interactions between fork length and MC (0.006**), RR (0.004*) and TC (0.006**)

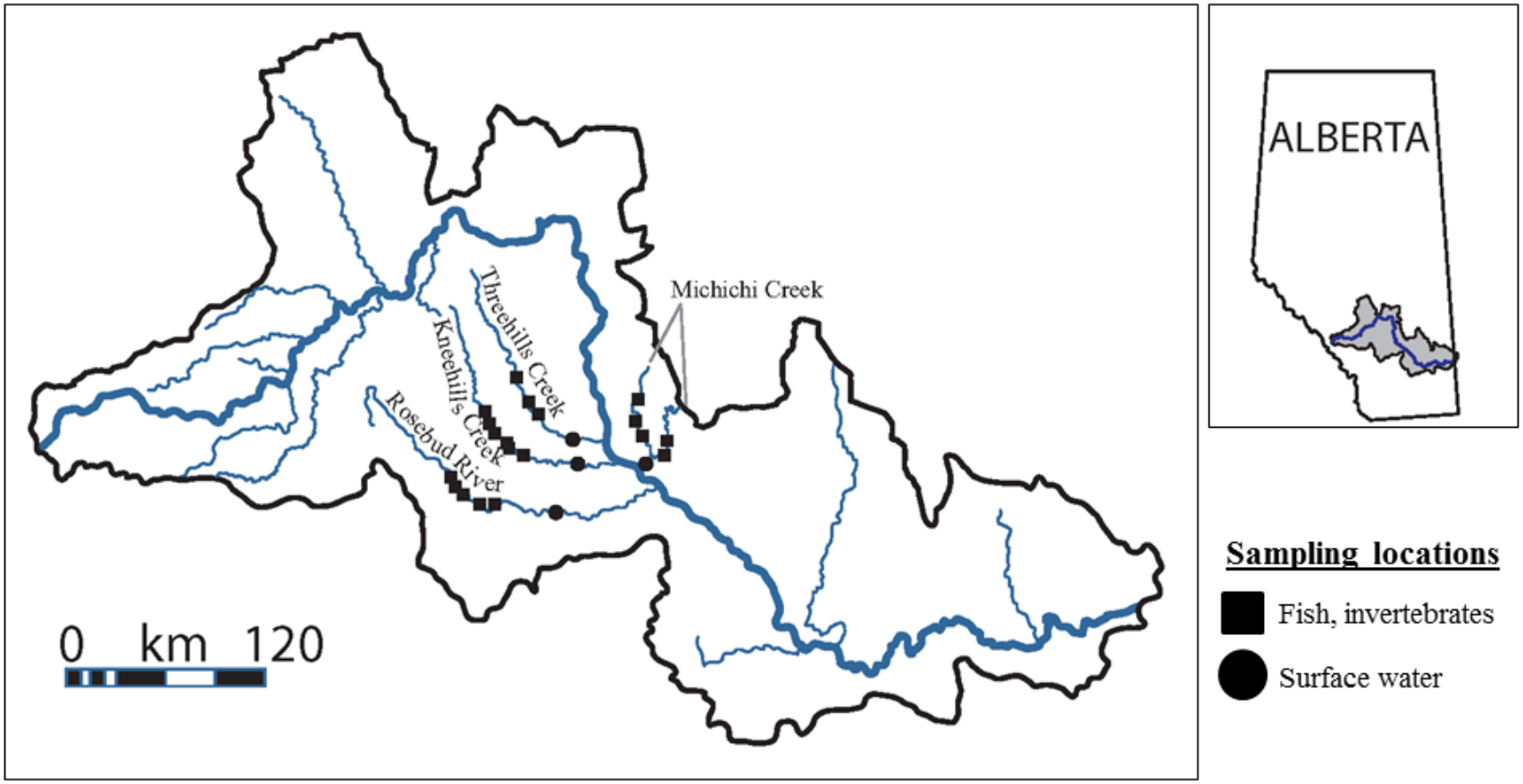

Figure 2.1. Map of sampling locations in the Red Deer River Watershed, AB, Canada. Samples were taken from four tributaries: Rosebud River, Kneehills Creek, Threehills Creek and Michichi Creek. Fish and invertebrates were sampled at 19 sites from June to August 2017 (squares). Surface water samples were collected monthly at one site on each tributary from April 2016 to August 2017 (circles).

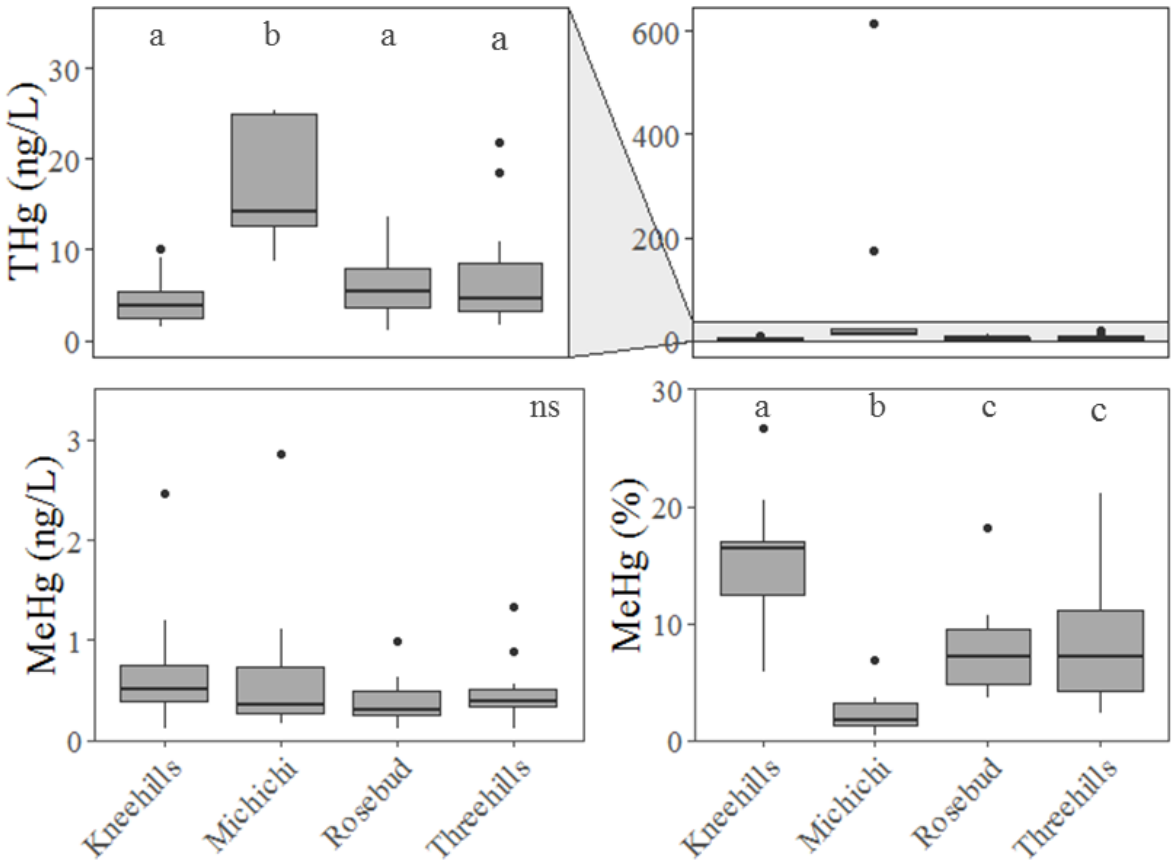

Figure 2.2. Concentrations of total mercury (THg), methylmercury (MeHg), and the percentage of THg as MeHg (%MeHg) in surface water sampled between April 2016 and August 2017. Boxplots represent the median and quartile ranges (25^{th} and 75^{th}), whiskers represent ± 1.5*inter-quartile range from the 25^{th} and 75^{th} quartiles. Tributaries with different letters above the boxplots are significantly different ($p<0.05$), those with the same letter are not significantly different, and those with "ns" have no differences among tributaries.

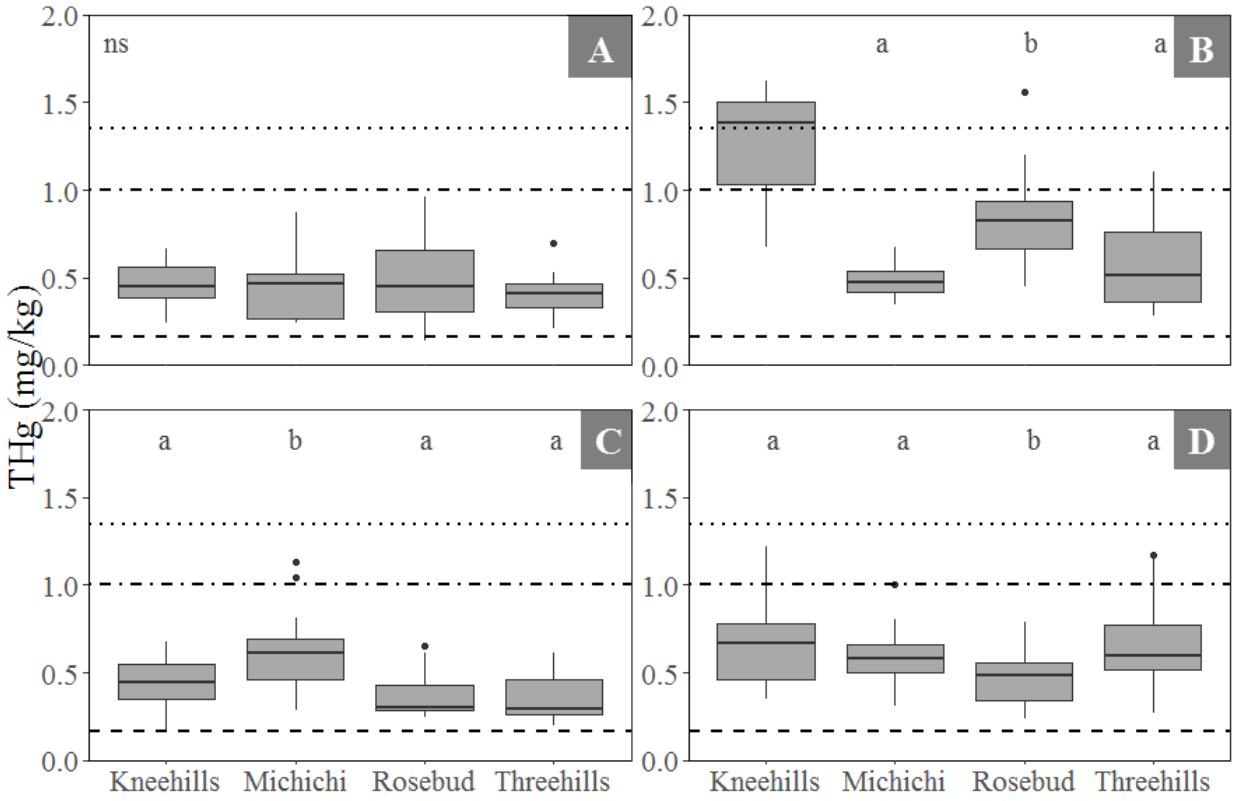

Figure 2.3. Total mercury concentrations in fathead minnow (A), lake chub (B), Prussian carp (C) and white sucker (D). Lines within panels represent mercury concentration criteria: a fish tissue residue guideline for the protection of wildlife consumers of aquatic biota (0.165 mg/kg – dashed line); a criterion for issuing consumption advice for subsistence consumers of fish (1.00 mg/kg – dot/dash line;) and an estimated threshold associated with diminished fish health (1.35 mg/kg – dotted line;). These concentration criteria were modified to compare to wet weight concentration values in muscle tissue. Lake chub from Kneehills Creek were not included in the statistical comparison due to low sample size (light grey, n = 3). Boxplots represent the median and quartile ranges (25th and 75th), whiskers represent ± 1.5*inter-quartile range from the 25th and 75th quartiles. Fish from tributaries with different letters above the boxplots are significantly different ($p<0.05$), those with the same letter are not significantly different, and those with "ns" have no differences among tributaries.

