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1 **ASSESSING THE IMPACTS OF MINING ACTIVITIES ON FISH HEALTH**
2 **IN NORTHERN QUÉBEC**

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10

ABSTRACT

11 For several decades, Northern Québec has been exploited by mining companies for its mineral
12 resources, yet, research documenting the effects of toxic stress on fish health in subarctic
13 environments remains limited. In this study, one lake directly affected by mining activities in the
14 Schefferville area, two lakes close to mining facilities in the Fermont and Schefferville areas, and
15 one reference lake were sampled for water, sediment and fish. Our results suggest that
16 manganese bioaccumulation induced an oxidative stress in *Catostominae*, as demonstrated by
17 the positive relationships between manganese concentrations and catalase activity and 8-
18 hydroxy-2'-deoxyguanosine concentrations, as well as the negative relationship with glutathione
19 peroxidase activity (both catalase and glutathione peroxidase activities being biomarkers of
20 antioxidant capacities and 8-hydroxy-2'-deoxyguanosine an indicator of DNA oxidative damage).
21 Similarly, selenium bioaccumulation was positively correlated with 8-hydroxy-2'-deoxyguanosine
22 concentrations in *Salmoninae*. These results suggest a prooxidant role of excess selenium and
23 manganese, and highlight the interspecific variability of fish responses to contaminated areas
24 around historical and current iron ore mining operations.

25

26 **Keywords:** Northern fish, metal contamination, oxidative stress, Fermont, Schefferville

27 **1 INTRODUCTION**

28 Fish are ectothermic organisms, meaning that energy allocation is modulated in part by the
29 environmental temperature (Jobling et al., 1997; Power, 1978). Generally, in cold environments,
30 the age at maturity is delayed and the growth and reproductive seasons are shorter than in a
31 warmer climate (Duchesne and Magnan, 1997; Houde, 1989; Stewart et al., 2007). Energy
32 accumulated is allocated towards the maintenance of homeostasis, growth, reproduction, energy
33 storage and sometimes migration, though this is species, life stage and habitat dependent
34 (Jørgensen and Fiksen, 2006). However, when fish are facing a toxic stress such as metal
35 contamination, detoxification of these stressors and repair of affected biological tissues and
36 functions represent additional energetic costs. As a consequence, this will reduce allocation of
37 energy to other vital functions, potentially leading to physiological (Couture and Kumar, 2003;
38 Maes et al., 2005; McPhee and Janz, 2014) and behavioral (Berntssen et al., 2003; Scott and
39 Sloman, 2004; Sloman and McNeil, 2012) disruptions. Uptake and internal handling of trace
40 elements vary according to environmental (e.g. temperature, pH, water hardness) (Karthikeyan
41 et al., 2007; Köck et al., 1996) and individual variables (e.g. age, length, growth) (Farkas et al.,
42 2003; Greenfield et al., 2001) that in turn modulate fish health. Additionally, depending on their
43 trophic position, fish can be exposed to high metal concentrations through their diet (Kuchapski
44 and Rasmussen, 2015; van der Velden et al., 2013).

45 Metals are naturally occurring at the Earth's surface, but environmental concentrations can
46 be significantly amplified by recent human activities. Mining operations (from the exploration
47 phase to after the end of exploitation) increase the input of contaminants into natural
48 environments which often include aquatic ecosystems. Although some elements are essential for
49 fish, playing a key role in various metabolic reactions (e.g. barium (Ba), cobalt (Co), copper (Cu),
50 iron (Fe), manganese (Mn), selenium (Se) and zinc (Zn)), others are classified as non-essential

51 (e.g. aluminum (Al), arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb)). However, the
52 essentiality of certain elements, such as chromium (Cr) or nickel (Ni) remains controversial
53 (Wood, 2011). While essential elements can lead to toxic stress when occurring in elevated
54 concentrations (Wood et al., 2011a), non-essential elements can result in negative effects on fish
55 health even at very low concentrations (Wood et al., 2011b). Many physiological disruptions have
56 been associated with elevated elemental concentrations, including impairment of metabolic
57 activities (Couture and Pyle, 2012) and hormonal responses (Laflamme et al., 2000; Levesque et
58 al., 2002), reproduction (Friedmann et al., 1996), reduction of growth (Eastwood and Couture,
59 2002) and longevity (Couture and Pyle, 2008; Pyle et al., 2008) or impacts on the antioxidant
60 defence system, as highlighted by the observation of biomarkers of reactive oxygen species
61 (ROS) (Sevcikova et al., 2011).

62 The ROS are naturally produced by aerobic metabolism (Winston and Di Giulio, 1991) and
63 are regulated by two defense systems: the activity of antioxidant enzymes, such as superoxide
64 dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and antioxidant molecules
65 such as reduced glutathione (GSH) (Di Giulio et al., 1989; Lackner, 1998; Livingstone, 2001).
66 Generally, metals are associated with the production of ROS in two ways (Sevcikova et al., 2011).
67 First, some elements such as Fe, Cu, or Cr generate ROS through their redox potential, while Hg,
68 Pb, Cd, or Ni can directly impact antioxidant mechanisms. Oxidative stress is what occurs when
69 these antioxidant capacities are exceeded, which then disrupts cellular homeostasis and
70 culminates in accumulated ROS. This leads to associated oxidative damage, such as lipid
71 peroxidation and deoxyribonucleic acid (DNA) damage (Halliwell, 1999; Lackner, 1998). Although
72 fish responses to mining and metal contamination have been well documented in more Southern
73 regions of Canada (Couture and Pyle, 2012; Sherwood et al., 2000), literature evaluating such
74 impacts in more Northern locations remains limited to a few scientific studies (Martyniuk et al.,
75 2020b) and small scale regional environmental monitoring programs (Makivik Coproration, 2021).

76 Even though literature is scarce on the particular impacts of iron ore mining on fish health,
77 one study case stands out: the Samarco disaster (southeast Brazil). In 2015, roughly 50 million
78 m³ of iron mine tailing were discharged in the environment after a dam break. Since then, several
79 studies have highlighted an increase of metal concentrations (Ba, Co, Cr, Cd, Cu, Fe, Mn, Ni) in
80 the aquatic ecosystem downstream (da Silva et al., 2022; Passos et al., 2020; Siqueira et al.,
81 2022). Assessing fish responses to metal contamination, Gabriel et al. (2020) showed that two
82 demersal fish species captured in the estuary (*Cathorops spixii* and *Genidens genidens*)
83 expressed high values of As, Cr, Mn, Hg and Se in both liver and muscle tissues. Additionally,
84 trace metal bioaccumulation was significantly correlated with the expression of metallothioneins
85 (MT) and GSH, two biomarkers of detoxification processes. Another study from Weber et al.
86 (2020) demonstrated that two fish species (*Hoplias intermedius* and *Hypostomus affinis*), sampled
87 along the Doce River in a contaminated area, showed liver damage (vacuolization, necrosis and
88 hypertrophy of hepatocytes) and a significant response of cytochrome P450 1A (CYP1A), MT and
89 CAT. *Hoplias intermedius* also expressed significantly higher level of SOD than their counterparts
90 from the reference site. Together, these results reinforce the conclusion of a long-term chronic
91 contamination which can be observed in the surrounding ecosystem of iron ore mining operations
92 (Baeten et al., 2018). Nevertheless, fish response to environmental contamination from iron
93 mining operations could markedly differ between warm-water species and cold-water species. In
94 their study, Weber et al. (2020) selected two species that are known to be compliant to strong
95 environmental changes, yet, they still observed negative effects due to metal contamination.
96 Northern fish species could be more sensible to such toxic stressors since they experience shorter
97 periods of high energy accumulation and metabolic activity compared to individuals from Southern
98 locations. Thus, their tolerance range (i.e. detoxification capacities, behavioural adaptations,
99 reproductive success) to environmental contamination from mining activities might be more
100 limited than warm-water species.

