



# Mercury bioaccumulation in stream fish from an agriculturally-dominated watershed

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## HIGHLIGHTS

- Four species were sampled from streams characterized by high THg concentrations.
- Fish THg concentrations exceeded federal guideline for wildlife consumers.
- Differences in stream THg did not determine fish THg concentrations.
- Trophic level and body size were correlated with THg in two species.

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## ABSTRACT

Bioaccumulation of mercury in freshwater fish is a complex process driven by environmental and biological factors. In this study, we assessed mercury in fish from four tributaries to the Red Deer River, Alberta, Canada, which are characterized by high surface water mercury concentrations. We used carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes to examine relationships between fish total mercury (THg) concentrations, food web dynamics and patterns in unfiltered THg and methylmercury (MeHg) concentrations. We found that THg concentrations exceeded the tissue residue quality guideline for the protection of wildlife consumers in 99.7% of fish sampled. However, while the surface water THg concentration was highest in Michichi Creek and the MeHg concentration was consistent across streams, patterns of fish THg concentrations varied depending on species. Furthermore, body size and trophic level were only correlated with THg concentrations in white sucker (*Catostomus commersoni*) and Prussian carp (*Carrasius gibelio*). The results of this study suggest that mercury poses a risk to the health of piscivorous wildlife in the Red Deer River watershed. Despite high THg concentrations in these streams, mercury bioaccumulation is not driven by environmental inorganic mercury concentrations. Additionally, commonly cited factors associated with mercury concentrations in fish, such as body size and trophic level, may not strongly influence bioaccumulation in these stream ecosystems.

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## 1. Introduction

Mercury, specifically methylmercury (MeHg), is a neurotoxin which can be transferred to humans and wildlife through the consumption of contaminated fish. Mercury is emitted into the atmosphere through natural and anthropogenic sources, and 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). The disconnection between trends in emissions and bioaccumulation presents a gap in our knowledge that is crucial to address.

Mercury is commonly found in both inorganic and organic (e.g., MeHg) forms in the environment. Inorganic mercury is converted to MeHg predominantly by sulphate- and iron-reducing bacteria (Lin et al., 2011). The primary site of mercury methylation occurs in

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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.

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). Mercury concentrations in fish are often associated with body size (Eagles-Smith et al., 2016a; Razavi et al., 2019), age (Redmayne et al., 2000; Donald et al., 2015), and trophic level (Donald et al., 2015; Pandey et al., 2017). Analysis of stable nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) isotope ratios can be used to determine fish trophic level and sources of dietary carbon, respectively (Vander Zanden and Rasmussen, 2001). In fish,  $\delta^{15}\text{N}$  values typically increase with higher positions in the food chain (Vander Zanden and Rasmussen, 2001) and more lower  $\delta^{13}\text{C}$  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 values in combination with mercury analysis can provide an effective way to investigate the influence of biological factors on fish mercury concentrations.

Streams provide 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). Bioaccumulation in streams has been studied 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 mobilized in agriculturally dominated watersheds (Balogh et al., 1998; Brinkmann and Rasmussen, 2012). 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 have been reported in association with high levels of suspended sediment in the water column, especially in certain streams (i.e. Michichi Creek; Kerr and Cooke, 2017). In this study, we sought to evaluate mercury accumulation in fish from this watershed. Our objectives were to: 1) determine fish mercury concentrations and the potential risk to wildlife and human consumers, 2) ascertain if fish mercury concentrations would reflect differences in environmental mercury concentrations among streams, and 3) quantify the relationships between biological characteristics (age, body length, and food web dynamics) and fish mercury concentrations. We predicted that fish mercury concentrations would reflect patterns in aqueous THg concentrations among the streams, but would also be positively correlated to age, body size, trophic level and in-stream dietary carbon source use.