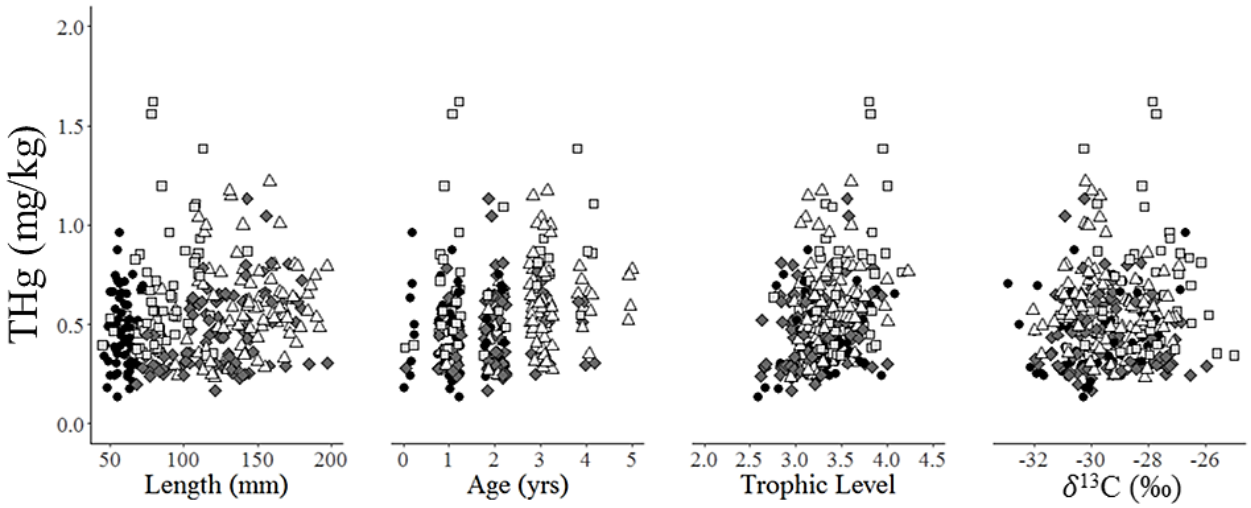

Figure 2.4. Fish mercury concentrations versus fork length, trophic position, age and $\delta^{13}C$. Symbol shapes represent fathead minnow (circles), lake chub (squares), Prussian carp (diamonds) and white sucker (triangles). Note, symbols denoting fish age have been offset in the figure for visualization, but all fish ages are recorded in whole number values.

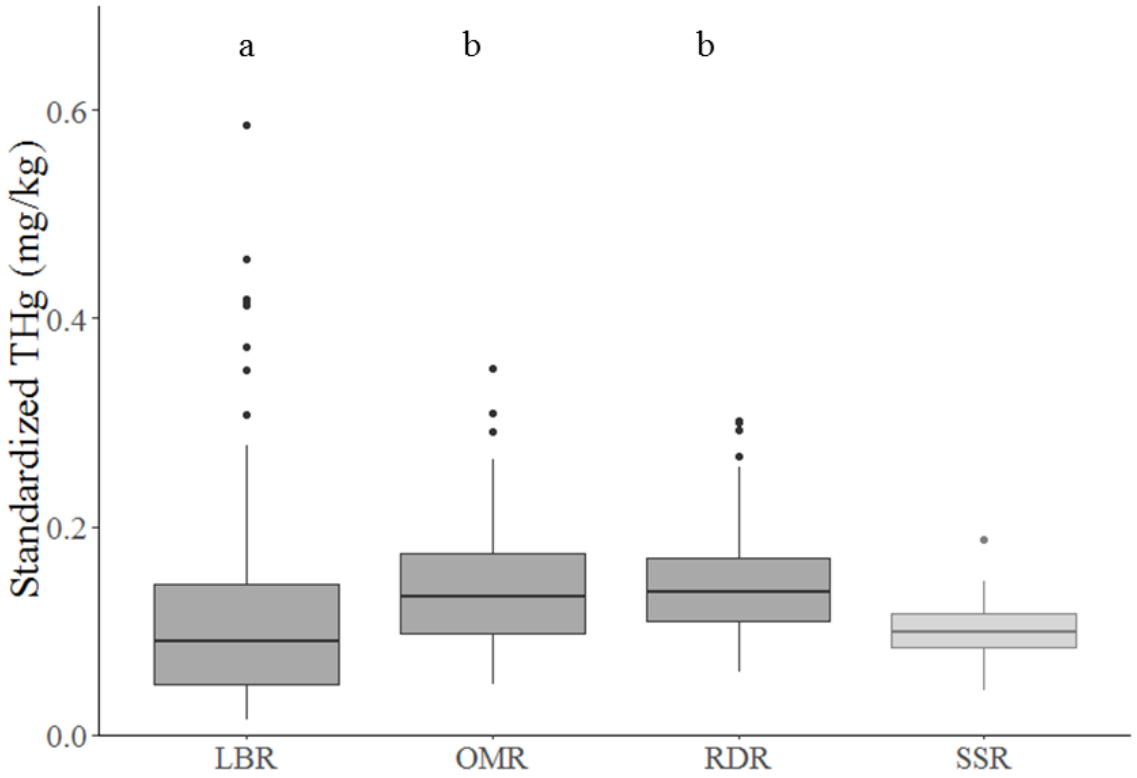

Figure 2.5. A comparison of standardized total mercury concentrations (THg; wet weight) in white suckers sampled from various waterbodies in southern Alberta between 1997 and 2017. Waterbodies are grouped into Little Bow River (LBR), Oldman River (OLM), Red Deer River (RDR) and South Saskatchewan River (SSR). Boxplots represent the median and quartile ranges (25^{th} and 75^{th}), whiskers represent ± 1.5*inter-quartile range from the 25^{th} and 75^{th} quartiles. Fish from waterbodies with the same letter above boxplots are not significantly different ($p>0.05$). SSR (light grey) was not included in statistical comparison due to low sample size ($n = 10$). Chapter 3: Biological factors moderate trace element accumulation in fish along an environmental concentration gradient

Chapter 3: Biological factors moderate trace element accumulation in fish along an environmental concentration gradient

3.1 Executive Summary

Trace elements in freshwater systems can accumulate in fish, becoming potentially hazardous to fish health as well as piscivorous wildlife and human consumers. High surface water concentrations of heavy metals in the Red Deer River, Alberta, Canada have raised concern for potential accumulation in aquatic biota, particularly in the badlands region of the watershed. The objectives of this research were to evaluate trace element concentrations in fish tissue, assess the influence of food web dynamics on concentrations, and examine potential spatial patterns in fish tissue concentrations along the river. Fish muscle tissue was analyzed for 20 elements and $\delta^{15}N$ and $\delta^{13}C$ were used as indicators of trophic position and dietary carbon source, respectively. Mercury, zinc, and selenium were detected in 100% of fish (mean 0.38 ± 0.32 mg/kg, 6.83 ± 3.0 mg/kg, 0.76 ± 0.34 mg/kg, respectively), arsenic in 99% (mean 0.05 ± 0.03 mg/kg,), chromium in 89% (mean 0.03 ± 0.11 mg/kg), and nickel in 91% (mean 0.02 ± 0.11 mg/kg). A principal component analysis revealed fish trophic position and dietary carbon source were correlated with mercury and arsenic concentrations, but not zinc, selenium, nickel, or chromium. However, fish body size was correlated to trace element concentrations in many species and the results suggest mercury and selenium biomagnify, whereas arsenic and zinc biodiminsh. Fish mercury concentrations raised concerns as 64% exceeded the criterion for issuing consumption advice for subsistence consumers of fish, 53% exceeded a threshold associated with potentially diminished fish health and 100% exceeded a tissue residue quality guideline for the protection of piscivorous wildlife. Very few fish exceeded suggested concentration criteria for other elements. Lastly, fish trace element concentrations showed no generalizable spatial patterns and were not reflective of

differences in surface water concentrations. My results indicate biological factors influence trace element accumulation, and trace element concentrations in fish are not locally restricted to areas of relatively high aqueous concentrations in riverine environments.

3.2 Introduction

Fresh waters provide critical habitat and services to living organisms, but can become impaired through the introduction of contaminants such as trace elements, plastics, nutrients, suspended sediments, and pesticides. Contaminants are difficult to control once in the aquatic ecosystem due to the complex interactions between the contaminant and the environment (Schwarzenbach et al. 2006). Trace elements are naturally-occurring substances that can be mobilized by anthropogenic activities (Younger 1997, Domagalski 2001, Schwarzenbach et al. 2006). Many trace elements are notorious aquatic contaminants because they can persist in the environment for long periods of time, even after the external source has been eliminated (Harada 1995, Younger 1997). Once in the freshwater system, trace elements can become incorporated into fish tissues, creating a potential health hazard to the fish, as well as the humans and wildlife who may consume them. Trace elements in the environment may be present in high enough concentrations to cause fish mortality, but most often occur at lower concentrations resulting in sublethal effects (Beckvar et al. 2005, Kumari et al. 2017).

Aquatic organisms may incorporate bioavailable trace elements into their tissues from the water column. Fish assimilate contaminants over time, making them useful as indicators of long-term changes in water quality. However, biological factors may influence the assimilation of bioavailable trace elements into fish tissues from the surrounding environment. Trace element concentrations in fish tissues often differ significantly among species (Penland et al. 2018), and can be related to factors including but not limited to body size (Al-Yousuf et al. 2000, Eagles-

Smith et al. 2016a), feeding guild (Ali and Kahn 2018), trophic position (Sakata et al. 2015), growth rate (Sandheinrich and Drevnick 2016), and sex (Madenjian et al. 2015). Dietary uptake through the gut is a major pathway of contaminant accumulation in fish tissues. Trace elements that are transferred via dietary interactions in the food chain may be subject to biomagnification. Biomagnification occurs when organisms have increasing concentrations of a contaminant relative to prey sources because the dietary absorption of the contaminant exceeds the rate of elimination (Gobas and Morrison 2000, Ali and Khan 2018). Stable isotopes can be used to estimate fish trophic position and dietary carbon source (Vander Zanden and Rasmussen 2001). Pairing trace element and stable isotope analysis can provide insight into fish trace element accumulation in food webs (Reinhart et al. 2018, Griboff et al. 2018). Food webs are dynamic, and can differ among fresh water systems. Therefore, examining biological factors that influence levels of trace elements in fish are needed alongside environmental data to understand patterns of contamination.

The Red Deer River is a popular angling destination in southern Alberta, Canada. A recent study found high concentrations of trace elements, such as mercury, lead, cadmium, and copper, in the Red Deer River at peak concentrations comparable to industrially-impacted rivers globally (Kerr and Cooke 2017), causing concerns related to fish consumption and potential risk to human health. These high trace element concentrations were linked to suspended sediment supply from the erosion of the badlands, and trace element concentrations increased from upstream (outside of the badlands) to downstream (within the badlands) (Kerr and Cooke 2017). In this study, I sought to understand if these high concentrations in the Red Deer River have influenced trace element concentrations in fish, and to investigate the effect of environmental and biological factors on fish tissue trace element concentrations. My objectives were 1) determine the trace

element concentrations in fish muscle tissue and assess whether they exceed suggested concentration criteria, 2) utilize stable isotope analysis (SIA) to examine the influence of trophic position and dietary carbon source on fish trace element concentrations, and 3) conduct an upstream to downstream spatial analysis on fish tissue to determine if fish trace element concentrations are influenced by environmental concentrations. I hypothesized that fish body size and δ^{15}N would be strongly associated with fish contaminants levels. Additionally, more negative δ^{13}C signatures (indicative of instream carbon source) would be associated with higher levels of contaminants. Finally, while differences in contaminant levels among species are expected, I hypothesized that fish sampled from downstream badlands sites would have elevated concentrations of contaminants compared to individuals of the same species sampled upstream of the badlands.