101 The Labrador Trough is a region of Mid-Northern Québec recognized for its mining potential,
102 especially iron (Simard, 2018) in the Fermont and Schefferville areas. The first mining operations
103 began in Schefferville in the 1950s under the direction of the Iron Ore Company of Canada (IOC)
104 (Boutet, 2012) while in Fermont, development started in 1970 with the Compagnie minière
105 Québec Cartier (CMQC) (CLD de Caniapiscau, 2015). Historically, several companies have
106 exploited claims in the two regions, which has had a major impact on the environment, particularly
107 the surrounding aquatic ecosystems (Aebischer et al., 2015; Laperrière et al., 2007). More
108 recently in 2014, further environmental concerns were raised in the region after several incidents.
109 These included the failure of a dam at the Bloom Lake mine site in Fermont (Gouvernement du
110 Canada, 2014) and red coloured water identified by Schefferville residents entering aquatic
111 systems in areas proximate to several of the surrounding mines (Blais, 2019). The objective of
112 this study was to investigate the effects of past and present mining activities on fish health in both
113 locations. To assess elemental accumulation in fish with similar feeding strategies and trophic
114 position, six fish species further grouped in three subfamilies were sampled in three contaminated
115 lakes from Fermont and Schefferville in addition to a reference lake in the same region, but far
116 from potential mining influence. Relationships between liver trace element concentrations and
117 condition indices (Fulton's condition factor (FCF), hepatosomatic index (HSI)) and biomarkers of
118 antioxidant capacities (CAT, SOD and GPx) as well as oxidative damage (malondialdehyde
119 (MDA), a lipid peroxide, and 8-hydroxy-2'-deoxyguanosine (8-OHdG), an indicator of DNA
120 oxidative damage) were investigated to evaluate effects of contamination from mining operations
121 on fish health.

122 **2 MATERIALS AND METHODS**

123 **2.1 Study area**

124 The towns of Fermont (52°46'59"N, 67°05'00"W) and Schefferville (54°47'59"N,
125 66°50'00"W) are located in Northern Québec on the provincial boundary between Québec and
126 Labrador (Figure 1). Geologically, Fermont and Schefferville are located in the Labrador Trough
127 of the Canadian Shield, which has been globally identified as an exceptionally productive area for
128 metal mining (Simard, 2018). These two regions are characterized by a subarctic climate with a
129 short growing season and vegetation transitioning between boreal forest and arctic tundra
130 (Vowinkel et al., 1975). Recorded average temperatures range from -22.1°C in January to
131 13.5°C in July for Fermont (Ministère de l'Environnement et de la Lutte contre les changements
132 climatiques, 2020). In Schefferville, the average temperatures for January and July are -23.3°C
133 (Gouvernement du Canada, 2020a) and 13.8°C, respectively (Gouvernement du Canada,
134 2020b). Mining operations, and more specifically iron ore exploitation, have occurred in the region
135 since the 1950's and have been a dominant force in the local economy of both Fermont and
136 Schefferville (Boutet, 2012; Simard and Brisson, 2013). Two main iron ore exploitation sites are
137 currently operational in Fermont: the Mont-Wright Mine (16 Mt/year) owned by ArcelorMittal
138 Mining Canada G.P. (AMEM), and the Bloom Lake Mine (7.6 Mt/year) by Minerai de fer Québec
139 (a subsidiary of Champion Iron). Tata Steel Minerals Canada (TSMC) has been the sole mining
140 operation profiting from iron ore deposit exploitation (1 Mt/year) in Schefferville since 2014.
141 Historical mining operations in Schefferville involves two companies: IOC which exploited iron ore
142 from 1954 to 1982 (from 2 to 12 Mt/ year) and Labrador Iron Mine Limited (LIM) from 2011 to
143 2013 (3.6 Mt produced during the three years of exploitation). Schefferville is also positioned in
144 the traditional territories of two Indigenous Nations: the Naskapi Nation of Kawawachikamach and
145 Innu community of Matimekush-Lac-John (Simard and Brisson, 2016). Individuals from both

146 communities still practice traditional hunting and fishing activities on land situated near both
147 historic and current mining operations.

148 In summer 2019, three lakes reported to have received input from both historical and
149 present mining operations (Table 1) were sampled to assess the effects of mining activities on
150 fish health and their environment (Figure 1). In Schefferville, lakes were chosen with the
151 assistance of an Innu guide, for their proximity to past and present mining activity and identified
152 as of potential concern from the local community. In addition to be a primary food item, fish
153 represent important cultural, economical and social resources for Indigenous Nations. As such,
154 there have been growing concerns since local guides identified mine tailings contaminating the
155 lakes (Table 1). Lake Perroche ($54^{\circ}1'40.11''\text{N}$ and $69^{\circ}37'30.72''\text{W}$), located nearly 200 km from
156 both Fermont and Schefferville and free from any mining influences, was chosen as a reference
157 site due to its ecological and physicochemical similarity with the aforementioned sampling sites.
158 In Fermont, Lake Cherny ($52^{\circ}52'40.02''\text{N}$ and $67^{\circ}26'0.64''\text{W}$) is located about 6 km downstream
159 from the Bloom Lake mining site. In Schefferville, Lake Denault ($54^{\circ}50'13.75''\text{N}$ and
160 $66^{\circ}53'26.79''\text{W}$) is directly adjacent to a historical site of IOC exploitation, while Lake Houston
161 ($54^{\circ}43'57.31''\text{N}$ and $66^{\circ}41'5.43''\text{W}$) is approximately 10 km from this location, but closer to past
162 Labrador Iron Mines (LIM) and current TSMC exploitation sites. Sampling occurred between July
163 15th and August 8th in Fermont area and in the reference site, and between August 25th and
164 September 3th in Schefferville.

165 **2.2 Sample collection**

166 Water variables, including pH (Waterproof pHep@5 pH/Temperature Tester – H198128,
167 Hanna Instruments, Laval, Québec, Canada), temperature ($^{\circ}\text{C}$) and conductivity ($\mu\text{S}/\text{cm}$) (YSI
168 Model 85, YSI Incorporated, Yellow Springs, Ohio, USA) were measured at each sampling
169 location. The probe of both instruments was recording approximately 30 cm underwater. Two
170 surface water and sediment samples (Ekman Bottom Grabs 225 cm^2 , KC Denmark A/S,

171 Silkeborg, Denmark) were also collected at each site to establish trace element concentrations in
172 addition to water field and transport blanks. Ultrapure water was used to prepare water field and
173 transport blanks to evaluate potential procedural and material contamination. To sample surface
174 water, the syringe was rinsed five times consecutively with surface water before setting the filter
175 (25 mm PES 0.45 μm , Canadian Life Science, Peterborough, ON, Canada). Then, 20 mL of
176 surface water were sampled and 5 mL were discarded. The remaining 15 mL were stored in trace
177 metal free tubes at 4°C.