## 2. Methods

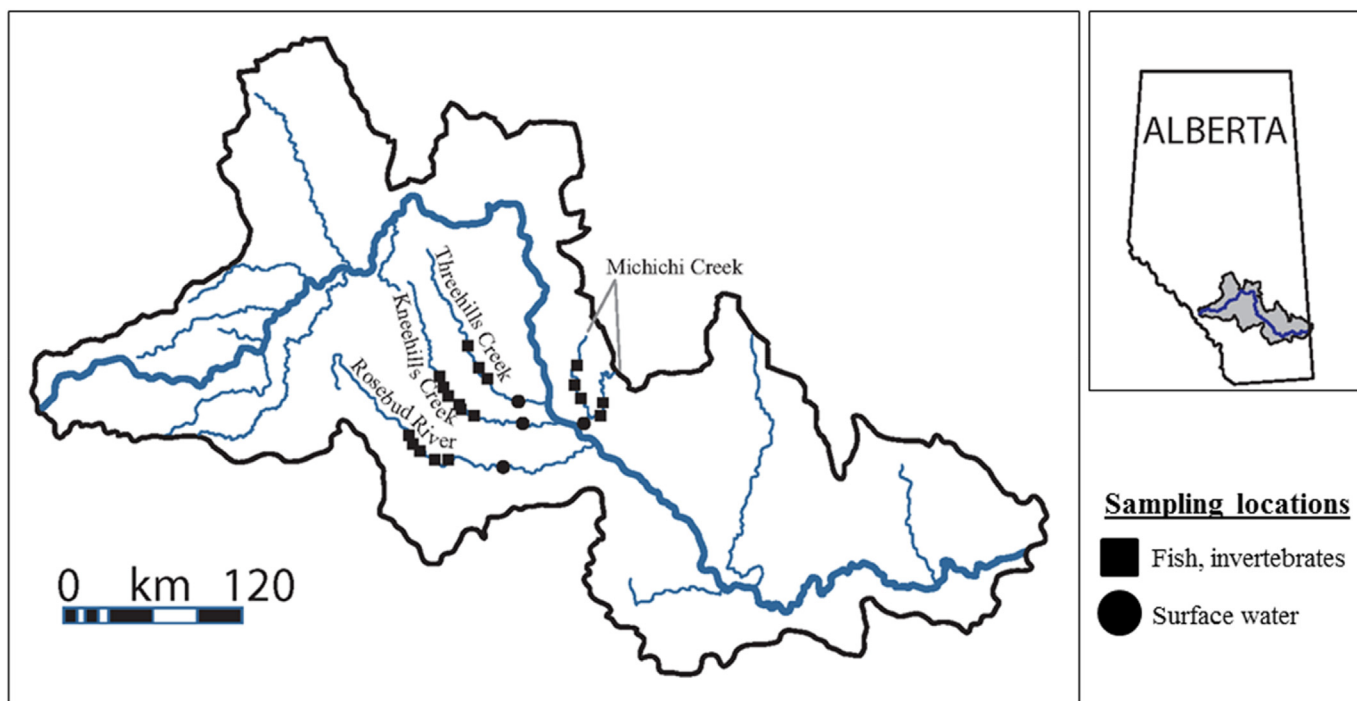
### 2.1. Study area

The Red Deer River flows from its headwaters in the Rocky Mountains of Alberta to Saskatchewan, where it joins the South Saskatchewan River (Campbell, 1977, Fig. 1). The south-eastern corner of Alberta is a semi-arid region and the river is flanked by badlands (Campbell, 1977). 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 ironstone 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 subwatersheds of the four streams range in size from 2735 km<sup>2</sup> (Kneehills Creek) to 6204 km<sup>2</sup> (Michichi Creek) (Aquality Environmental Consulting Ltd, 2009). Land use around the streams is predominantly agricultural (proportional area: 64–77%; Kerr and Cooke, 2017), with minimal wetland cover (0.67–5.53%; Aquality Environmental Consulting Ltd, 2009).