3.3 Methods

3.3.1 Study area

The Red Deer River originates in the Rocky Mountains of Alberta and flows southeast through the foothills and grassland into Saskatchewan where it joins the South Saskatchewan River (Campbell 1977a). The 780 km long river is a part of a watershed which occupies 49 650 km^2; and farmland and pastures cover approximately 75% of the watershed (Campbell 1977a, Kerr and Cooke 2017). The cities of Red Deer (the largest urban center, population 100, 418; Statistics Canada 2017) and Drumheller (population 7,982; Statistics Canada 2017) are located along the river. Oil and gas activity have a significant presence throughout the watershed (Aquality Environmental Consulting Ltd, 2009). A flow regulation dam, the Dickson Dam, impounds the Red Deer River approximately 50 km upstream of the City of Red Deer creating Gleniffer Lake. The underlying geology of the watershed changes abruptly with the erosion of

the badlands. Upstream of the Badlands, the bedrock geology is dominated by Quaternary clay-rich alluvium, while the outcropping Badlands are Cretaceous in age (Edmonton Formation) and are primarily clays and bentonite (Allan 1922). The Badlands also contain ironstone bands as well as coal (Allan 1922). The majority of sediment in the Red Deer River is contributed by the badlands, which make up two percent of the basin area (Campbell, 1977b).

3.3.2 Sampling design and fish collection

Fish from eight study sites were sampled along the Red Deer River in 2014, 2015, and 2017. Sites located upstream of the City of Red Deer were considered upstream of the badlands region. Unfortunately, an outbreak of whirling disease in 2017 prevented resampling of upstream areas, so only sites downstream of Red Deer were sampled in 2017. Fish were sampled by boat electrofishing, identified to species and measured to fork length. In 2014–2015, large sport fish were targeted for collection by the Government of Alberta for the purpose of generating fish consumption advice for anglers. To gain a more complete understanding of contaminant levels in fish and drivers of accumulation, a broader range of fish size classes was collected in 2017.

3.3.3 Sample processing

Skinless, boneless muscle tissue samples were collected for analysis. Muscle tissue sampled from fish in 2014 and 2015 was analyzed wet, whereas fish tissues from 2017 were freeze-dried before analysis. For freeze-dried samples, muscle tissue was placed into clean glass vials and then freeze dried. Muscle samples were weighed before and after freeze-drying to determine percent moisture of the tissue and convert trace elements detected in tissues to wet weight concentrations. Freeze-dried muscle samples were homogenized using a stainless steel pulverizing instrument or ceramic mortar and pestle until a consistent powder was reached. Once

dry fish material was analyzed, these values were converted to wet weight concentrations using the approach of Magalhães et al. (2007) as follows:

$C_W = C_D * ((100-\%H)/100)$

Where C_w is the concentration of the trace element in wet weight, C_D is the concentration of the trace element in the dry tissue. %H is the moisture percentage and calculated for each muscle sample to reduce error associated with using an assumed percentage (Cresson et al. 2017). Due to time constraints, about 25% of freeze-dried samples did not have individual moisture values measured. Fish tissue moisture values were consistent (80% ± 0.02, mean ± standard deviation) and similar to literature values used to calculate contaminant wet-weight concentrations (approximately 80%; Scudder-Eikenberry et al. 2015, Carr et al. 2017). Therefore the moisture content was replaced with the average from the previously measured individuals of the same species for wet weight trace element calculations.

3.3.4 Mercury analysis

Total mercury concentrations were analyzed in 321 fish muscle tissue samples. Total mercury here refers to all organic and inorganic species of mercury in a sample and here is simply referred to as "mercury". Fish mercury concentrations were obtained following EPA method 7473 (U.S. EPA 1998). Tissue was analyzed with a Milestone Direct Mercury Analyzer (DMA-80) where samples were thermally decomposed, and the vapor was trapped on a gold amalgamator for quantitation using atomic absorption detection. Quality control was conducted using certified reference material (CRM) controls within ±10% of certified values, and with duplicates within ±10% value of each other (Table A3.1). The first value was reported for duplicate samples.

3.3.5 Trace elements analysis

A total of 204 fish were examined for a suite of 20 elements (Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sb, Ba, Tl, Pb, Th, and U) using inductively coupled plasma mass spectrometry (ICP-MS). Fish samples were weighed into a microwave digestion vessel. Fish tissue was digested in five milliliters nitric acid (Optima grade, Fisher) completely using microwave digestion (CEM Mars 6 acid digestion system) before analysis. The vessels were kept loosely capped overnight in the fume hood. Five mL of de-ionized water was added into each sample. Extra acid was removed after digestion by transferring the sample into a 50 mL beaker and evaporated at 200 °C. Prior to instrumental analysis, the solution was diluted to 10 mL with 2% nitric acid. The total trace elements analyses were conducted using a CETAC autosampler coupled with an Agilent ICP-MS. The ICP-MS detection was operated at radio frequency 1550 W. The carrier gas flow rate was 0.9 to 1.0 L/min, and m/z 75 was monitored for arsenic. Quality assurance and quality control protocols included duplicate procedural blanks during each run, re-analysis of the calibration curve every 10 to 15 samples and comparison of measured values to standard reference material 1566b Oyster tissue (Table A3.1).

3.3.6 Stable isotope analysis

Sample weights were often not sufficient to conduct all analyses on the same individual; therefore only 148 fish were analyzed for stable isotopes. Fish muscle tissue was analyzed for $\delta^{15}N$ and $\delta^{13}C$ ratios using a Vario Pyrocube elemental analyzer coupled to an Elementar IsoPrime visIon continuous-flow isotope ratio mass spectrometer. Isotope ratios are reported in per mil (‰) and were determined as follows:

$$\delta R‰ = ((R_{sample}/R_{standard})-1) \times 1000$$

Where δR‰ is the heavy isotope, R_{sample} indicates the ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample, and $R_{standard}$ for $\delta^{15}N$ or $\delta^{13}C$ is referenced to that in Vienna Pee Dee Belemite and air, respectively. Every 20 samples throughout analyses, NIST 8415 whole egg powder SRM was also used as an in-house $\delta^{15}N$ (6.89‰) and $\delta^{13}C$ (-23.99‰) QA/QC check with a precision of $\delta^{15}N$ ±0.2‰ and $\delta^{13}C$ ±0.01‰, respectively. Tissue weights for river shiners were low due to small body size (18 individuals with less than 0.60 mg of tissue). However, ranges of $\delta^{13}C$ and $\delta^{15}N$ compared to river shiner samples with higher tissue weights showed comparable values ($\delta^{13}C$: -24.07 to -26.80 low weight compared to -24.95 to -26.80 high weight; $\delta^{15}N$: 8.8 to 14.2 low weight, 10.4 to 14.4 high weight).

3.3.7 Data analyses

Data analysis was conducted in R (version 3.5.3; R Core Team 2019). Data manipulation and visualization was done using dplyr (Wickham et al. 2019), ggplot2 (Wickham 2019), ggpubr (Kassambara 2019), ggforce (Pedersen 2019), ggthemes (Arnold 2019) and rcompanion (Mangiafico 2019) packages.

Our analysis focused on goldeye (*Hiodon alosoides*), sauger (*Sander Canadensis*), mountain whitefish (*Prosopium williamsoni*), longnose sucker (*Catostomus catostomus*), shorthead redhorse (*Moxostoma macrolepidotum*), white sucker (*Catsostomus comersonii*) and river shiner (*Nortropis blennius*) where at least 10 individuals had each type of chemical analysis completed (Table 3.1). One exception, walleye (*Sander vitreus*), was included despite only a small number of individuals with completed stable isotope analysis (n = 3) because it is a species commonly consumed by anglers. Of the 21 elements analyzed, all were detected in fish tissue but some at very low rates (e.g., U, Pb, and Be; Table A3.2). Therefore, only a subset of trace elements was

included in data analyses. Trace elements considered in analyses were found on U.S. EPA list of priority pollutants (U.S. EPA 2014) and were detected in at least 85% of fish analyzed (mercury, zinc, arsenic, chromium, selenium, nickel). The detection rate of 85% was selected to avoid misleading conclusions due to differences in methodology between sampling years which may have resulted in differing ability to detect trace metals in fish tissue (e.g., wet vs. dry analysis). For samples where the concentrations were below the method detection limit, the concentration was assumed to be one half the detection limit.

Comparisons of fish contaminant concentrations were made among species to identify patterns in accumulation linked to species specific factors. The data did not satisfy the assumptions of normality and homogeneity, therefore Kruskal-Wallis tests were used to compare between group differences (Gao et al. 2015, Burns and Riva-Murray 2018). Dunn's post hoc tests were conducted for pairwise comparisons among species with a holm p adjustment correction for multiple comparisons (Burns and Riva-Murray 2018). Kruskal-Wallis and Dunn's post hoc test were done using the FSA package in R (Ogle et al. 2018).

Principal component analysis (PCA) was conducted using the ggfortify package (Horikoshi and Tang 2016) to assess the influence of fish size, trophic position, and dietary carbon source. PCA is an ordination technique, which orients the data to maximize variation along an axis. Eigenvalues are calculated to represent the amount of variance explained by each axis and presented as principal components (PCs; Zuur et al. 2007). Loading values indicate correlations between variables and PCs and vary between +1 to -1, indicating the direction and strength of the correlation (Fletcher et al. 2014, Zuur et al. 2007). Important loadings were assessed as greater than or equal to ± 0.4 to aid interpretability of the results. Component loadings can be visualized by being overlain on PCA biplot as vectors over the data, where the direction and angle between

loadings indicate correlation of variables. Unfortunately, missing values cannot be included in PCA, therefore only fish that had all relevant analyses were considered (n = 120). Variables were normalized prior to PCA by computing z-scores. Outliers can drive PCA output (Zuur et al. 2007), therefore two outliers were removed before analysis due to high values. PC1, 2, and 3 were selected for presentation because they had eigenvalues greater than one, and a scree plot was used to determine a cutoff point based on the "elbow effect" (Zuur et al. 2007).

To further examine the potential for contaminant biomagnification in the aquatic food web, a regression of log10 transformed fish tissue concentrations vs $\delta^{15}N$ was created for each trace element. Significant relationships with a positive slope indicate biomagnification in the aquatic food web, whereas negative slopes indicate a biodiminishing trend (Jardine et al. 2013, Sakata et al. 2015).

Certain trace elements are known to vary significantly with fish body size (e.g., mercury; Eagles-Smith et al. 2016a). Therefore, regression analysis was also used to examine trace element concentrations against fish fork length within species. As extreme values can have strong effects on regression analysis, two goldeye outliers were removed from the analysis due to being very small in size compared to the rest of goldeye sampled (z-score < - 4). Contaminants were log transformed where necessary to meet model assumptions of normality and homogeneity. Not all fish species were consistently sampled throughout the river reaches. In particular, mountain whitefish were only captured at upstream sites, whereas sauger, river shiner and white sucker were only collected in middle or downstream sites. Therefore, only walleye, goldeye, longnose sucker and shorthead redhorse were selected for spatial analysis because they were sampled throughout the river. For spatial comparisons of fish contaminants, sites were combined into upstream (two sites), middle (three sites) and downstream (three sites) reaches. If a significant

relationship between fish trace element concentrations and fork length was observed in the regression analysis, concentrations were length-standardized for that species and trace element. For length standardization, linear regression was used to predict fish trace element concentrations at the median length for each species, and residuals were added back to predicted values to calculate standardized values prior to spatial analysis. Trace element concentrations were then compared within each species, among river sections by Kruskal-Wallis test. Pairwise comparisons were made with Dunn's post hoc test.