178 To maximize diversity of fish capture, sampling was completed using experimental gill nets
179 (25 to 150 mm mesh panels, 120 m length, 2 m depth, Fipec Industries, Grande-Rivière, Québec,
180 Canada), bait traps and angling. A total of ten different species were caught: Northern pike (*Esox*
181 *lucius*), Lake charr (*Salvelinus namaycush*), Brook charr (*Salvelinus fontinalis*), White sucker
182 (*Catostomus commersonii*), Longnose sucker (*Catostomus catostomus*), Lake whitefish
183 (*Coregonus clupeaformis*), Round whitefish (*Prosopium cylindraceum*), Lake chub (*Couesius*
184 *plumbeus*), Burbot (*Lota lota*) and Slimy sculpin (*Cottus cognatus*). See Table 2 for the details of
185 capture data. A maximum of 20 individuals from each species were retained from each location.
186 However, considering the low sample numbers for Burbot ($n = 3$) and Slimy sculpin ($n = 4$), these
187 species were excluded from any further analysis. Only one Northern pike was captured in a
188 contaminated site (Lake Cherny) and Lake chub could only be analysed for whole fish metal
189 concentration, hence, both species were also excluded from this study. Our sampling protocol
190 was approved by the Comité Institutionnel de Protection des Animaux (CIPA) of the Institut
191 National de la Recherche Scientifique (INRS) and by the Ministère des Forêts, de la Faune et des
192 Parcs (MFFP) (SEG permit 18-08-06-061-09-S-P).

193 All fish captured were sacrificed by a sharp blow to the head before fork length (± 1 mm)
194 and mass (± 1 g) were measured for each individual. These measurements were then used to
195 calculate FCF as follows: $\text{FCF} = (W_T/L^3) \cdot 10^5$ where W_T represents the total weight of the fish (g)

196 and L the fork length (mm). Prior to further dissection and tissue sample collection, sex and
197 maturity were determined when possible. Gonad weight (± 1 g), as well as liver weight (± 1 g)
198 were also recorded to estimate gonadosomatic index (GSI) and HSI, respectively. The HSI, an
199 energy status indicator, was estimated as follows: $HSI = 100 \cdot W_L / W_T$ where W_L is the liver wet
200 weight (g). The GSI is used to assess sexual maturity of fish and was determined as follows: GSI
201 $= 100 \cdot W_G / W_T$ where W_G is the gonad weight (g). Otoliths, scales, and opercular bones for White
202 sucker and Longnose sucker were removed and evaluated by MFFP personnel to estimate fish
203 age. Two independent readings were performed on each structure and if results differed, a third
204 reading was completed. Otoliths were prioritized compared to other structures.

205 All captured fish were then dissected at the sampling site to collect liver tissue for trace
206 element analyses, as well as enzyme, lipid peroxidation and DNA damage analyses. Lake chub
207 were excluded from this procedure, as whole fish tissue was used to perform trace element
208 analyses for this species. Samples were immediately placed in 15 mL trace metal free tubes and
209 frozen at -20°C until subsequent trace element analyses could be performed. For enzyme, lipid
210 peroxidation, DNA damage analyses, and protein concentration determination, liver tissue was
211 sub-sampled and stored in cryogenic tubes at -80°C .

212 **2.3 Trace Element Analyses**

213 **Fish**

214 Trace element analyses for water, sediment and fish liver tissues were all performed at
215 the Institut National de la Recherche Scientifique – Centre Eau Terre Environnement (INRS-ETE)
216 in Québec (QC), Canada. Liver samples were lyophilized for a minimum of 48 h (FTS Systems
217 TMM, Kinetics Thermal Systems, Longueuil, QC, Canada), after which freeze-dried tissues were
218 divided between total mercury (THg) (approximately $50\text{-}100$ mg \pm 0.1 mg) and additional trace
219 element analyses (approximately $10\text{-}15$ mg \pm 0.1 mg) (XS205 DualRange, Mettler-Toledo GmbH,

220 Greifensee, Switzerland). In instances where sample weights were insufficient for both analyses,
221 trace element analyses were prioritized.

222 Dry weight (dw) THg results were determined with a Direct Mercury Analyzer (DMA-80,
223 Milestone Inc., Shelton, USA) according to US EPA method 7473 (EPA, 1998) before conversion
224 to final wet weight concentrations using percent moisture calculations (van der Velden et al.,
225 2013). As THg concentrations are generally highly correlated with age and/or length (Gantner et
226 al., 2010; Muir et al., 2005), obtained data were tested for significant relationships with these
227 variables using linear regressions ($\log_{10}\text{THg}$ vs. $\log_{10}\text{fork length}$, fork length, or $\log_{10}\text{age}$, and
228 age) (Martyniuk et al., 2020a; Tran et al., 2015) in an effort to determine the necessity of data
229 length standardization. Certified reference materials TORT-3 (Lobster hepatopancreas, National
230 Research Council of Canada, NRCC, Halifax, NS, Canada) and DOLT-5 (Dogfish liver, National
231 Research Council of Canada, NRCC, Halifax, NS, Canada) from the National Research Council
232 of Canada (NRCC), as well as blanks, were assessed in conjunction with every fifth tissue sample
233 in the same analytical cycle to establish accuracy and recovery. The average recoveries
234 (expressed as % of certified values \pm standard error) were $97.4 \pm 0.9\%$ and $90.4 \pm 0.7\%$ for TORT-
235 3 and DOLT-5 respectively.

236 Liver samples dedicated for additional trace element analyses were digested in 1500 μL
237 of Optima grade nitric acid (70%, v/v, Optima grade, Fisher Scientific, Whitby, ON, Canada) for 3
238 days at room temperature, before being heated for 2 hours at 60°C. After samples were cooled
239 to room temperature, 750 μL of hydrogen peroxide (30%, v/v, Optima grade, Fisher Scientific,
240 Whitby, ON, Canada) and 15 μL of hydrofluoric acid (47 – 51%, v/v, Optima grade, Fischer
241 Scientific, Whitby, ON, Canada) were added and samples were subsequently digested for an
242 additional 24 hours. Volume was adjusted to 15 mL with ultrapure water for a final digestion
243 volume of 10% nitric acid. Regarding trace element analyses of whole fish, a similar protocol was
244 applied with the following modifications: 5000 μL nitric acid, 2500 μL hydrogen peroxide, 50 μL