### 2.2. Field sampling

Fish and benthic macroinvertebrates were collected to compare mercury bioaccumulation among four tributaries to the Red Deer River. Ethics approval for this research was received from the University of Alberta Research Ethics Board, Animal Care and Use Committee “Stream Assessment” AUP 00000757 and provincial Fish Research License 17-3012. Fish were collected from 19 sites on Kneehills Creek, Michichi Creek, Rosebud River and Threehills Creek during June to August 2017 (Fig. 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). White sucker, lake chub and fathead minnow spawn in the spring (Scott and Crossman, 1973), whereas Prussian carp can reproduce asexually multiple times throughout the year through gynogenesis (Lamatsch and Stöck, 2009). The behavior and feeding habits of native species are described in detail by Scott and Crossman (1973). In brief, white sucker are primarily bottom feeders consuming macroinvertebrates. Lake chub are primarily invertivores, but may also consume zooplankton and sometimes other fish. Prussian carp and fathead minnow are omnivorous, consuming vegetable matter, detritus and macroinvertebrates (Özdilek and Jones, 2014). Benthic macroinvertebrates were collected by a 2-min 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.

Surface water samples were collected from the streams 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



**Fig. 1.** Map of sampling locations in the Red Deer River Watershed, AB, Canada. Samples were taken from four streams: 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 stream from April 2016 to August 2017 (circles). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

stream located downstream of biota collection sites (Fig. 1). Samples were taken just below the surface, approximately at the midpoint between the banks of each stream 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 S1.

### 2.3. 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.

### 2.4. 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 ( $n = 115$ ).

### 2.5. 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 (Elementar Inc., Hanau, Germany) and an IsoPrime vislon continuous-flow isotope ratio mass spectrometer (Elementar Inc. Stockport, England) for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  ratios. Isotope ratios were determined as follows:

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

Where X is the heavy isotope,  $R_{\text{sample}}$  indicates the ratio of  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$  in the sample, and  $R_{\text{standard}}$  is a certified value determined in air ( $\delta^{15}\text{N}$ ) or Vienna Pee Dee Belemnite ( $\delta^{13}\text{C}$ ). Certified values of nitrogen and carbon were from the US Geological survey (Reston Stable Isotope Laboratory, Reston, USA) and the International Atomic Energy Agency (Vienna, Austria). An additional QA/QC check was conducted (NIST SRM, 1845 whole egg powder, National Institute of Standards and Technology, Gaithersburg, USA) every 20 samples for  $\delta^{15}\text{N}$  (6.89‰) and  $\delta^{13}\text{C}$  (−23.99‰) with a precision of  $\delta^{15}\text{N} \pm 0.2\text{‰}$  and  $\delta^{13}\text{C} \pm 0.01\text{‰}$ , respectively.

### 2.6. 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). 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  $\pm 10\%$  of certified values, and duplicates were within  $\pm 10\%$  value of each other. The first value was reported for duplicate samples and the method detection limit (MDL) was 0.003 mg/kg.