3.4 Results

3.4.1 Fish trace element concentrations and comparison to suggested protective criteria

Trace element concentrations in fish tissue were highly variable among species (Figure 3.1). Mercury, zinc and selenium were detected in 100% of fish analyzed, arsenic in 99%, chromium in 89% and nickel in 91%. Further information on elements detected in less than 85% of fish sampled is summarized in Table A3.2. Mercury, selenium, and zinc concentrations in fish tissue averaged 0.38 ± 0.32 mg/kg, 0.76 ± 0.34 mg/kg, and 6.83 ± 3.0 mg/kg, respectively. In contrast, lower average concentrations of nickel (0.02 ± 0.11 mg/kg), arsenic (0.05 ± 0.03 mg/kg), and chromium (0.03 ± 0.11 mg/kg) were noted. The highest mercury concentrations were found in popular sport fish species: walleye, sauger, and goldeye (Figure 3.1). High concentrations of selenium and arsenic were also found in some sport fish species such as mountain whitefish and goldeye, whereas zinc and arsenic concentrations were elevated in mostly non-sportfish species occupying low trophic levels. There was considerable overlap in chromium, nickel, and zinc concentrations among species.

Mercury suggested concentration criteria for humans, piscivorous wildlife and fish health were exceeded by 29 to 100% (Table 3.2). When only considering sport fish species, 83% exceeded a

criterion at which issued fish consumption advice suggests limiting consumption for subsistence consumers (0.2 mg/kg, n=145; Government of Alberta 2019a). Mercury concentrations in fish also exceeded a tissue residue quality guideline for wildlife consumers, as well as a threshold suggested for the protection of fish health. Criteria for protection of humans were not exceeded by selenium in fish tissue. Selenium concentrations in less than one percent of fish exceeded suggested criteria for the protection of aquatic life. No current consumption guidance or protective criteria for nickel, zinc or chromium concentrations could be found for fish muscle tissue.

3.4.2 Fish biological factors and influence on trace element concentrations

Analysis of $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios illustrated two general trophic tiers occupied by the fish species sampled (Table 3.1). Average $\delta^{15}N$ values were highest in goldeye, followed closely by sauger and walleye (all around 15 ‰), indicating occupation of top-trophic levels in the food chain. Sucker and cyprinid species shared lower $\delta^{15}N$ values (~11–12 ‰), thus occupying a lower trophic level. A biplot of fish $\delta^{13}C$ and $\delta^{15}N$ values was constructed to portray the position of each species in the aquatic food web, as illustrated respectively by the signature of their potential carbon source ($\delta^{13}C$) and food chain location ($\delta^{15}N$) (Donald et al. 2015, Vander Zanden et al. 1999; Figure 3.2). While sucker and cyprinid $\delta^{15}N$ values indicated they occupy a similar trophic position, a large range in $\delta^{13}C$ values suggested they utilize a diversity of dietary sources. Similarly, sauger and walleye $\delta^{13}C$ values indicated differing dietary sources to goldeye despite all species occupying a top trophic level. Omnivory is suspected in species whose range of $\delta^{15}N$ values exceed the fractionation value (3.4‰; Vander Zanden and Rasmussen 1996) representative of one trophic level (Jespsen and Winemiller 2002). Species

with ranges of $\delta^{15}N$ which exceeded 3.4 in this study are mountain whitefish (5.5), river shiner (5.6), shorthead redhorse (6.2), longnose sucker (7.6), sauger (8.3) and goldeye (8.7). Only white sucker and walleye did not show indications of omnivory (3.1 and 2.9, respectively), although conclusions for walleye may be limited due to a small sample size (n = 3).

Biological factors, such as dietary carbon source, position on the food chain and body size, were highly correlated with some trace elements in the PCA (Figure 3.3, Table 3.3). Taken together, PC1, 2, and 3 captured ~73% of the variability of the data. PC1 explained ~32% of the variation in the data and was positively correlated with fish fork length. When the relationship between trace element concentration and body length within species was examined, some significant relationships were detected (Table 3.4). In particular, mercury was strongly related to fish body size, and accounted for a large proportion of the variation in the mercury concentration data for most species. PC2 captured ~27% of the variation in the data, and was driven by mercury, arsenic, $\delta^{15}N$ and $\delta^{13}C$ variables (Figure 3.3, Table 3.3). Mercury, $\delta^{15}N$, and $\delta^{13}C$ all shared negative loading values along PC2 indicating correlation among these variables, and there is a high degree of correlation between mercury and $\delta^{15}N$. A positive arsenic loading value indicated a negative correlation with mercury, $\delta^{15}N$ and particularly $\delta^{13}C$ values, suggesting a potential dietary influence on fish arsenic concentrations which operates in the opposite direction to mercury. PC3 was not driven by fish biological factors, instead by selenium and zinc loadings. Significant relationships with $\delta^{15}N$ were found between mercury, selenium, arsenic, and zinc. $\delta^{15}N$ values only explained a substantial proportion of the variation for mercury ($R^2 = 0.48$; Table 3.5). For selenium, arsenic, and zinc, the proportion of variance explained was less than 0.10. For mercury and selenium, the resulting slope was positive, indicating these trace elements

biomagnify with increasing trophic level. Negative slopes for zinc and arsenic, indicate these trace elements biodiminish.

3.4.3 Spatial comparison of fish trace element concentrations

Contrary to expectations, fish trace element concentrations did not increase from upstream to downstream but instead differed depending on the species or trace element being examined. Most trace element concentrations showed no significant differences when comparing within a species, upstream to downstream (Figure 3.4). Furthermore, where significant differences were found, the concentrations were elevated in upstream and/or middle regions of the river in comparison to downstream sites. Thus, the fish tissue results differ from the clear spatial pattern in water quality (Kerr and Cooke 2017), which shows an increase in trace element concentrations downstream of the Badlands.

3.5 Discussion

In contrast to expectations, only five priority pollutants were detected in the majority of fish sampled. In their study of water quality in the Red Deer River, Kerr and Cooke (2017) highlighted concerns about elevated concentrations and loads of mercury, cadmium, copper and lead. Mercury was detected at elevated concentrations in all fish sampled, but cadmium, copper and lead were detected at much lower rates (57% for copper, 44% for cadmium and 0% for lead) despite their high concentrations in the water column. One of the key objectives of this study was to evaluate fish trace element concentrations as they relate to risk to human health, therefore fish muscle tissue was examined. Unlike mercury, many other trace elements do not display a high affinity for accumulation in fish muscle tissue (Goldstein et al. 1996, Squadrone et al. 2013, Fletcher et al.2014, Dhanakumar et al. 2015). Nevertheless, given the elevated concentrations of contaminants in the river system, it is likely that accumulation of these metals is occurring in

other organs of the fish. Like in humans, fish kidneys and liver play a role in the detoxification of contaminants (Hamilton and Mehrle 1986). Therefore, these organs may sequester elements, impeding their accumulation in muscle tissues. Fletcher et al. (2014) found cadmium, copper, and lead concentrations in fish liver tissue at much higher concentrations than muscle from the same individuals. Dhanakumar et al. (2015) documented similar results for lead and copper concentrations, where concentrations in kidney and liver samples were often orders of magnitude higher than muscle tissue. The accumulation of metals may cause damage to these important detoxifying organs in fish (Abalaka 2015). Thus, because this study was initiated to investigate concerns related to human consumers, the extent to which the fish themselves may be impacted requires further investigation. Moreover, piscivorous wildlife often consume whole fish, and would be exposed to contaminants sequestered in detoxifying organs. Further studies of trace element concentrations in other organs are necessary not only to assess fish health, but also the risk to wildlife consumers.

Mercury was often detected in fish muscle at elevated concentrations in this study. Mercury is one of the most widespread and toxic contaminants in freshwater systems. Inorganic mercury can be converted to methylmercury, an organic form with known neurotoxic effects in humans (Harada 1995) in the environment by sulphate and iron-reducing bacteria (Lin et al. 2011). Methylmercury has a strong tendency to biomagnify in aquatic food chains, thus almost all mercury accumulated in the tissues of fish is methylmercury (Bloom 1992). Consuming fish is generally encouraged because they provide an important nutritional source of omega-3 fatty acids, vitamins and minerals (Sidhu 2003). However, fish mercury concentrations in this study regularly exceed criteria at which issued fish consumption advice suggests limiting consumption for subsistence consumers and for the protection of aquatic biota. In humans, consumption of

mercury in excess can result in cognitive impairments, balance issues, and sensory disturbances, tremors, ataxia and death (Harada 1995). The high mercury concentrations in several sought after sport-fish species sampled in this study could pose a risk to the health of human consumers, particularly children and pregnant women, if these fish are consumed at rates greater than those recommended (e.g., Government of Alberta 2019a). Individuals should refer to public resources when deciding to consume fish from the Red Deer River (e.g., Government of Alberta 2019b). Some fish species, such as mountain whitefish and goldeye, were also elevated in selenium, which may have a protective effect against mercury toxicity (Ralston 2008, Burger et al. 2012). Methylmercury binds selenium in the body, inhibiting selenoenzyme activity in the brain (Ralston 2008). Increased selenium in the diet can replace that which is lost by methylmercury binding, providing protection against mercury toxicity (Ralston 2008). However, selenium itself can also be toxic, therefore any consideration of selenium as a protective agent should be done with caution (Janz 2011). Currently, consumption advice in Alberta is issued based on mercury concentrations alone, not in regard to other trace elements concentrations present in the tissue. Potential interactions between multiple contaminants on the health of human and wildlife consumers is an important area of consideration for future scientific research, government monitoring and fish consumption advice programs.

Mercury and arsenic concentrations in fish tissue were often correlated to fish biological factors and dietary tracers in this study. These results support previous research that shows mercury strongly biomagnifies in the food web and is tied to an organism's trophic position and dietary carbon source (Lavoie et al. 2013, Riva-Murray et al. 2013a, Donald et al. 2015). Arsenic concentrations in fish tissue have also previously been linked to trophic processes. Like in this study, Chen and Folt (2000) found a biodiminishing pattern of arsenic in the food web when they

examined organisms in a contaminated lake, with zooplankton concentrations much higher than those in fish. In their study, a dietary effect was also seen where strictly planktivorous fish had significantly higher concentrations of arsenic in their tissues than other omnivorous species. While there are no strictly plankitvorous species sampled in this study, fish with more depleted $\delta^{13}C$ signature were associated with higher arsenic concentrations indicating a potential dietary influence. In contrast, zinc, chromium, and nickel were not strongly linked to dietary tracers. Although the results of this study do not show selenium strongly tied to dietary tracers, other research suggests that the gut is a major site of uptake for selenium (Janz 2011), and fish exposed to the same ambient concentrations accumulate differing tissue concentrations related to their foraging strategy (Stewart et al. 2004). Fish $\delta^{13}C$ signatures in this study do suggest a diversity of dietary carbon sources, therefore future studies should investigate whether trace element concentrations of basal resources differ in the river to help understand these results. However, another pathway may be responsible for accumulation of trace elements in fish tissue which was not accounted for in this study: uptake from the water column through the gills. Palermo et al. (2015) demonstrated that nickel can be accumulated in fish muscle tissue from ambient exposure in laboratory experiments. However, of the four concentrations of nickel fish were exposed to (0 µg/L, 25 µg/L, 250 µg/L, and 2500 µg/L) only the highest treatment resulted in muscle tissue nickel concentrations significantly different from the 0 µg/L treatment. Zinc and arsenic can also be taken up by both gills and diet (Giardina et al. 2009, McIntyre and Linton 2011). Additionally, unlike arsenic and mercury which are considered elements with no biological benefit, nickel, chromium, zinc, and selenium have some amount of essentiality for fish and their uptake and sequestration in the body can be regulated (Tuzen 2009, Janz 2011, Hogstrand 2011, Pyle and Couture 2011). Therefore, potential uptake from sources other than diet and possible

regulation of essential trace elements may be factors contributing to fish contaminant concentrations not examined in this study.