245 hydrofluoric acid and volume completed to 50 mL with ultrapure water. Concentrations of Al, As,
246 Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, and Zn were then quantified using either inductively
247 coupled atomic emission spectroscopy (ICP-AES) (Varian Vista XP Axial CCD Simultaneous ICP-
248 AES, Agilent Technologies, Santa Clara, California, USA) or inductively coupled plasma-mass
249 spectrometry (ICP-MS) (Model x-7, Thermo Elemental, Winsford, England, UK). The suite of trace
250 elements analyzed in this study has been chosen considering metals of concerns from iron mining
251 operations and from other mining industries (i.e. copper, gold, nickel) that took place in the
252 Canadian Shield. Certified reference materials TORT-3, DOLT-5 and NIST-1515 (Apple leaves,
253 National Institute of Standards and Technology, NIST, Gaithersburg, MD, USA) from the NRCC
254 and blanks were digested in triplicate with identical protocols to control for efficacy, accuracy, and
255 reproducibility. The average recoveries (expressed as % of certified values \pm standard error) of
256 TORT-3 were as follows: As ($99.2 \pm 5.0\%$), Cd ($92.1 \pm 2.8\%$), Co ($101.6 \pm 4.8\%$), Cr ($98.1 \pm$
257 6.5%), Cu ($88.0 \pm 3.7\%$), Fe ($87.7 \pm 5.6\%$), Mn ($89.4 \pm 5.4\%$), Ni ($84.5 \pm 4.9\%$), Pb ($97.3 \pm 10.4\%$),
258 Se ($95.3 \pm 5.9\%$) and Zn ($96.3 \pm 8.9\%$). The average recoveries of DOLT-5 were as follows: Al
259 ($98.1 \pm 16.4\%$), As ($82.4 \pm 3.3\%$), Cd ($91.9 \pm 3.9\%$), Co ($95.0 \pm 5.2\%$), Cr ($93.3 \pm 5.1\%$), Cu (75.2
260 $\pm 5.3\%$), Fe ($89.0 \pm 6.0\%$), Mn ($84.0 \pm 6.4\%$), Ni ($98.3 \pm 6.9\%$), Pb ($84.3 \pm 10.9\%$), Se ($91.1 \pm$
261 6.1%) and Zn ($100.4 \pm 9.6\%$). The average recoveries (expressed as % of certified values \pm
262 standard error) for NIST-1515 were as follows: Al ($88.1 \pm 7.1\%$), Ba ($81.6 \pm 4.2\%$) and Mn (83.5
263 $\pm 9.6\%$).

264 **Water and sediment**

265 Water samples for trace element analyses were acidified to a pH of 2 (Audet and Couture,
266 2003) using optima grade nitric acid (70%, v/v, Optima grade, Fisher Scientific, Whitby, ON,
267 Canada) and stored at 4°C until subsequent ICP-AES and ICP-MS analyses were performed.
268 Field and transport blanks were prepared following identical procedures. Sediment samples for
269 trace element analyses were digested following a protocol adapted from Tessier et al. (1979)

270 aimed at the extraction of bioavailable elements. Approximately 20 mL of $\text{NH}_2\text{OH}\cdot\text{HCl}$ 0.04 M
271 prepared in glacial acetic acid (99%, v/v, Optima grade, Fisher Scientific, Whitby, ON, Canada)
272 was added to 1 g of non-lyophilized sediment before samples were heated at 96°C ($\pm 3^\circ\text{C}$) for 6
273 hours. Samples were agitated every 30 min. Sample volume was then adjusted to 20 mL with
274 ultrapure water before a decantation period of 48 hours. Subsequently, 5 mL of supernatant was
275 removed and treated to the previously described digestion protocol.

276 **2.4 Enzyme activities, protein concentration, lipid peroxidation and DNA** 277 **damage analyses**

278 Enzyme activities included the quantification of CAT, SOD and GPx activity. Analyses were
279 performed using a UV/Vis spectrophotometer (Varian Cary 100, Varian Inc., Palo Alto, California,
280 USA) on 96-well microplates at room temperature (20°C). Liver samples were homogenized in a
281 buffer solution prepared with 20 mM HEPES, 1 mM EDTA and 0.10% Triton X-100 and activities
282 were measured as follows:

- 283 • *Catalase (CAT; EC 1.11.1.6)*: Catalase activity was measured using an assay kit (No.
284 707002 purchased from Cayman Chemical Company Inc. (Ann Arbor, Michigan USA)),
285 following the manufacturer's protocol at 540 nm.
- 286 • *Superoxide dismutase (SOD; EC 1.15.1.1)*: Superoxide dismutase activity was performed
287 using an assay kit (No. 706002 purchased from Cayman Chemical Company Inc. (Ann
288 Arbor, Michigan USA)), following the manufacturer's protocol at 450 nm.
- 289 • *Glutathione peroxidase (GPx; EC 1.11.1.9)*: Glutathione peroxidase activity was prepared
290 using an assay kit (No. 703102 purchased from Cayman Chemical Company Inc. (Ann
291 Arbor, Michigan USA)), according to the manufacturer's protocol at 340 nm.

292 Enzyme concentrations are expressed as nmol/min/mg protein for both CAT and GPx activities
293 and as U/mg protein for SOD activity (one unit of SOD is defined by the manufacturer as the
294 amount of enzyme needed to exhibit 50% dismutation of the superoxide radical). Protein

295 concentrations, used to normalize the enzyme activities, were determined on liver homogenates
296 before centrifugation using Coomassie (Bradford) Protein Assay Kit (No. 23200) at a wavelength
297 of 595 nm with protein concentrations expressed as mg protein per g of liver wet weight (Bradford,
298 1976).

299 Lipid peroxidation and DNA damage were estimated through the measurement of MDA
300 and 8-OHdG concentrations, respectively. Concentrations of MDA were estimated using
301 thiobarbituric acid reactive substances (TBARS) assay kits (No. 10009055) purchased from
302 Cayman Chemical Company Inc. (Ann Arbor, Michigan USA). Sample preparation and assays
303 were performed according to manufacturer's instructions with MDA concentrations measured at
304 540 nm and expressed as $\mu\text{mol MDA/g protein}$. The TBARS assay is not specific to MDA but it is
305 generally acknowledged that MDA represents the majority of TBARS in the liver. For quantification
306 of 8-OHdG concentrations, DNA was extracted from collected liver samples using Quick-DNA™
307 Miniprep Plus Kit (Nos. D4068 & D4069) purchased from Zymo Research (Irvine, CA, USA)
308 following the manufacturer's instructions. Initial DNA concentrations were then measured with a
309 NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific). Prior to the measurement of 8-
310 OHdG concentrations, DNA samples were prepared with the following protocol: (1) heat-
311 denaturation of the sample for 15 min at 100°C followed by 10 min on ice, (2) addition of 1 μL
312 nuclease P1 solution prepared at 0.175 $\mu\text{g}/\mu\text{L}$ in a buffer solution of 200 mM acetic acid and 10
313 mM CaCl_2 (pH = 6.5), (3) incubation for 1 hour at 37°C, (4) addition of 11.66 μL of alkaline
314 phosphatase solution at 3 $\mu\text{g}/\mu\text{L}$ in 1 M Tris buffer solution (pH = 8), (5) incubation for 30 min at
315 37°C, (6) heating for 10 min at 100°C before final storage on ice. A secondary DNA concentration
316 measurement was then taken to adjust the sample solution to 20 ng DNA/ μL . The concentrations
317 of 8-OHdG were then determined using DNA/RNA Oxidative Damage ELISA kit (No. 589320)
318 purchased from Cayman Chemical Company Inc. (Ann Arbor, Michigan USA) according to

319 manufacturer's protocols. Absorbance was measured at 410 nm and concentrations of 8-OHdG
320 are expressed as pg of 8-OHdG per μg of DNA.