THg in surface water samples was analyzed using EPA Method 1631E (U.S. EPA, 2002). In brief, Bromine Monochloride (Fisher Scientific, Fair Lawn, USA) was added to the sample to oxidize all forms of Hg. After a minimum of 12 h the BrCl was neutralized by addition of Hydroxylamine Hydrochloride (Fisher Scientific, Fair-lawn, USA). Following neutralization, Stannous Chloride (Fisher Scientific, Fair Lawn, USA) was added to the sample to reduce the Hg from the HgII to the Hg0 oxidation state, which was then purged onto gold-coated glass bead traps, thermally desorbed to a second gold trap, and analyzed using cold vapor atomic fluorescence spectrometry (CVAFS) on a Tekran 2600 mercury Analyzer (Tekran Inc, Toronto, Canada). The MDL was 0.05 ng/L. Every 20 samples, instrument and method blanks were used to check for system contamination. A second source reference material (Spex Certiprep, Metuchen, USA) was used to monitor accuracy and instrumental drift, and results were accepted if the reference was inside the 85%–115% recovery range. Every 20 samples, duplicates were used to monitor precision, and results were accepted if the relative percentage difference was below 20%. Additionally, every 20 samples a matrix spike (Fisher Scientific, Fair Lawn, USA) was used to monitor for any potential sample interference and results were accepted if the analyte fell within 80–120% of the recovery range. MeHg was analyzed with isotope dilution, purge and trap, and inductively coupled plasma mass spectrometry (ICP-MS). Water samples were spiked with  $^{201}\text{MeHg}$  Internal Standard in toluene (Trace Scientific, Richmond Hill, Ontario). The spiked samples were distilled at 127 °C by a Tekran 2750 Methyl Mercury Analyzer (Tekran Inc., Toronto, Canada) with the addition of ammonium 1-pyrrolidinedicarbodithioate (APDC) (JT Baker, Radnor, USA) and hydrochloric acid (Fisher Scientific, Ottawa, Canada) to remove matrices that may interfere with the ethylation process. Ascorbic acid (Fisher Scientific, Fair Lawn, USA) was added to distillate to remove any trace of free chlorine. In a glass vial, the distillate was adjusted to pH 4.9 with acetate buffer (Sigma Aldrich, Darmstadt, Germany). Sodium tetrathyl borate (Strem Chemicals, Newburyport, USA) was added to distillate for ethylation of MeHg to volatile MeHgEt. The ethylated samples were loaded on to the Tekran 2700 Methyl Mercury Analyzer coupled with Agilent 7900 ICP-Mass Spectrometer (Agilent Technologies, Tokyo, Japan). Volatile ethylated mercury compounds in the sample were stripped from the liquid phase with argon gas, trapped and desorbed on the Tenax trap, separated mercury species by capillary GC and temperature ramping GC oven, and then the pyrolytic break down of mercury compounds to Hg0 occurred. The elemental mercury was then introduced to ICP-MS to detect  $^{202}\text{Hg}$  and  $^{201}\text{Hg}$ . The concentrations were corrected based on the recovery of the internal standard. The MDL was 0.016 ng/L. Quality assurance for the single point calibration was conducted by analyzing the calibration standard (MeHg Chloride Stock Calibration Standard; Brooksrand, Seattle, USA) in 4 replicates where the abundance of mercury isotope 201 over 202 is calculated. The results were accepted if the relative standard deviation of the replicates is less than 5%. Every 20 samples, instrument and method blanks were used to check for system contamination. Additionally, a second source reference material (MeHg Hydroxide Stock Quality Control Calibration Standard Brooksrand, Seattle, USA) was used to monitor accuracy and instrumental drift, and results were accepted if the reference was inside the 85%–115% recovery range. Every 20 samples, duplicates were used to monitor precision, and results were accepted if the relative percentage difference was below 15%. Finally, every 24 samples a matrix spike (MeHg Chloride Stock Calibration Standard;

Brooksrand, Seattle, USA) was used to monitor for any potential sample interference and results were accepted if the analyte fell within 80–120% of the recovery range.

## 2.7. 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).

To assess the risk to wildlife and human health, fish mercury concentrations were compared to three criteria: 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, 2019), 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).

Comparisons of mercury concentrations in fish and surface water among the streams 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 streams were conducted with Dunn's post hoc test using the FSA package (Ogle et al., 2019). In fish, between-group differences were compared among the streams using ANOVA with type III sums of squares for unequal sample sizes and pairwise comparisons among streams 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 stream ( $n = 3$ ). 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).

Calculating fish trophic level using baseline primary consumers is suggested to correct for environmental variation in  $\delta^{15}\text{N}$  among sites (Post, 2002). Biota collected from the Rosebud River were elevated in  $\delta^{15}\text{N}$ , likely reflecting inputs from an external nitrogen source (Fig. S1; Brinkmann and Rasmussen, 2012). Therefore, trophic levels were calculated for all fish to correct for differences in fish  $\delta^{15}\text{N}$  related to potential external nitrogen sources. Collectors (Caenidae, Chironomidae, Corixidae) were the most widespread macroinvertebrate taxa in the streams, so they were used as baseline primary consumers for calculation of fish trophic level (Table S2). Trophic level was calculated within each stream following the approach of Post (2002):

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

Where  $\delta^{15}N_{\text{consumer}}$  was the fish value,  $\delta^{15}N_{\text{baseline}}$  was the average value of baseline primary consumers within a stream, 3.4 is the trophic enrichment value of one trophic level (Post, 2002) and  $\lambda$  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 stream, age, fork length,  $\delta^{13}C$  value, trophic level and a length – stream 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 through 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 with the *MuIn* package (Barton, 2019), between the two models. 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).