Fish trace element concentrations did not reflect increasing aqueous trace element concentrations from upstream to downstream. In fact, there were a few examples of the opposite trend in both sport and non-sport fishes, where trace element concentrations were highest at upstream sites. The lack of an upstream to downstream pattern in fish trace element concentrations may result from the movement of large-bodied fish within the Red Deer River, which would normalize any spatial gradients in exposure. Goldeye have been recorded travelling long distances in other Albertan rivers between their wintering habitat and spawning grounds (Munson 1978, Donald and Kooyman 1977). Furthermore, although another species examined in this study, walleye, often exhibit fidelity to spawning and nearby feeding grounds (Bozek et al. 2011), other studies have suggested that contaminant concentrations in walleye caught during or recently after migration may reflect different habitats than the ones they were sampled from (Carr et al. 2017). While fish may undertake long-distance migrations, many are relatively stationary during non-migratory periods (Rodriguez 2002). All species included in the spatial comparison spawn during the spring (Scott and Crossman 1973), and should be occupying their feeding grounds by the time of sampling in late summer. To further investigate contaminant accumulation by aquatic biota, future studies should target sessile, non-migratory species.

Mercury in fish has been well studied and summarized for many species from across Canada (Depew et al. 2013) and western North America (Eagles-Smith et al. 2016a). Mercury concentrations in goldeye, longnose sucker, mountain whitefish and shorthead redhorse were similar to those from elsewhere in Canada and western North America (Depew et al. 2013, Eagles-Smith et al. 2016a). Mercury concentrations in walleye from this study (median = 0.69

mg/kg) were generally elevated compared to those from across western North America (0.31 mg/kg; Eagles-Smith et al. 2016a) and Canada (0.41 mg/kg; Depew et al. 2013). However walleye sampled in this study were generally larger (median = 528 mm) compared to those from Canada (469 mm; Depew et al. 2013) or western North America (430 mm; Eagles-Smith et al. 2016a). Since body size is often correlated with mercury concentrations in fish, relatively high walleye mercury concentrations as a result of biological factors cannot be ruled out. In contrast, white sucker from the Red Deer River had comparable mercury concentrations (median =0.13 mg/kg) to those from Canada (0.12 mg/kg) and western North America (0.12 mg/kg; Eagles-Smith et al. 2016a) but were generally much smaller (median = 284 mm; this study, 413mm; Depew et al. 2013, 361 mm; Eagles-Smith et al. 2016a), suggesting mercury concentrations in white sucker from the Red Deer River might be elevated compared to the larger area.

Published records of arsenic, selenium, nickel, zinc and chromium concentrations in muscle tissue of these species are scarcer than mercury. Selenium concentrations in fish from the Red Deer River were similar (Orr et al. 2006, Muscatello et al. 2008, Ofukany et al. 2014) or slightly elevated (Donald and Sardella 2010, Essig 2010, Ofukany et al. 2014, Penland et al. 2018, Matwee and Peitrock 2019) compared to other studies with no identified point sources. Fish selenium concentrations from the Red Deer River were also comparable to sites associated with coal fired power plants (Reash et al. 2015). Like mercury, body size is often correlated with selenium concentrations for fish in this study so comparisons may be confounded. However, selenium concentrations were lower by an order of magnitude or more in fish from this study compared to those from sites of known contamination (Muscatello et al. 2008, Orr et al. 2006). Likewise, arsenic concentrations in fish sampled from this study were on average similar to those from other studies with no identified point source (Donald and Sardella 2010, Essig 2010,

Ofukany et al. 2014, Matwee and Pietrock 2019), and relatively low compared to those from Giant Mine which has a legacy of arsenic contamination (de Rosemond et al. 2008). Nickel, zinc, and chromium concentrations in fish were similar to those from other studies (Ofukany et al. 2014, Donald and Sardella 2010, Penland et al. 2018, Matwee and Pietrock 2019). Additionally, chromium concentrations in fish from this study were over an order of magnitude lower than those found in rainbow trout in a waterbody exposed to natural geologic sources (Watkins and Bodensteiner 2010). Overall, when compared to fish of the same species sampled from other waterbodies, trace element concentrations are commensurate with those from previous studies, and lower than those with known sources of contamination.

3.6 Conclusions

This study focussed on contaminant concentrations in fish species from the Red Deer River, an area of elevated trace element concentrations in surface water (Kerr and Cooke 2017). Overall, trace element concentrations varied depending on fish species and trace element being examined. In particular, I found mercury was elevated in many species highlighting potential concerns for the health of fish, as well as human and wildlife consumers. Dietary tracers were correlated to mercury and arsenic concentrations among fish species indicating the concentrations of these trace elements may be moderated by dietary carbon sources and trophic position. Relationships between tissue concentrations and trophic position, but not dietary carbon source, were also found for selenium and zinc. Trace element concentrations in fish tissue were correlated with fish body size depending on fish species and the element being examined. While contamination from other sites may be a factor due to migratory movements of large-bodied fish, contaminants strongly tied to dietary sources should reflect the general area from which fish were sampled. Trace element concentrations in fish did not reflect surface water concentrations in this system

and my results suggest biological factors are strong moderators of contaminant accumulation. When compared to other studies, trace element concentrations in fish from the Red Deer River were overall similar to those without identified point sources, and lower than those with a known source of contamination. These results provide evidence that trace metal accumulation in fish is dependent on the trace metal being considered and species-specific biological factors, but not localized areas in river environments.

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3.8 Figures and Tables

Table 3.1. Summary statistics. Mean values (±) standard deviation of fork length (mm), wet weight trace element concentration (mg/kg) and stable isotope analysis (‰) for goldeye (GOLD), longnose sucker (LNSC), mountain whitefish (MNWH), river shiner (RVSH), sauger (SAUG), shorthead redhorse (SHRD), walleye (WALL) and white sucker (WHSC). Sample sizes (n) for each species are listed for each analysis.

Species	n	Fork Length	Hg	n	As	Se	Cr	Ni	Zn	n	$\delta^{13}C$	$\delta^{15}N$
GOLD	55	333 ± 53	0.44 ± 0.14	17	0.03 ± 0.01	1.27 ± 0.54	0.01 ± 0.01	0.02 ± 0.02	7.12 ± 2.7	11	-28.87 ± 0.89	15.5 ± 3.0
LNSC	40	355 ± 119	0.18 ± 0.11	35	0.06 ± 0.04	0.6 ± 0.15	0.01 ± 0.01	0.03 ± 0.04	6.68 ± 2.1	25	-28.58 ± 2.41	11.3 ± 2.2
MNWH	30	367 ± 32	0.16 ± 0.08	10	0.07 ± 0.01	1.52 ± 0.36	0.01 ± 0.01	0.01 ± 0.01	6.21 ± 0.94	10	-30.77 ± 0.85	12.1 ± 2.0
RVSH	40	68 ± 8	0.15 ± 0.06	26	0.04 ± 0.02	0.86 ± 0.12	0.08 ± 0.25	0.35 ± 1.14	12.35 ± 3.16	22	-25.97 ± 0.74	11.2 ± 1.3
SAUG	51	307 ± 76	0.64 ± 0.36	29	0.02 ± 0.01	0.64 ± 0.1	0.01 ± 0	0.03 ± 0.03	5.02 ± 1.17	28	-25.55 ± 0.70	14.5 ± 1.9
SHRD	50	291 ± 136	0.28 ± 0.19	49	0.07 ± 0.03	0.71 ± 0.19	0.03 ± 0.11	0.12 ± 0.56	6.44 ± 2.07	32	-27.52 ± 2.03	11.4 ± 1.7
WALL	38	545 ± 135	0.8 ± 0.41	22	0.03 ± 0.01	0.66 ± 0.12	0.01 ± 0.01	0.01 ± 0.01	5.1 ± 1.61	3	-24.68 ± 0.44	14.3 ± 1.7
WHSC	17	282 ± 94	0.18 ± 0.1	16	0.04 ± 0.02	0.41 ± 0.11	0.04 ± 0.1	0.21 ± 0.5	5.09 ± 1.28	17	-26.64 ± 1.00	11.5 ± 1.0

Table 3.2. Fish trace element concentrations compared to suggested criteria for the protection of fish, wildlife and humans. Recommended concentration criteria in fish tissue (Concentration) for the protection of aquatic biota and fish consumers (Receptor) are stated for each trace element, as well as the percentage and sample size (n) of fish in this study which exceed these criteria (Percent exceeded). Some concentration criteria have been modified from whole body (wb) or dry weight (dw) concentrations for comparison to muscle tissue wet weight (ww) concentrations. For modified criteria, the original concentration value is stated in brackets.

Trace element	Concentration (mg/kg ww)	Receptor	Reference	Percent exceeded (n)
Mercury	0.20	Humans – limit consumption of wild fish for subsistence consumers	Government of Alberta (2019a)	64 (204)
Mercury	0.50	Humans – avoid consumption of wild fish	Government of Alberta (2019a)	29 (93)
Mercury	0.033	Wildlife consumer of aquatic biota	CCME (2000)	100 (321)
Mercury	0.27[a], (0.2 wb)	Fish health	Beckvar et al. (2005)	53 (169)
Selenium	2.5	Humans – subsistence fishers	U.S. EPA (2000)	0
Selenium	20	Humans – recreational anglers	U.S. EPA (2000)	0
Selenium	2.26[b], (11.3 dw)	Aquatic Life Ambient Water Quality Criterion	U.S. EPA (2016)	0.5 (1)

[a]Converted from whole body to muscle concentration by dividing by 0.74 following Eagles-Smith et al. (2016a)
[b]Converted from dry weight to wet weight assuming 80% moisture content

Table 3.3. Loading values for the principal component analysis. Correlations are displayed between fish fork length (length), dietary tracer signature ($\delta^{13}C$, $\delta^{15}N$), trace element concentration (mercury, arsenic, selenium, nickel, chromium and zinc) and principal components (PC1, PC2, and PC3). Influential variables (correlation greater than +/- 0.4) are italicized.

	PC1	**PC2**	**PC3**
Length	*0.60*	0.04	0.16
$\delta^{13}C$	-0.37	*-0.49*	-0.07
$\delta^{15}N$	0.36	*-0.41*	-0.37
Mercury	0.37	*-0.47*	-0.08
Arsenic	0.20	*0.52*	0.11
Selenium	0.31	0.25	*-0.63*
Nickel	-0.03	0.01	0.06
Chromium	-0.04	-0.02	-0.02
Zinc	-0.33	0.20	*-0.64*

Table 3.4. Regression correlation matrix between fish length and trace element concentrations. The coefficients of determination (R^2) from regression analysis between fish length and trace element concentration are displayed for each species. Bolded values indicate significant relationships ($P<0.05$).

Species	Mercury	Selenium	Arsenic	Nickel	Chromium	Zinc
Walleye	**0.60**	-0.05	-0.05	0.01	0.004	-0.02
Goldeye	**0.38**	**0.59**	-0.06	-0.06	0.12	-0.01
Longnose sucker	**0.76**	**0.20**	0.05	**0.16**	0.02	**0.21**
Shorthead redhorse	**0.90**	**0.12**	**0.14**	-0.01	0.05	**0.43**
White sucker	**0.70**	**0.62**	**0.45**	0.06	-0.03	**0.38**
Mountain whitefish	**0.26**	-0.06	0.14	-0.12	0.13	-0.12
River shiner	**0.26**	**0.16**	-0.03	0.04	**0.26**	**0.17**
Sauger	**0.67**	0.02	0.02	0.07	-0.004	-0.04

Table 3.5. Regression results for fish trace element concentrations vs $\delta^{15}N$. Trace element concentrations were compared to $\delta^{15}N$ to determine the potential for trace element biomagnification in the food web. Significant relationships ($p<0.05$) were found for mercury, selenium, arsenic and zinc. Positive slopes indicate biomagnification (mercury and selenium), and negative slopes indicate a biodiminishing relationship (arsenic and zinc).