321 **2.5 Statistical analyses**

322 Statistical analyses were performed using the R (Version 4.0.3, 2020) software package
323 and RStudio (Version 1.3.1093, © 2009-2020 RStudio, PBC) with a type I error set to $\alpha = 0.05$.
324 To better identify trends of elemental accumulation in ecologically similar fish, species were
325 grouped by subfamily. Thus, we gathered White sucker and Longnose sucker in the *Catostominae*
326 subfamily, Lake whitefish and Round whitefish in the *Coregoninae* subfamily and Lake charr and
327 Brook charr in the *Salmoninae* subfamily. Grouping the species into subfamilies was necessary to
328 perform statistically valid comparisons of patterns of bioaccumulation and investigate their
329 relationships with indicators of fish health, due to the low sample size for some species in some
330 lakes. However, this grouping clearly influences the metal bioaccumulation values for each
331 subfamily, depending on the relative number of individuals from different species within these
332 subfamilies and differences in habitat preference and diet of each species. For example, Lake
333 charr and Brook charr may occupy different ecological niche or forage on different prey types.
334 Data interpretation took this limitation into consideration.

335 Comparisons between mean values of biometric and biochemical variables and trace
336 element concentrations were performed using one-way analysis of variance (ANOVA). Data
337 normality was assessed using residual diagnostic histograms, Q-Q plots and the Shapiro-Wilk W
338 test (Shapiro and Wilk, 1965). Homoscedasticity was verified using the Bartlett's test (Bartlett,
339 1937) or the Levene's test (Levene, 1961). Data that did not respect normality or homoscedasticity
340 conditions were \log_{10} transformed and potential outliers were evaluated using Cook's Distance
341 statistic (Zar, 2007). A Tukey-Kramer honestly significant difference post-hoc test was completed
342 after each significant ANOVA to evaluate significant differences among means of studied groups.
343 When data were nonparametric, a Kruskal-Wallis test (Kruskal and Wallis, 1952) was performed.

344 Student's t-tests or Wilcoxon/Mann-Whitney test for nonparametric data were used for
345 *Catostominae*. Principal component analyses (PCA) were computed for each subfamily to
346 investigate relationships among liver trace element concentrations and biomarkers to reduce the
347 dimensionality of the dataset. In order not to bias the PCAs with missing values, the *MissMDA*
348 package was used to impute values that do not have effects in the calculation of the dimensions.
349 Results of PCAs are represented by biplots that project the dispersion of the variables on two
350 principal components. The significance of studied parameters is characterized by arrow length
351 and direction in relation to the two principal components. Major outputs from the PCA were then
352 examined with Spearman correlations (r_s) to identify significant relationships between trace
353 element concentrations and biomarkers. A Bonferroni correction was applied to account for the
354 multiple comparisons performed in the correlation matrices.

355 **3 RESULTS**

356 **3.1 Lake characteristics**

357 **Water parameters**

358 Water chemistry parameters for all sampled lakes are available in Table 1. Each location
359 expressed very similar values for temperature, while pH varied slightly between the most acidic
360 lake studied (Perroche, pH 6.6) and Lake Denault (pH 7.7). Values of conductivity showed a
361 greater variability among lakes but still remained similar to those detailed in a previous study from
362 Larocque et al. (2006) where the authors reported the conductivity of sixty lakes (mean: 28.7; min:
363 10.0; max: 170.0 $\mu\text{S}/\text{cm}$) distributed from latitude 49°48'N to 59°32'N in northwestern Québec.

364 **Water and sediment trace element concentrations**

365 Considering the high variability and the substantial number of values below the limits of
366 detection for As, Ba and Ni concentrations in fish tissues, those elements were excluded from
367 further analyses. Detection limits are listed in Table S1. Trace element concentrations of Al, Cd,
368 Co, Cr, Cu, Fe, Mn, Pb, Se and Zn for water and sediment are presented in Figure 2. Aqueous
369 and sediment concentrations of As, Ba and Ni can be found in Table S2. Aqueous concentrations
370 of Al and Fe expressed a very similar trend among sampled lakes, with mean values being
371 approximately 5-fold higher for Al and 10-fold higher for Fe in the reference lake than in lakes
372 Cherny and Houston. Waterborne concentrations of Cd and Co were greater in Lake Cherny
373 compared to the reference site and Lake Houston. The concentration of Cr was only determined
374 in Lake Houston samples, since values from the reference lake, lakes Cherny and Denault were
375 below the detection limits. Mean values of Cu concentrations appeared to be roughly 2-fold higher
376 in lakes Cherny and Houston compared to the reference site and Lake Denault. Aqueous
377 concentrations of Mn and Pb were higher in Lake Houston when compared to the reference lake,
378 lakes Cherny and Denault. Waterborne Se concentration did not differ among sampled lakes. Zinc

379 concentrations were higher in Lake Houston samples, being approximately 4-fold higher than in
380 the reference lake and Lake Cherny and 15-fold higher than in Lake Denault. In general, aqueous
381 element concentrations were low in all lakes sampled and never exceeded the Provincial Water
382 Quality Objectives (PWQO) (Ministère de l'Environnement et de la Lutte contre les changements
383 climatiques, 2021).

384 In sediment, Lake Houston exhibited generally higher element concentrations compared
385 to other lakes. The average value of Cd concentration in Lake Houston was roughly 3-fold higher
386 than in Lake Cherny, 10-fold higher than in the reference site and 45-fold higher than in Lake
387 Denault. Sediment Cu concentrations were higher in lakes Houston and Denault compared to the
388 reference lake and Lake Cherny. Concentrations of Co, Se and Zn all exhibited comparable
389 patterns among study sites, with mean values being the highest in Lake Houston and the lowest
390 in Lake Denault. Additionally, sediment Fe and Mn concentrations also showed higher elemental
391 values in Lake Houston when compared to the reference site and Lake Cherny with Fe
392 concentrations being 6- to 12-fold higher in Lake Houston compared to other lakes. Sediment Mn
393 concentrations further varied greatly among sampled lakes, with lakes Denault and Houston
394 demonstrating values 10- to 30-fold higher than in the reference lake and Lake Cherny.
395 Concentrations of Al and Cr also differed among study sites, with average sediment
396 concentrations of these elements in Lake Houston being 18-fold higher than in Lake Denault.
397 Finally, sediment concentrations of Pb were higher in lakes Cherny and Houston than in the
398 reference site and Lake Denault. Overall, sediment elemental concentrations were consistently
399 higher than waterborne elemental concentrations.