Bioaccumulation factors were determined for each fish species in the four streams. The average mercury concentration for each species in a stream was converted to wet weight concentrations assuming an 80% moisture content (Magalhães et al., 2007). BAFs were calculated with average fish mercury concentration in the numerator and the average stream filtered methylmercury concentration in the denominator to give a result in  $L\text{ kg}^{-1}\text{-tissue}$ .

### 3. Results

#### 3.1. Risk to human and wildlife health

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%; Fig. 2).

#### 3.2. Mercury in the water

THg concentrations in water were highly variable (range: 1.14–615.33 ng/L; Fig. 3), and there were significant differences among the streams (Kruskal-Wallis test,  $\chi^2 = 20.2$ ,  $df = 3$ ,  $p < 0.001$ ). 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 stream 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 streams (Kruskal-Wallis test,  $\chi^2 = 29.4$ ,  $df = 3$ ,  $p < 0.001$ ). 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).

#### 3.3. Mercury in the fish

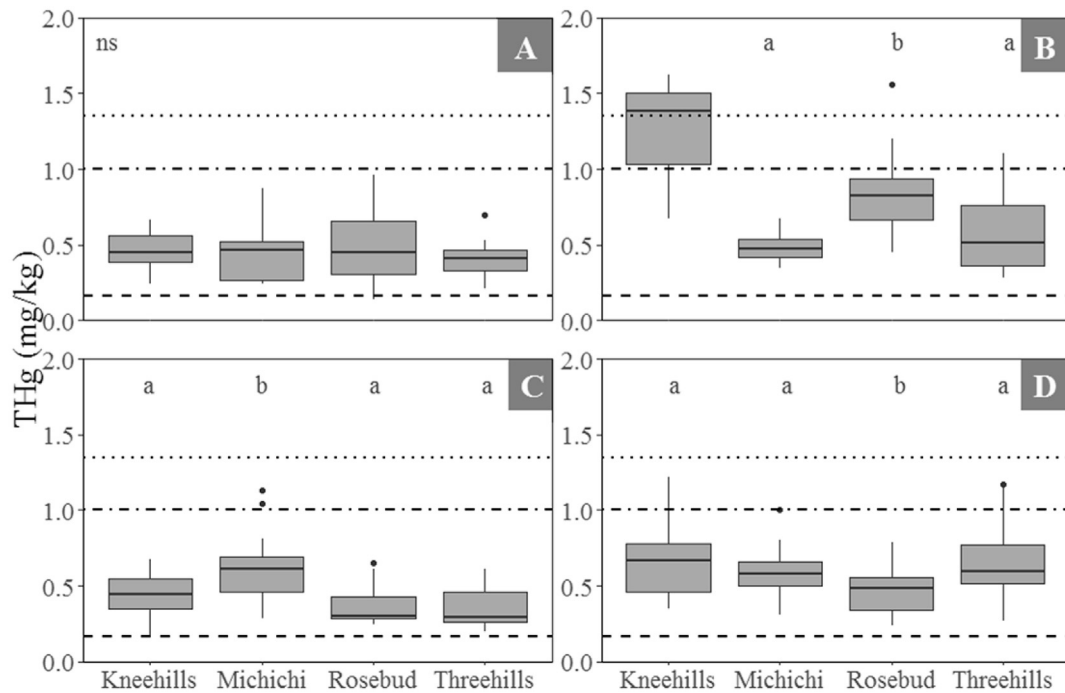
Mercury concentrations differed significantly among species (ANOVA test,  $F = 15.2$ ,  $df = 3$ ,  $p < 0.001$ ), where Prussian carp and fathead minnow had lower mercury concentrations than lake chub and white sucker, but were not different from each other. Fish mercury concentrations were significantly different among streams 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 (Fig. 2). However, patterns of fish mercury concentrations between the streams 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 streams (Tukey test, Kneehills Creek:  $p = 0.002$ , Michichi Creek:  $p = 0.046$ , Threehills Creek:  $p = 0.006$ ).