Trace Element	Regression Equation	R^2	p-value
Mercury	$\log10[Hg] = 0.114*\delta^{15}N - 2.161$	0.48	**2e-16**
Selenium	$\log10[Se] = 0.019*\delta^{15}N - 0.434$	0.07	**0.002**
Arsenic	$\log10[As] = -0.034*\delta^{15}N - 1.079$	0.02	**0.049**
Nickel	$\log10[Ni] = -0.039*\delta^{15}N - 1.700$	0.02	0.072
Chromium	$\log10[Cr] = 0.033*\delta^{15}N - 2.508$	0.01	0.184
Zinc	$\log10[Zn] = -0.014*\delta^{15}N + 0.953$	0.04	**0.013**

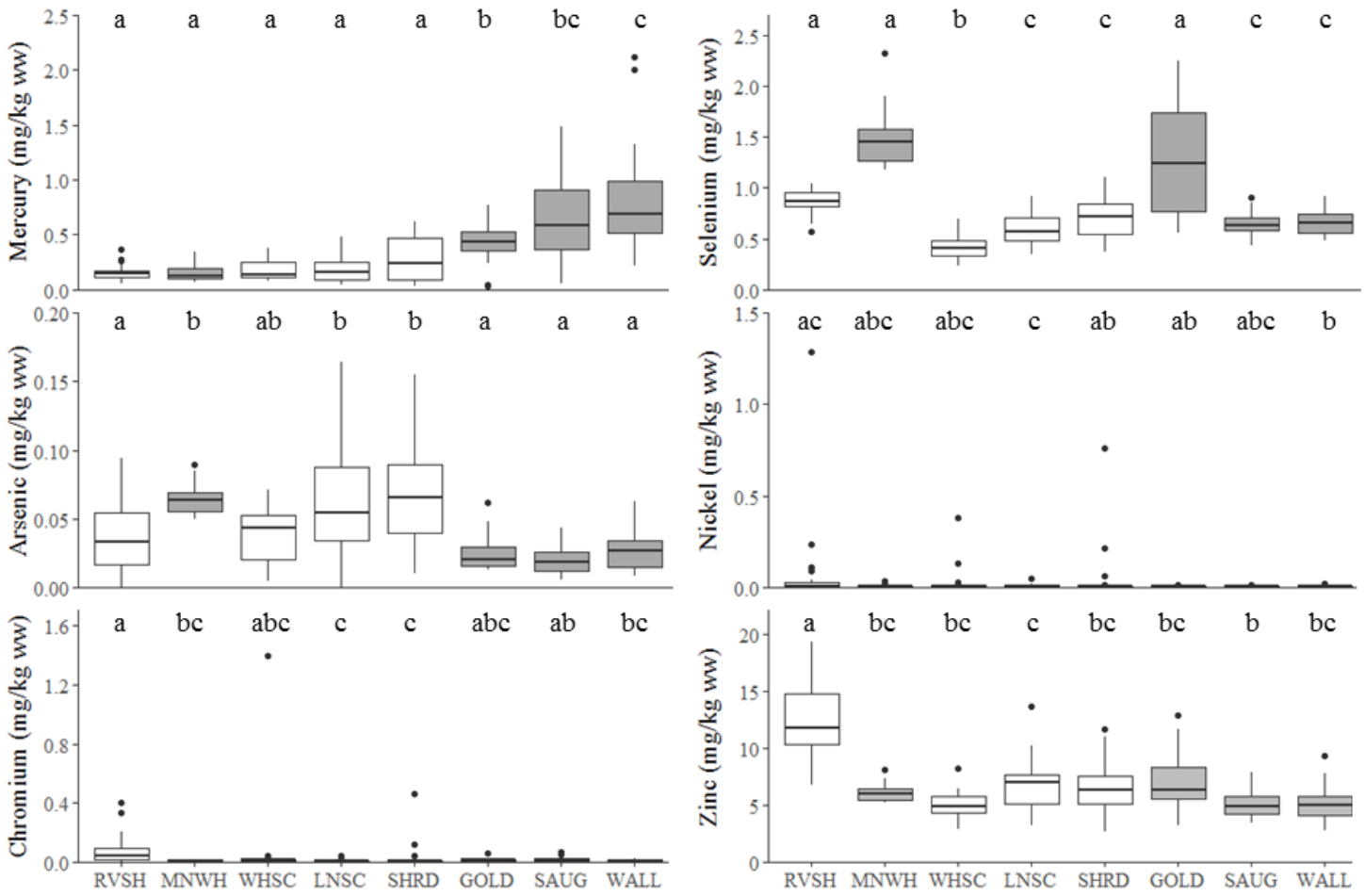

Figure 3.1. Comparison of trace element concentrations among fish species. Wet weight concentrations of each trace element were compared among eight species: river shiner (RVSH), mountain whitefish (MNWH), white sucker (WHSC), longnose sucker (LNSC), shorthead redhorse (SHRD), goldeye (GOLD), sauger (SAUG) and walleye (WALL). Shaded boxplots indicate sport-fish species. Significant differences were detected using a Kruskal-Wallis test and Dunn's post hoc pairwise comparisons. Boxplots sharing the same letter indicate no significant differences, whereas differing letters indicate differences between species.

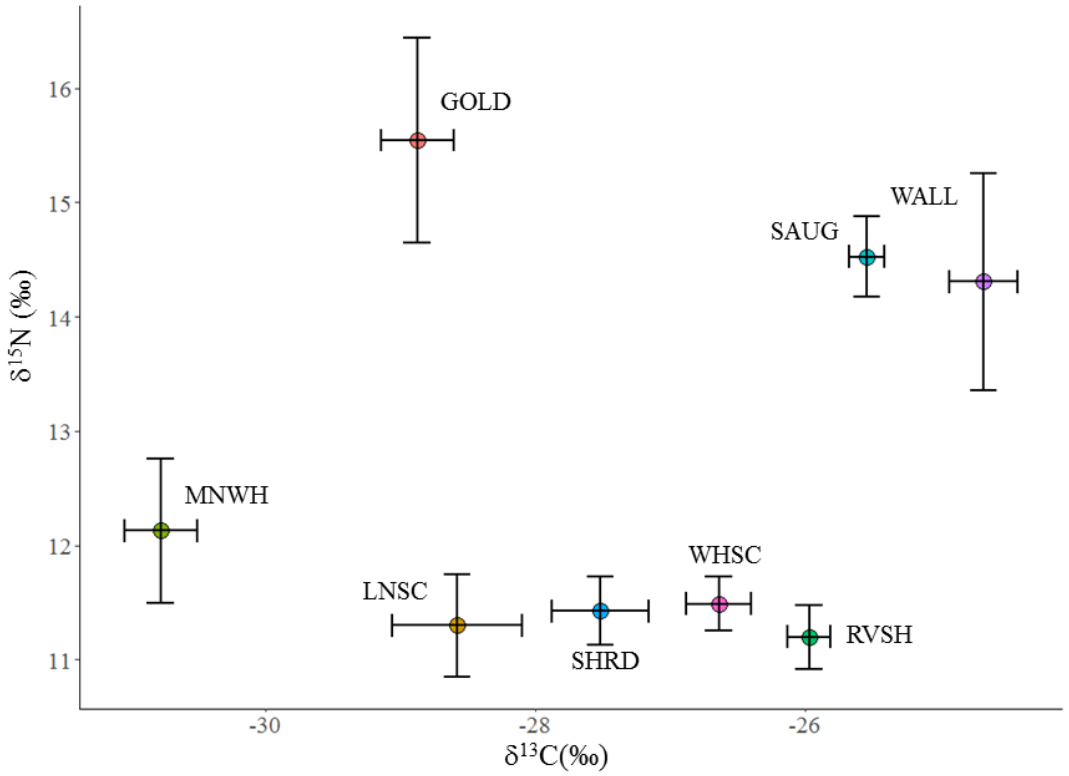

Figure 3.2. Stable isotope biplot of fish species. Dietary tracer ($\delta^{13}C$ and $\delta^{15}N$) signatures for river shiner (RVSH), mountain whitefish (MNWH), white sucker (WHSC), longnose sucker (LNSC), shorthead redhorse (SHRD), goldeye (GOLD), sauger (SAUG) and walleye (WALL) are displayed. $\delta^{15}N$ signatures indicate trophic position, and $\delta^{13}C$ signatures indicate source of dietary carbon. Mean values are indicated by points, and error bars represent standard error.

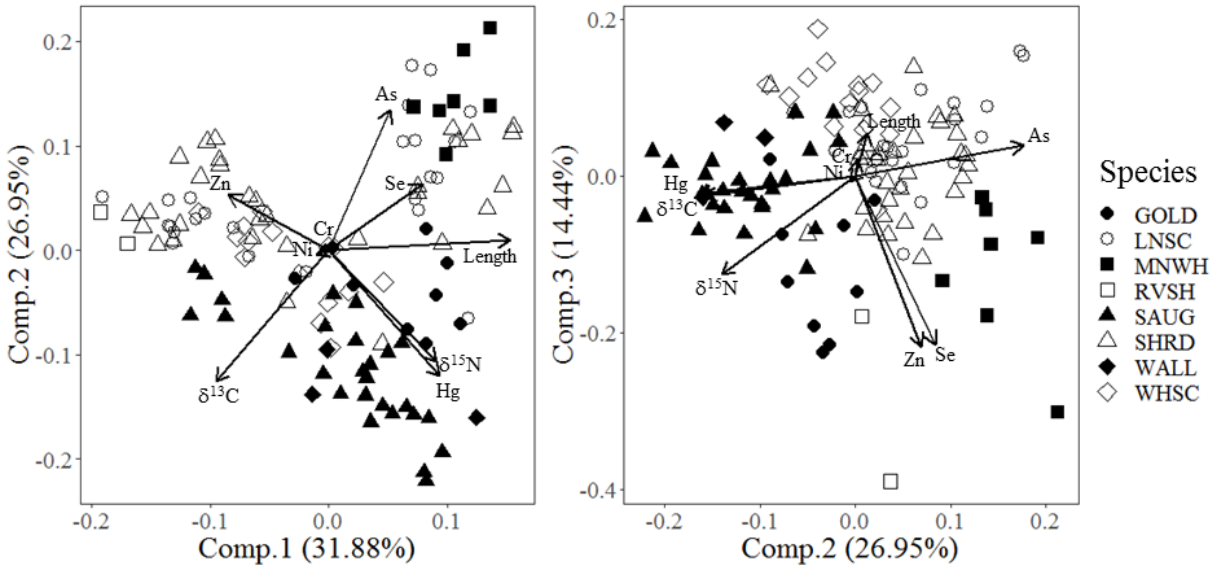

Figure 3.3. Principal component analysis (PCA) of fish trace element concentrations and biological factors. Correlations between fish body size (Length), trace element concentrations (As, Se, Hg, Cr, Ni and Zn), and dietary tracers ($\delta^{13}C$, $\delta^{15}N$) were examined using PCA among fish species river shiner (RVSH), mountain whitefish (MNWH), white sucker (WHSC), longnose sucker (LNSC), shorthead redhorse (SHRD), goldeye (GOLD), sauger (SAUG) and walleye (WALL). Percentages indicate percent of variation in the data explained by each principal component.