400 **3.2 Biometric variables**

401 Biometric variables and sex ratios of *Salmoninæ*, *Catostominæ* and *Coregoninæ* are
402 presented in Table 3, except for GSI, presented in Table S3. Observed fish diversity was greatest
403 in Lake Denault, but success of captures was highly variable among lakes. *Salmoninæ* were

404 collected in the reference lake (Brook charr and Lake charr), Lake Cherny (Lake charr), Lake
405 Houston (Brook charr) and in Lake Denault (Brook charr and Lake charr). *Catostominæ* were
406 caught in the reference site (White sucker) and Lake Denault (White sucker and Longnose
407 sucker). *Coregoninæ* were captured in the reference lake (Lake whitefish), Lake Cherny (Lake
408 whitefish and Round whitefish) and Lake Denault (Lake whitefish and Round whitefish). Fish
409 sampled at the reference site were generally longer, heavier and older than fish collected from
410 contaminated sites. Fulton's condition factor and HSI were also mostly greater in fish from the
411 reference lake compared to fish from other lakes. *Salmoninæ* from the reference site, especially
412 Brook charr, exhibited higher values for fork length ($X^2_{(1,39)} = 36.00, p < 0.0001$), whole weight
413 ($X^2_{(1,39)} = 32.50, p < 0.0001$) and FCF ($t_{(1,39)} = -4.10, p = 0.0002$) when compared to individuals
414 from Lake Houston. Nevertheless, *Salmoninæ* collected in Lake Houston were older ($X^2_{(1,39)} =$
415 $8.42, p = 0.0148$) than individuals from the reference site. Similarly, *Catostominæ* demonstrated
416 higher values for fork length ($X^2_{(1,20)} = 1.00, p = 0.0004$), whole weight ($X^2_{(1,20)} = 1.00, p = 0.0004$),
417 FCF ($X^2_{(1,20)} = 8.00, p = 0.0011$), HSI ($X^2_{(1,20)} = 6.00, p = 0.0213$) and age ($X^2_{(1,20)} = 11.50, p =$
418 0.0044) in the reference lake compared to individuals from Lake Denault. *Coregoninæ*
419 (particularly Lake whitefish) collected in Lake Cherny displayed higher values of HSI ($F_{(2,39)} =$
420 $13.34, p < 0.0001$) compared to HSI values of individuals collected in the reference lake ($p =$
421 0.0006) and Lake Denault ($p = 0.0006$).

422 3.3 Trace element bioaccumulation

423 Trace element concentrations of Al, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, Se and Zn in the liver
424 of *Salmoninæ*, *Catostominæ* and *Coregoninæ* are shown in Table 4, while liver concentrations of
425 As, Ba and Ni are reported in Table S4.

426 ***Salmoninæ***

427 In *Salmoninæ*, liver concentrations of Al ($X^2 = 28.20$, $p < 0.0001$), Cd ($F_{(3,44)} = 21.3$, p
428 < 0.0001), Co ($F_{(3,44)} = 4.07$, $p = 0.0124$), Cr ($F_{(3,44)} = 29.88$, $p < 0.0001$), Cu ($F_{(3,44)} = 7.16$, $p =$
429 0.0005), THg ($X^2 = 13.33$, $p = 0.0040$), Pb ($F_{(3,44)} = 6.46$, $p = 0.0010$), Se ($F_{(3,44)} = 66.64$, $p <$
430 0.0001) and Zn ($F_{(3,40)} = 12.98$, $p < 0.0001$) varied by lake. However, trace element concentrations
431 were generally higher in *Salmoninæ* from Lake Houston compared to other lakes. For example,
432 concentrations of Al, Cd and Cr were higher in fish collected from Lake Houston compared to
433 *Salmoninæ* captured in the reference lake ($p = 0.0001$, $p < 0.0001$ and $p < 0.0001$ respectively)
434 and lakes Cherny ($p = 0.0011$, $p = 0.0007$ and $p = 0.0004$ respectively) and Denault ($p < 0.0001$,
435 $p = 0.0122$ and $p < 0.0001$ respectively). Liver Co concentrations were higher in *Salmoninæ*
436 collected from Lake Houston compared to *Salmoninæ* sampled from Lake Denault ($p = 0.0242$).
437 The concentrations of Cu were greater in liver tissues sampled from Lake Houston than in those
438 from the reference site ($p = 0.0008$). Individuals captured from Lake Cherny exhibited higher
439 values of THg than fish from the reference lake ($p = 0.0076$) and Lake Houston ($p = 0.0058$), while
440 those collected in Lake Denault displayed greater THg concentrations than fish from the reference
441 lake ($p = 0.0042$) and Lake Houston ($p = 0.0050$). Liver Pb concentrations were greater in fish
442 collected from the reference lake ($p = 0.0026$) and Lake Houston ($p = 0.0017$) than those from
443 Lake Denault. However, individuals from Lake Denault showed higher Se concentrations than
444 *Salmoninæ* collected in the reference site ($p < 0.0001$) and lakes Cherny ($p = 0.0042$) and
445 Houston ($p < 0.0001$). The concentrations of Se were also greater in fish sampled in lakes
446 Houston ($p < 0.0001$) and Cherny ($p = 0.0012$) compared to the reference lake. Finally, the
447 concentrations of Zn were higher in fish captured in Lake Houston compared to the reference lake
448 ($p = 0.0013$), lakes Cherny ($p = 0.0216$) and Denault ($p < 0.0001$), while Zn concentrations of
449 *Salmoninæ* from the reference site were greater than in fish from Lake Denault ($p = 0.0056$).

450 *Catostominæ*

451 In *Catostominæ*, concentrations of Cr, Pb and Zn did not vary significantly by site. Concentrations
452 of Al ($t_{(1,37)} = 6.01$, $p < 0.0001$), Mn ($X^2_{(1,37)} = 217.00$, $p < 0.0001$) and Se ($t_{(1,37)} = 2.48$, $p = 0.0299$)
453 differed by sampling location, with fish from Lake Denault exhibiting significantly greater
454 concentrations than *Catostominæ* from the reference site. In contrast, concentrations of Cd
455 ($X^2_{(1,37)} = 7.00$, $p < 0.0001$), Co ($t_{(1,37)} = -4.82$, $p = 0.0011$), Cu ($t_{(1,37)} = -2.83$, $p = 0.0154$), Fe ($t_{(1,37)}$
456 $= -4.59$, $p = 0.0005$) and THg ($X^2_{(1,33)} = 0.00$, $p < 0.0001$) were higher in *Catostominæ* collected
457 in the reference lake than in fish from Lake Denault.