#### 3.4. Fish biological characteristics and mercury bioaccumulation

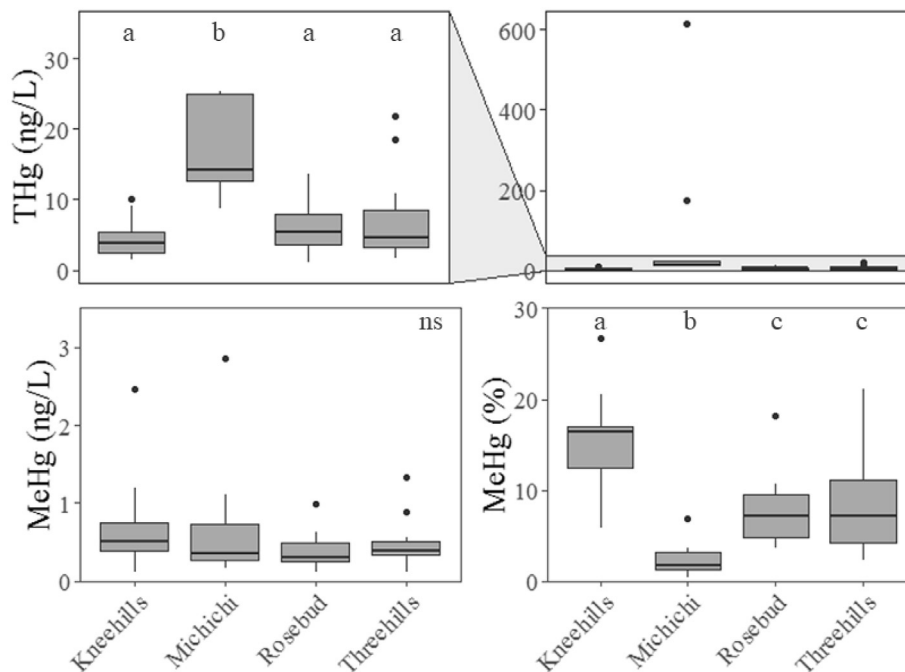
The largest fish sampled were white sucker (maximum fork length = 240 mm), followed by Prussian carp (maximum fork length = 201 mm), with maximum ages of 5 years old for both (Fig. 4). Fathead minnow were generally smaller (<75 mm) and younger (<2 years) than other fish species (Table 1, Fig. 4). All fish species had similar  $\delta^{13}C$  values (~29‰) and trophic levels (~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 (stream) was a significant explanatory variable in multiple regression models for Prussian carp, white sucker and lake chub (Table 2). Fish length was a significant factor for white sucker, and also Prussian carp. A significant length – stream 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 (Fig. 4). Log10 BAFs were consistent among streams and species, ranging from 5.43 to 5.99 (Table 3).

### 4. Discussion

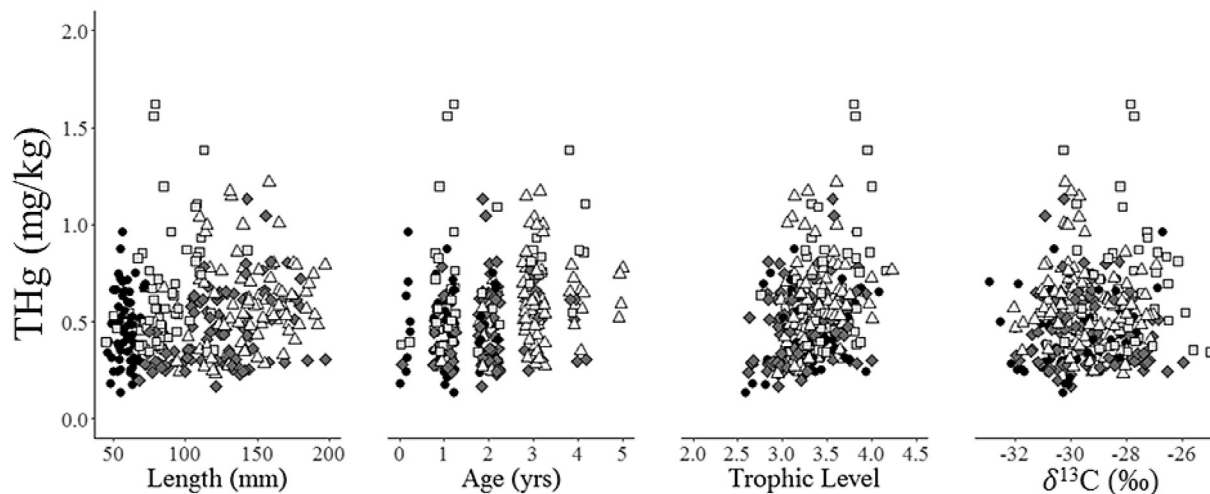
Understanding the extent to which mercury in the water column is accumulated by fish is necessary to identify the potential risk to human and wildlife consumers. Mercury concentrations in surface water of the Red Deer River have been shown to exceed surface water guidelines for the protection of freshwater 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 streams. MeHg concentrations in the streams were comparable to others with historical mining activity and naturally high geologic deposits (Domagalski, 2001), contaminated liquid effluents (Xu et al., 2019), as well as industrial spills