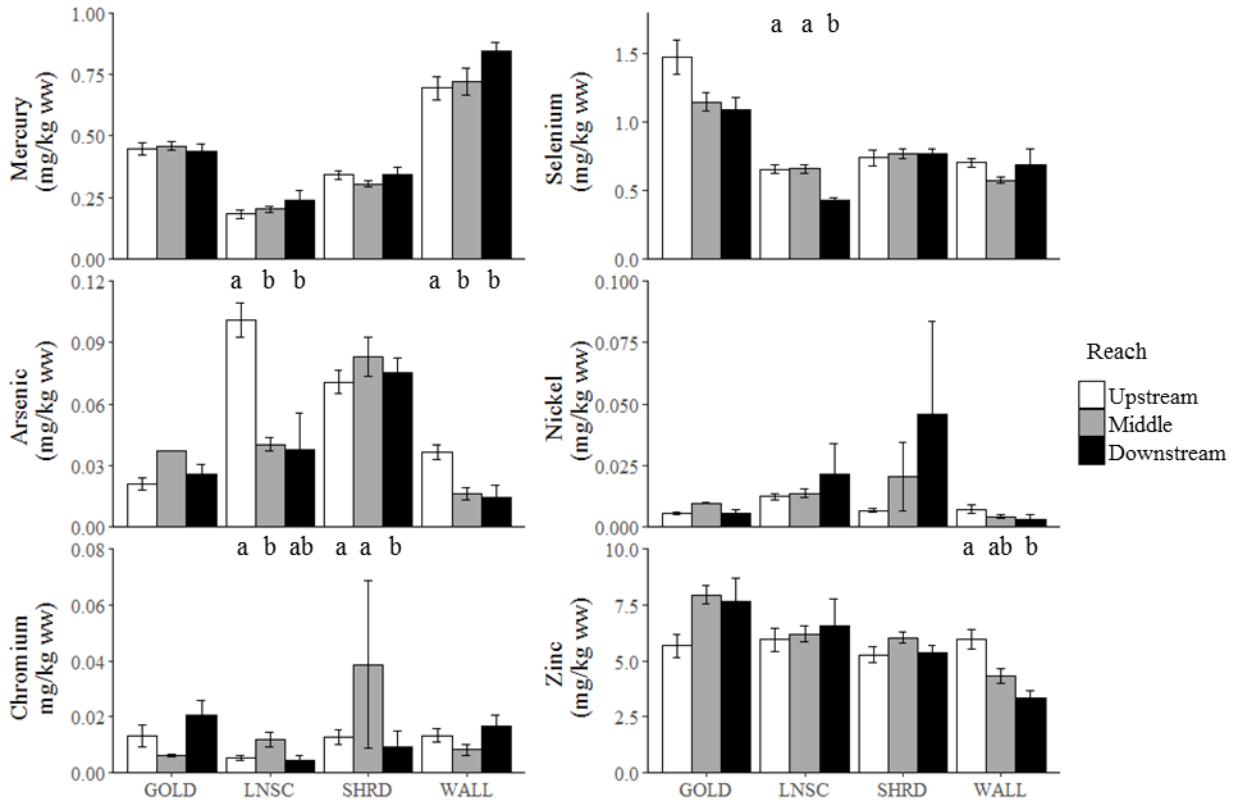

Figure 3.4. Spatial comparison of fish trace element concentrations among river reaches. Trace element concentrations were compared within species Goldeye (GOLD), Longnose Sucker (LNSC), Shorthead Redhorse (SHRD) and Walleye (WALL) collected from upstream to downstream reaches. Bars represent mean concentrations from upstream, middle, and downstream reaches. Trace element concentrations were standardized in species which showed a significant correlation between fork length and trace element concentration. Differences were detected using Kruskal-Wallis test and Dunn's post hoc pairwise comparison. Differing letters above bars indicate significant differences between reaches within each species; shared letters indicate no significant differences. Bars without letters indicate no significant differences among reaches. Error bars represent standard error.

Chapter 4: General Conclusion

The unifying goal of my research was to examine the relationship between patterns of trace element concentrations in fish and their environment. In both chapters, I assessed fish biological factors (trophic level, dietary carbon signature and body size) alongside patterns in surface water trace element concentrations to understand patterns of trace element accumulation in fish. I studied the Red Deer River watershed where previous research has identified trace elements in surface water at concentrations which raise concerns for the health of humans and wildlife (Kerr and Cooke 2017). My thesis focused on two lotic habitats within the Red Deer River watershed to address my research goal. In Chapter 2, I studied mercury in fish from small streams with variable surface water mercury concentrations. In Chapter 3 I examined mercury, in addition to other trace elements, in fish from the river mainstem, where surface water concentrations increase from upstream to downstream (Kerr and Cooke 2017).

Although mercury in the environment has been studied for several decades, how diet, physical characteristics and environmental factors drive mercury accumulation in riverine fish is still an area of emerging research (Pandey et al. 2017, Roxanna Razavi et al. 2019, Broadley et al. 2019). My thesis adds to our knowledge on fish mercury dynamics by examining factors that influence bioaccumulation in both stream environments (Chapter 2) and a larger mainstem community (Chapter 3). I found that patterns in fish mercury concentrations did not match those in surface water total mercury concentrations. My results complement findings by Eagles-Smith et al. (2016b), who found that fish mercury concentrations are not closely related to inorganic mercury contamination of the environment. In contrast, my results show that biological factors were often correlated to fish mercury concentrations. Body size and trophic position were significant factors linked to fish mercury concentrations in the river mainstem. These results

support previous findings, where trophic position and body size explain a large amount of variation in fish mercury concentrations (Donald et al. 2015, Riva-Murray et al. 2013b, Roxanna Razavi et al. 2019). Surprisingly, trophic position and body size were not strongly correlated to fish mercury concentrations in stream fish. This finding may reflect the smaller ranges in body size and trophic levels captured in stream fish. However, further investigation is needed to explain the variation in fish mercury concentrations not accounted for by both surface water mercury concentrations and the selected biological factors.

Insight on the influence of stream water chemistry variables on fish mercury concentrations could further our understanding of mercury biomagnification in this watershed. For example, in New Hampshire, USA, Broadley et al. (2019) found that concentrations of mercury in stream fish peak at intermediate levels of dissolved organic carbon (DOC) and mercury concentrations, suggesting that DOC inhibits mercury biomagnification at high concentrations. The method used in my thesis (Chapter 2) is an example of how researchers can utilize existing data from larger government programs to answer questions that might otherwise be inhibited by cost for a single project. I utilized water quality data from a larger monitoring program by Alberta Environment and Parks (AEP) (Kerr and Cooke 2019), in conjunction with fish sampling. This research can be taken even further as this program analyzes surface water for a large suite of water chemisty and trace element variables at monitoring stations in rivers and tributaries across Alberta (Kerr and Cooke 2019). For future studies, this dataset could be a powerful tool if paired alongside sampling of biota to investigate the influence of water quality variables, like DOC, and potential interactions on trace element biomagnification.

My research shows that in the Red Deer River watershed, fish mercury concentrations frequently exceeded criteria for the protection of human and wildlife consumers. For wildlife consumers,

the risk is also not localized to areas of high mercury concentrations in surface water, but present in fish throughout the river and its tributaries. Human consumers, both subsistence and recreational, can make informed consumption decisions based on current fish consumption guidance (Government of Alberta 2019a, b). The results of my thesis are in-line with this fish consumption guidance, which suggests avoiding frequent consumption of large, top predator fish. However, wildlife in the Red Deer River watershed which rely on aquatic biota as a food source could also be at risk. I demonstrated that mercury in the environment is becoming incorporated into aquatic biota. This has implications for not only fish eating wildlife, but also insectivorous wildlife which feed on emergent aquatic invertebrates (e.g., Bats; Becker et al. 2018). Future studies should assess mercury concentrations in wildlife species which consume aquatic biota in the Red Deer River watershed to determine potential risks to their health.

In addition to mercury, the diverse fish community in the river mainstem provided an opportunity to examine the relationship between environmental concentrations, fish biological factors and concentrations in fish for a suite of other trace elements. Conflicting results have emerged regarding the role of biological factors, such as trophic position, feeding guild or dietary carbon source, and body size, in trace element accumulation in fish (Ali and Kahn 2018). Given this uncertainty, my research expands our understanding on fish trace element accumulation in the Red Deer River watershed. Like mercury, my results indicated that patterns in fish trace elements concentrations did not follow an environmental concentration gradient from upstream to downstream. Additionally, my research shows that concentration of trace elements in fish and the relationship with biological factors were species-specific. For example, in contrast to mercury which accumulated in higher concentrations with increasing trophic level and length, arsenic concentrations were higher in fish occupying lower trophic levels and rarely correlated

with fish body size. Fletcher et al. (2014) studied a stream impacted by a coal-fired power plant and also found species-specific concentrations of trace metals in fish and the biological factors which influenced their accumulation. However, the ability to characterize relationships between fish biological characteristics, environmental concentrations and fish tissue concentrations could be limited by the use of total trace element concentrations in my research. Huang (2016) reviewed studies of arsenic in food webs across terrestrial, marine and freshwater ecosystems and found evidence that although total arsenic concentrations generally do not increase with trophic level, organic arsenic often biomagnifies. Future endeavors should incorporate chemical speciation into their analyses to address potential differences in biomagnification.

Some trace elements identified at concentrations posing a potential risk to the health of aquatic biota in surface water (e.g., Lead; Kerr and Cooke 2017) were rarely detected in fish in my research. Low or undetected concentrations of some trace elements in fish from the Red Deer River may result from selecting muscle tissue for analysis, as many trace elements accumulate in other organs such as the liver and kidneys (Fletcher et al. 2014, Dhanakumar et al. 2015). Muscle tissue was selected to assess the risk to human consumers, but this tissue may not be ideal for assessing potential risks for the health of fish and wildlife consumers. As a part of laboratory protocol for this project, liver samples were also collected during fish dissection, although it was outside of the scope of this thesis to conduct analysis on these samples. Investigating trace element concentrations in liver from these fish could serve as an informative next-step to identify potential risks to wildlife consumers and the fish themselves for trace elements not detected in muscle tissue.

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Appendices

Table A2.1. Summary of sample size (n), raw total mercury concentrations (THg) and body size (weight) of white suckers sampled from various waterbodies in southern Alberta. Mercury concentrations and weights are represented by the median ± standard deviation.

Waterbody	Sampling Site (LAT, LONG)	Sampling Years	n	THg (mg/kg ww)	Weight (g)
Oldman River (OMR)	Mainstem (49.6833, -112.856)	2006	10	0.27 ± 0.17	1112 ± 365
	Willow Creek (50.121128, -113.776474)	1997 - 2007	140	0.14 ± 0.12	141 ± 276
	Pine Coulee Reservoir (50.1683, -113.7364)	2007	6	0.21 ± 0.08	905 ± 271
	Chain Lakes (50.24501, -114.211)	1997	7	0.09 ± 0.02	221 ± 91
Summary		1997 - 2007	163	0.14 ± 0.13	201 ± 357
South Saskatchewan River (SSR)	Mainstem (50.06199, -111.15)	2006	10	0.29 ± 0.14	1106 ± 246
Little Bow River (LBR)	Downstream of Carmangay Weir (50.14257, -113.10024)	2002	20	0.19 ± 0.11	615 ± 367
	Upstream of Carmangay Weir (50.13268, -113.16416)	2002	20	0.08 ± 0.08	546 ± 522
	Twin Valley Upstream (50.32683, -113.50765)	2004 - 2006	56	0.11 ± 0.08	791 ± 459
	Twin Valley Downstream (50.22041, -113.39161)	2004 - 2006	50	0.30 ± 0.2	1004 ± 374

	Twin Valley Reservoir (50.26872, -113.45581)	2004 - 2006	41	0.19 ± 0.14	300 ± 490
Summary		2002-2006	187	0.17 ± 0.16	792 ± 477
Red Deer River (RDR)	Tolman Bridge (51.838099, -113.016501)	2017	11	0.12 ± 0.05	134 ± 229
	Jenner (50.847569, -110.696815)	2017	6	0.26 ± 0.1	791 ± 180
	Kneehills Creek	2017	23	0.13 ± 0.04	12 ± 17
	Michichi Creek	2017	26	0.12 ± 0.03	43 ± 20
	Rosebud River	2017	32	0.10 ± 0.03	37 ± 20
	Threehills Creek	2017	24	0.12 ± 0.05	21 ± 45
Summary		2017	122	0.12 ± 0.06	36 ± 179

Table A2.2. Summary of water quality metrics in the tributaries. Water samples were collected between April 2016 and August 2017. Mean values ± standard deviation are summarized for pH, dissolved organic carbon (DOC), turbidity, dissolved oxygen (DO) and dissolved sulphate (DS), total mercury (THg), filtered mercury (FHg), methylmercury (MeHg) and filtered methylmercury (FMeHg). Filtered mercury samples were obtained by passing water though 0.45 micron acetate filter prior to analysis. Water chemistry measurements were taken following Alberta Environment field sampling protocols (Alberta Environment 2006). DOC was determined by sparging the sample with nitrogen gas or aeration with acid medium, followed by in-line acid-persulfate-UV digestion. DOC was then assessed by colourimic analysis of the remaining CO_2 concentration of organic carbon. An electronic meter was used to measure pH, with a glass pH and porous junction electrode. Turbidity was measured based on the amount of light from a tungsten filament lamp that is scattered by suspended particles in a sample and dissolved oxygen was measured using an electronic meter with luminescent sensor (Hach LDO probe). To determine dissolved sulphate in samples, a photometer was used to measure extinction of $BaSO_4$ following the precipitation of the sample with HCl and Barium Chloride ($BaCl_2$). The extinction was compared to a standard curve measured photometrically at 420 nm to determine the SO_4^{2-} concentration.