458 *Coregoninæ*

459 In *Coregoninæ*, liver Al ($F_{(2,42)} = 7.84$, $p = 0.0013$), Cd ($F_{(2,42)} = 19.84$, $p < 0.0001$), Co ($F_{(2,43)}$
460 $= 13.01$, $p < 0.0001$), Cr ($F_{(2,43)} = 21.12$, $p < 0.0001$), Cu ($F_{(2,42)} = 14.90$, $p < 0.0001$), Fe ($F_{(2,43)} =$
461 4.45 , $p = 0.0176$), THg ($F_{(2,41)} = 11.41$, $p = 0.0001$), Mn ($F_{(2,43)} = 14.36$, $p < 0.0001$), Pb ($F_{(2,36)} =$
462 5.31 , $p = 0.0096$), Se ($F_{(2,43)} = 29.98$, $p < 0.0001$) and Zn ($F_{(2,40)} = 25.4$, $p < 0.0001$) concentrations
463 significantly differed by sampling locations. The concentrations of Al, Cd and Co were higher in
464 samples from lakes Denault ($p = 0.0038$, $p = 0.0018$ and $p < 0.0001$ respectively) and the
465 reference site ($p = 0.0133$, $p < 0.0001$ and $p = 0.0059$ respectively) than in liver tissue sampled
466 from Lake Cherny. Liver Cr and Zn concentrations in sampled *Coregoninæ* from the reference
467 lake were greater than in fish from lakes Cherny ($p < 0.0001$ and $p < 0.0001$ respectively) and
468 Denault ($p = 0.0096$ and $p < 0.0001$ respectively). The concentrations of Cu and THg were higher
469 in individuals captured in the reference lake ($p < 0.0001$ and $p = 0.0001$ respectively) and Lake
470 Cherny ($p = 0.0004$ and $p = 0.0002$ respectively) than *Coregoninæ* captured in Lake Denault. The
471 concentrations of Fe were greater in *Coregoninæ* from Lake Denault ($p = 0.0278$) compared to
472 *Coregoninæ* from Lake Cherny and *Coregoninæ* collected in the reference lake ($p = 0.0017$) and
473 Lake Denault ($p < 0.0001$) expressed higher Mn concentrations than those from Lake Cherny.
474 Individuals caught in the reference site exhibited higher Pb concentrations than individuals from

475 Lake Cherny ($p = 0.0069$). The concentrations of Se in *Coregoninæ* from Lake Denault were
476 greater than in *Coregoninæ* captured in the reference lake ($p < 0.0001$) and Lake Cherny ($p <$
477 0.0001), while Se concentrations in liver tissue sampled from Lake Cherny were higher than in
478 samples from the reference site ($p = 0.0040$).

479 **3.4 Relationships between trace element concentrations and biomarkers**

480 The PCAs and Spearman correlations performed to assess the relationships between
481 trace element concentrations and biomarkers are presented in Figure 3 and Table 5 for
482 *Salmoninæ*, Figure 4 and Table 6 for *Catostominæ* and Figure 5 and Table 7 for *Coregoninæ*,
483 respectively. See Table S5 for the results of enzyme activities (CAT, SOD and GPx activities) and
484 biomarkers of oxidative stress (MDA and 8-OHdG concentrations).

485 ***Salmoninæ***

486 The PCA computed for *Salmoninæ* led to two principal components explaining 60.6% of
487 the total variability. However, an ellipse could not be drawn for *Salmoninæ* collected in Lake
488 Cherny despite individuals having been collected in all four lakes as a result of a low sample size
489 ($n = 2$) in this location. The first dimension (Dim1) explained 33.3% of the overall variance and
490 distinctly separated *Salmoninæ* captured in Lake Denault (on the left side of the axis) from
491 *Salmoninæ* sampled in Lake Houston (on the right side of the axis). Individuals collected in the
492 reference site (Lake Perroche) represent an overlap of other two populations. Fork length, total
493 weight, THg and Se concentrations, age, FCF, HSI, and GPx activity correlated negatively with
494 Dim1. On the other hand, the activity of CAT, MDA and 8-OHdG concentrations as well as
495 concentrations of Fe, Cu, Co, Cd, Cr, Mn, Al and Zn correlated positively with Dim1. This first axis
496 appeared to detail relationships dependent on fish growth, as Se and THg concentrations were
497 highly associated to biometric variables (fork length, total weight, age and HSI) and GPx activity.

498 Results obtained from Spearman correlations further supported this trend, as positive
499 correlations were observed between concentrations of THg and length, weight and age.
500 Additionally, Se concentrations were positively correlated to age and 8-OHdG concentrations.
501 The concentrations of MDA and 8-OHdG appeared to be linked with concentrations of Fe, Cu,
502 Co, Cd and Cr, while CAT activity was associated with concentrations of Mn, Al and Zn. Spearman
503 correlations also revealed that Cd concentrations displayed a negative correlation with FCF, while
504 the MDA and 8-OHdG concentrations were positively correlated.

505 The second principal component (Dim2) explained 27.3% of the total variability observed
506 for the examined variables. Fulton's condition factor and concentrations of Pb and Zn correlated
507 negatively with Dim2 while all the other variables (except Al concentrations) correlated positively
508 with Dim2. A particularly high loading of SOD activity was additionally observed. This second
509 principal component separated *Salmoninæ* sampled in contaminated sites (lakes Denault and
510 Houston) on the top of the axis from *Salmoninæ* collected at the reference site on the bottom of
511 the axis.

512 ***Catostominæ***

513 The PCA performed to evaluate relationships between trace element concentrations and
514 biomarkers for *Catostominæ* led to two principal components explaining 54.6% of the total
515 variability. Since *Catostominæ* were caught in two sampling sites only (the reference lake and
516 Lake Denault), two ellipses are displayed on the biplot. The first principal component (Dim1)
517 explained 36.8% of the total variability and strongly separated both populations of *Catostominæ*.
518 The positive (right) side of Dim1 is defined by the high loading of FCF, fork length, total weight,
519 age and concentrations of Cd and THg. With the exemption of Cd concentrations, these results
520 mirror the similar pattern determined for *Salmoninæ*.

521 Spearman correlations support the strong positive relationship between THg and Cd
522 concentrations and all biometric variables (total weight, fork length, FCF and age). Hepatosomatic

523 index, GPx and SOD activities, MDA concentrations and Co concentrations were also associated
524 positively with Dim1. In contrast to our observations for *Salmoninæ*, Se concentrations did not
525 express any significant correlations with biometric or biochemical biomarkers in *Catostominæ*.
526 Concentrations of Mn, CAT activity and 8-OHdG concentrations loaded negatively on Dim1. The
527 concentrations of Mn were negatively correlated to fork length and total weight. Additionally, Mn
528 concentrations were strongly and negatively correlated with Cd and THg concentrations. Finally,
529 the activity of GPx was positively correlated to MDA concentrations.

530 The second principal component (Dim2) explained 17.8% of the total variability and was
531 characterized by the high positive loading of concentrations of Fe, Cr, Al and Pb. The
532 concentrations of these metals were higher in *Catostominæ* from Lake Denault than in fish from
533 the reference site, however, they did not appear to covary with biometric or biochemical
534 biomarkers.

535 ***Coregoninæ***

536 The PCA performed for *Coregoninæ* led to two principal components explaining 52.2% of
537 the overall variability. Three ellipses were determined representing each sampling site where
538 *Coregoninæ* were captured (the reference site, lakes Cherny and Denault). The first principal
539 component (Dim1) explained 29.9% of the total variance and patterns in the data appeared to be
540 dictated by parameters indicative of fish growth, as total weight, fork length, age, FCF and THg,
541 Cu, Cr and Cd concentrations were all positively correlated. Spearman correlations further
542 support these observations as positive correlations were determined for both Cu and THg
543 concentrations with fork length, total weight and age, in addition to FCF for THg concentrations.
544 Concentrations of Se did not tend to generally covary with biometric variables. However, Mn
545 concentrations were negatively correlated to fork length, total weight and FCF, as mostly identified
546 for *Salmoninæ* and *Catostominæ*.