**Fig. 2.** Total mercury concentrations (dry weight) 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 dry weight concentration values in muscle tissue. Lake chub from Kneehills Creek were not included in the statistical comparison due to low sample size (n = 3). Boxplots represent the median and quartile ranges (25th and 75th), whiskers represent  $\pm 1.5$  \*inter-quartile range from the 25th and 75th quartiles. Fish from streams 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 streams.



**Fig. 3.** 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 (25th and 75th), whiskers represent  $\pm 1.5$  \*inter-quartile range from the 25th and 75th quartiles. Streams 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 streams.



**Fig. 4.** Fish total mercury concentrations (dry weight) versus fork length, trophic level, age and  $\delta^{13}\text{C}$ . 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.

**Table 1**

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

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

**Table 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,  $\delta^{13}\text{C}$ , trophic level (TL) and the stream they were sampled from as explanatory variables. The streams are Kneehills Creek (KC), Michichi Creek (MC), Rosebud River (RR) and Threehills Creek (TC). Mercury concentrations were log10 transformed.

Species	Biological Characteristics				Stream				Adj. $r^2$	p	n
	Age (yrs)	Length (mm)	$\delta^{13}\text{C}$ (‰)	TL	KC <sup>a</sup>	MC	RR	TC			
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 <sup>b</sup>	-	-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.

<sup>a</sup> Intercept.

<sup>b</sup> Significant interactions between fork length and MC (0.006\*\*), RR (0.004\*) and TC (0.006\*\*).

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 streams, 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

**Table 3**

Mercury bioaccumulation factors (BAFs;  $L\ kg^{-1}\text{-tissue}$ ).  $\log_{10}$ BAFs were calculated for each species and stream in fish using average fish mercury concentration (converted to ww) in the numerator and the average stream filtered methylmercury concentration in the denominator. Fish species are fathead minnow (FTMN), lake chub (LKCH), Prussian carp (PRCR) and white sucker (WHSC).

	Kneehills	Michichi	Rosebud	Threehills
FTMN	5.58	5.57	5.57	5.53
LKCH	5.99	5.58	5.82	5.70
PRCR	5.55	5.68	5.46	5.43
WHSC	5.75	5.68	5.57	5.74

efficiency (Gilmour et al., 1998). Due to the vast differences in patterns of THg and MeHg concentrations among these streams, THg measurements should not be considered a reliable indicator of MeHg present in the water column.