Tributary	n	DOC (mg L^{-1})	pH	Turbidity (NTU)	DO (mg L^{-1})	DS (mg L^{-1})	THg (ng L^{-1})	FHg (ng L^{-1})	MeHg (ng L^{-1})	FMeHg (ng L^{-1})
Kneehills	13	17.6 ± 3.9	8.5 ± 0.1	27.3 ± 25.6	10.2 ± 1.4	17.6 ± 3.9	4.61 ± 2.95	1.4 ± 0.82	0.7 ± 0.59	0.25 ± 0.13
Michichi	11	22.6 ± 3.9a	8.5 ± 0.1	791.9 ± 1586.3b	10.2 ± 1.8	22.6 ± 3.9	84.34 ± 182.69	11.68 ± 20.57	0.67 ± 0.79	0.2 ± 0.16
Rosebud	13	11.1 ± 4.5	8.4 ± 0.2	38.6 ± 23.4	10.3 ± 1.9	11.1 ± 4.5	6.13 ± 3.93	1.02 ± 0.56	0.39 ± 0.24	0.13 ± 0.07
Threehills	13	19.2 ± 4.5	8.4 ± 0.2	143.1 ± 271.4	9.9 ± 1.3	19.2 ± 4.5	7.24 ± 6.32	1.77 ± 1.16	0.47 ± 0.33	0.18 ± 0.11

[a]One sample <MDL of 50 mg/L, replaced with ½ MDL

[b]Two samples above Max detection limit (4000 NTU), replaced with 4000 NTU

Table A2.3. Stable isotope analysis results for all sampled invertebrates. Mean values ± standard deviation are summarized for invertebrate $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰).

ID	Functional Feeding Group	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Chironomidae	Collector-Gatherer	18	-30.53 ± 1.31	8.7 ± 3
Corixidae	Collector-Predator	12	-30.94 ± 0.78	6.6 ± 1.6
Hyallelidae	Scraper-Predator	9	-29.12 ± 1.22	6.7 ± 3.5
Caenidae	Collector-Gatherer	8	-31.73 ± 1.3	6.6 ± 2.2
Physidae	Scraper	8	-29.71 ± 0.72	7.7 ± 2.6
Gammaridae	Scraper-Predator	7	-29.41 ± 1.44	7.8 ± 4.2
Lymnaeidae	Scraper	6	-27.43 ± 2.86	9.1 ± 3.9
Elmidae	Shredder-Scraper	5	-31.21 ± 0.51	8.8 ± 3
Simuliidae	Collector-Filterer	5	-32.63 ± 1.96	7.1 ± 2
Cladocera	Collector-Filterer	4	-33.44 ± 1.44	6.4 ± 3.6
Hydropsychidae	Collector-Filterer	4	-31.86 ± 0.31	9.3 ± 1.3
Limnephelidae	Scraper	4	-32.59 ± 1.46	7.1 ± 3.1
Aeshnidae	Predator	2	-31.29 ± 0.6	7.9 ± 2.4
Baetidae	Collector-Gatherer	2	-30.98 ± 0.54	7.4 ± 1.5
Coenagrionidae	Predator	2	-31.75 ± 0.38	8 ± 2.3
Dytiscidae	Predator	2	-28.8 ± 0.81	8.1 ± 2.6
Ephemerelidae	Collector	2	-30.44 ± 1.69	12.6 ± 5.2
Belostomatidae	Piercer-Predator	1	-31.79	8.7
Corduliidae	Predator	1	-32.82	6
Curculionidae	Collector	1	-25.36	3.6
Haliplidae	Shredder	1	-33.96	5.6
Hydrophilidae	Filterer	1	-26.29	6.8
Leptoceridae	Shredder	1	-28.6	3.3
Libelluidae	Predator	1	-32.57	5.6
Limnephilidae	Shredder	1	-33	5
Planorbidae	Scraper	1	-28.68	5.9
Sialidae	Predator	1	-30.51	4.2
Sphaeriidae	Filterer	1	-30.71	11.4
Tabonidae	Predator	1	-32.61	7

Table A3.1. Chemical analysis and quality control information for fish sampled in 2014, 2015 and 2017.

Year	Analysis	Sample State	Sample Weight (g)	Lab	Method Detection Limit	Quality Control
2014	Mercury (DMA-80)	wet	0.09 – 0.11	University of Calgary (ACFT)	0.005 mg/kg	• Certified Reference Material: (NIST SRM 2976, DORM-3, DOLT-4/BCR-463) • Duplicates • Blanks • Every 15 samples and at the end of the run
2015	Mercury (DMA-80)	wet	0.09 – 0.11	University of Calgary (ACFT)	0.005 mg/kg	• Certified Reference Material: (NIST SRM 2976, DORM-3, DOLT-4/BCR-463) • Duplicates • Blanks • Every 15 samples and at the end of the run
2015	Trace elements (ICP-MS)	wet	~0.1	University of Alberta Dr. Chris Le (AETL)	Varies by element, described in Table S1	• Duplicate procedural blanks for each run (values obtained subtracted from sample values) • Calibration curve re-analyzed every 10 to 15 samples • SRM 1566b "Oyster Tissue" compared to measured values (see email for certified values and % recovery
2015	Stable Isotope Analysis	Freeze-dried	~0.001	University of Alberta (BASL)	NA	• $\delta^{13}C$ or $\delta^{15}N$ is referenced to that in Vienna Pee Dee Belemite (VPDB) and air every 20 samples

						• NIST 8415 whole egg powder SRM as an in-house $\delta^{15}N$ and $\delta^{13}C$ QA/QC check every 20 samples
2017	Mercury (DMA-80)	Freeze-dried	0.02 – 0.08	University of Alberta (BASL)	0.0032 mg/kg	• Certified Reference Material: DORM-3, DORM-4 dogfish muscle (NRC, Ottawa, Canada) • Duplicates • Blanks • Every 10 samples
2017	Trace elements (ICP-MS)	Freeze-dried	~0.1	University of Alberta Dr. Chris Le (AETL)	Varies by element, described in Table S1	• Duplicate procedural blanks for each run (values obtained subtracted from sample values) • Calibration curve re-analyzed every 10 to 15 samples • SRM 1566b "Oyster Tissue" compared to measured values
2017	Stable Isotope Analysis	Freeze-dried	0.00002 – 0.00105	University of Alberta (BASL)	NA	• $\delta^{13}C$ or $\delta^{15}N$ is referenced to that in Vienna Pee Dee Belemite (VPDB) and air every 20 samples • NIST 8415 whole egg powder SRM as an in-house $\delta^{15}N$ and $\delta^{13}C$ QA/QC check every 20 samples

Table A3.2. Summary of trace element detection levels in fish tissue by ICP-MS. Fish collected in 2015 and 2017 were analyzed for 20 trace elements by ICP-MS (n = 204). The method detection limit (MDL) is provided for each element and the percentage of fish samples analyzed above the MDL is indicated in the "Detections" column.

Element	MDL (µg/g)	Detections (%)
Be	0.003	0.5
Al	0.8	19
V	0.001	57
Cr	0.003	89
Mn	0.003	100
Co	0.01	74
Ni	0.003	91
Cu	0.9	57
Zn	0.9	100
As	0.001	99
Se	0.002	100
Mo	0.001	80
Ag	0.005	7
Cd	0.001	44
Sb	0.002	3
Ba	0.03	91
Tl	0.002	54
Pb	0.3	0
Th	0.007	13
U	0.003	0.5

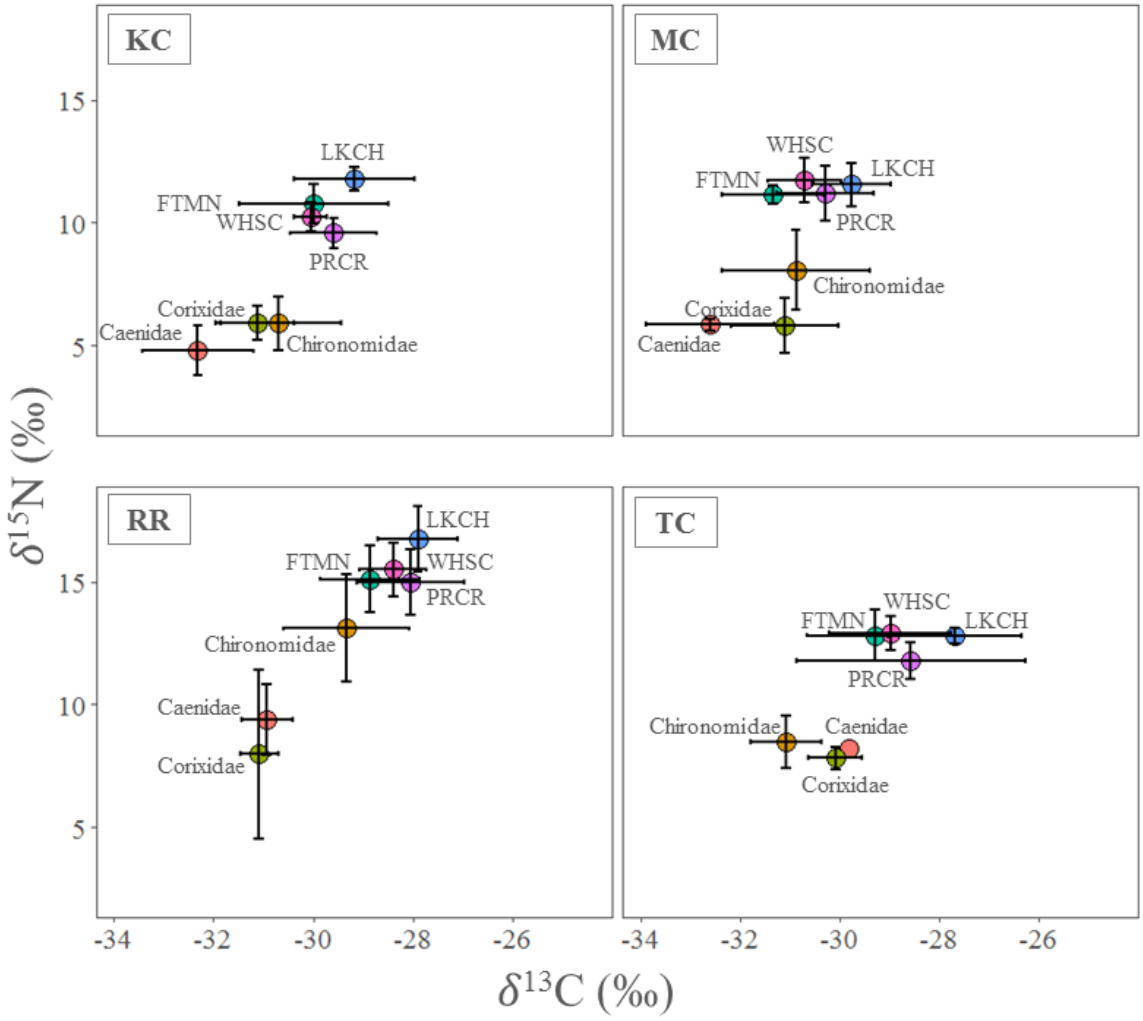

Figure A2.1. Stable isotope biplot of raw δ^{13}C (‰) and δ^{15}N (‰) values of fish species and macroinvertebrate families. Fish species are fathead minnow (FTMN), lake chub (LKCH), Prussian carp (PRCR) and white sucker (WHSC). A biplot was made for Kneehills Creek (KC), Michichi Creek (MC), Rosebud River (RR) and Threehills Creek (TC) to examine variation in stable isotope values among tributaries. Points represent mean values and errorbars represent standard deviation.