Small fishes, like the ones targeted in this study, form a key component of the ecosystem. Studies of mercury bioaccumulation in fish often target game fish species, which are generally top predators. However, elevated mercury in small fishes can negatively impact the larger ecosystem as they are important food sources for a variety of predators (Evers et al., 2008). Mercury concentrations in almost all fish collected in this study exceeded a federal guideline for the protection of wildlife consumers, raising concerns for the health of wildlife in the Red Deer River watershed. The Red Deer River basin is home to a variety of wildlife that may consume fish, including birds such as the common loon (*Gavia immer*), great blue heron (*Ardea herodias*) and belted kingfisher (*Megaceryle alcyon*). Additionally, reptiles such as red-sided garter snake (*Thamnophis sirtalis*) and wandering garter snake (*Thamnophis elegans*) consume fish and other aquatic biota. Fish-eating mammals such as American mink (*Neovison vison*) also occupy riverbank habitats in the Red Deer River basin. Additionally, small fish can be incorporated into the diet of species targeted by anglers, and through biomagnification, lead to potentially hazardous levels of mercury consumption.

While mercury concentrations in most fish species differed among the streams, they did not reflect the same pattern as surface water 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 streams either. Nonetheless, the dissimilarity between patterns of fish mercury and aqueous MeHg concentrations among the streams may result from the limited range of aqueous MeHg concentrations captured in the study. 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; Mathews 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 streams and therefore seem to be determined largely by environmental MeHg concentrations. However, for two species, length and trophic level were correlated with fish mercury levels. Growth efficiency (Ward et al., 2010a; Sandheinrich and Drevnick, 2016) and sex (Madenjian et al., 2015) can also influence fish mercury concentrations. It is likely that fish mercury concentrations in the Red Deer River are influenced by MeHg exposure at the base of the food web, in combination with the species-specific influence of biological factors, rather than aqueous THg concentrations. The lack of correlation between stream THg and fish mercury concentrations could also result from stream chemistry and mercury bioavailability. Despite consistent  $\log_{10}$  BAFs, comparable to other trophic level 3 fish (Scudder-Eikenberry et al., 2015), fish mercury concentrations do not reflect the significant variation in total mercury concentrations among streams in the present study. Dissolved organic carbon (DOC) concentrations have been found to have a non-linear relationship with mercury concentrations in biota, suggesting that high DOC concentrations may limit mercury bioavailability (Broadley et al., 2019). Broadley et al. (2019) reported low mercury concentrations in fish in streams with 0–5 mg/L DOC, peak fish mercury concentrations at 5–10 mg/L and decreasing fish mercury concentrations above 10 mg/L. Although the concentration of MeHg is higher in Kneehills Creek and Michichi Creek, they are also relatively high in DOC concentration (average DOC greater than 17 mg/L) which may have inhibited the bioaccumulation of mercury in fish from these streams. Additionally, mercury concentrations in the Red Deer River tributaries are associated with large volumes of suspended solids transported into the aquatic environment through erosion (Kerr and Cook 2017). While mercury methylation does occur on settling particles (Xu et al., 2019), the proportion of bioavailable mercury (methylmercury and acid-labile Hg) is often only a small proportion of the total mercury concentration (Reash, 2019) and this proportion is not consistent among streams (Broadley et al., 2019). Therefore, although Michichi creek has high levels of THg, the majority may not be incorporated into the food chain.

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, the white sucker we sampled 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. 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 in eastern Canada (Evers et al., 2007), and are characterized by 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). Despite lacking many qualities associated with high mercury concentrations in biota, our results suggest that stream fish from agriculturally-dominated watersheds such as the Red Deer River may still accumulate high concentrations of mercury.



## 5. Conclusion

Stream systems in southern Alberta support aquatic and terrestrial wildlife but contain high environmental mercury concentrations. This study showed that THg concentrations were elevated in Michichi Creek, but MeHg concentrations were not different among the streams. 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. Patterns of mercury concentrations in fish among the streams were species-specific and not reflective of variation in aqueous THg concentrations. Although 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 streams are likely influenced by a combination of environmental MeHg concentrations and biological factors. 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 mercury in fish from the Red Deer River may be elevated compared to the broader region. The results from this study indicate that bioaccumulation of mercury levels potentially hazardous to piscivorous wildlife is not limited to areas of high environmental inorganic mercury concentrations.

## Credit author statement

**Caitlyn Donadt:** Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Colin A. Cooke:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition. **Jennifer A. Graydon:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition. **Mark S. Poesch:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision, Project administration.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2020.128059>.

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