547 The activities of CAT, GPx and SOD, 8-OHdG and MDA concentrations, as well as
548 concentrations of Al, Co, Fe, Mn, Pb and Se were negatively associated to Dim1. Additionally,
549 CAT activity was positively correlated with MDA concentrations. Although Mn concentrations and
550 CAT activity loaded similarly on the negative (left) side of Dim1, no significant correlation could
551 be determined between these two variables.

552 The second principal component (Dim2) explained 22.3% of the observed total variability.
553 Similar to observations for *Salmoninæ*, the majority of examined variables were positioned on the
554 positive side (top) of the axis. Only HSI correlated negatively. In addition, Spearman values
555 detailed negative correlation between HSI and concentrations of Cd. Finally, *Coregoninæ*
556 collected in Lake Cherny appeared to be distinctly separated from fish collected in Lake Denault,
557 though overlap with *Coregoninæ* from the reference lake was observed.

558 **4 DISCUSSION**

559 While a small sub-set of studies have described the environmental contamination resulting
560 from iron mining operations in the Fermont (Jacques et al., 2020) and Schefferville (Aebischer et
561 al., 2015; Laperrière et al., 2007) areas, as well as the effects of trace metal contamination from
562 mining activities on fish elsewhere in the province of Québec (Couture et al., 2008a; Gauthier et
563 al., 2009; Martyniuk et al., 2020b; Ponton et al., 2016; Pyle et al., 2008), scientific research
564 detailing these subjects still remains limited in our region of study. Furthermore, with the
565 exemption of a few studies that documented trace metal accumulation and the associated impacts
566 (Amundsen et al., 2011; Moiseenko and Kudryavtseva, 2001) in other Northern regions, to the
567 best of our knowledge there are no reports in the scientific literature reporting on the effects of
568 trace element contamination on fish health and condition, especially antioxidant capacities and
569 oxidative damage, near past and present iron ore exploitation sites in subarctic regions. Our study
570 addresses this knowledge gap.

571 **4.1 Element concentrations in water and sediment among sampling sites**

572 Aqueous element concentrations varied among our study sites. The highest
573 concentrations of Al and Fe were observed in Lake Perroche, our reference location. However,
574 this can potentially be explained by the lower pH value measured in that lake, leading to greater
575 trace metal solubility (Lofts et al., 2008). The relationship between pH and aqueous trace metal
576 solubility may also explain the higher concentrations of Mn, Pb and Zn in Lake Houston in
577 comparison to those recorded at other locations. Nevertheless, all aqueous element
578 concentrations reported in any of our study lakes remained below the PWQO guidelines for
579 Québec, which has been designed to protect aquatic organisms (Ministère de l'Environnement et
580 de la Lutte contre les changements climatiques, 2021).

581 In sediment, concentrations of Cd, Cu, Se and Zn were higher in Lake Houston compared
582 to the reference lake, where values for these elements were comparable with sediment
583 concentrations determined by Couture et al. (2008b) at their reference sites. However, they
584 reported element concentrations 20-fold higher for Cd, Se and Zn and over 600-fold higher for Cu
585 in contaminated lakes from the Rouyn-Noranda area compared to Lake Houston. Mean values of
586 Fe in Lake Houston were 10-fold higher than in the reference site and 5-fold greater than the
587 highest values determined in twelve lakes by Pyle et al. (2005) in the Sudbury area. Accumulation
588 of Fe in the sediment of Lake Houston clearly reflects the impact of iron mining activities in the
589 Schefferville area, whereas Rouyn-Noranda and Sudbury areas were affected by copper and
590 nickel mining operations. In addition to Fe contamination, lakes Houston and Denault displayed
591 significantly higher concentrations of Mn in sediment compared to the reference site. A past study
592 has indicated that iron smelting in Schefferville is associated with Mn, as Leslie and Bourque
593 (1984) indicated that iron ore extracted from this location contained from trace amounts up to 10%
594 Mn. Undoubtedly, Mn concentrations determined in sediment in both lakes can be attributed to
595 iron ore exploitations in Schefferville. The comparison between element concentrations in
596 sediment and water samples among our study sites provides insight into the long-term deposition
597 processes of elements in sediment while aqueous element concentrations indicate short-term
598 inputs, which is also supported by Pyle et al. (2005).

599 Overall, lakes from Schefferville (Denault and Houston) appeared to be more negatively
600 affected by mining operations than Lake Cherny in Fermont and the reference location. Our data
601 also clearly indicates the effect of distance on aqueous habitat contamination by trace elements
602 from mining operations, as Lake Cherny is located farther from past and current mining operation
603 sites than lakes Denault and Houston. In Lake Denault, while other elemental concentrations were
604 similar to the reference lake, the high sediment concentrations of Mn reflect the legacy of past
605 iron ore exploitations (IOC, closed in 1982). However, as Lake Houston is affected by current

606 (TSMC) and more recent (LIM, closed in 2014) mining activities, we observed higher
607 concentrations of Cd, Cu, Fe, Mn, Se and Zn than in sediment from the reference lake. With this
608 location, apparent short-term inputs, manifested by surface water from watersheds upstream and
609 atmospheric transport are less pronounced.

610 **4.2 Element bioaccumulation among fish subfamilies and relationships with** 611 **biometric and biochemical biomarkers**

612 ***Salmoninæ***

613 In *Salmoninæ*, higher liver concentrations of THg were measured in fish from lakes
614 Denault (Schefferville) and Cherny (Fermont) compared to the reference location. Low THg
615 concentrations in the liver of *Salmoninæ* from Lake Houston were expected given the associations
616 between THg and species dependent trophic position. Total mercury, and more specifically MeHg,
617 is known to bioaccumulate and reach elevated concentrations in top predatory fish, such as Lake
618 charr (Chételat et al., 2015; Lockhart et al., 2005). However, only Brook charr, a smaller salmonid,
619 was present in Lake Houston. A shift in dietary preferences toward larger prey may explain the
620 elevated hepatic THg concentrations observed in *Salmoninæ* from Lake Cherny. In our study,
621 THg accumulation itself was not associated with any significant effect.

622 In Lake Houston, metal concentrations of Al, Cd, Co, Cr, Cu and Pb were more elevated
623 in comparison to concentrations measured in *Salmoninæ* from the reference site. Furthermore,
624 observed concentrations were greater than those detailed in previous reports of trace metal
625 concentrations in the liver of another *Salmoninæ* species, Arctic charr (*Salvelinus alpinus*),
626 collected in the Deception Bay region of Northern Québec (Martyniuk et al., 2020b) and other
627 Northern regions of the circumpolar Arctic (Allen-Gil et al., 2003). These elevated concentrations
628 may be explained by dietary metal contamination or by a slower growth in Lake Houston
629 *Salmoninæ* due to a decrease in food availability. Both alternatives would be consistent with the
630 heavy contamination of the sediment in this lake. In a study presented by Besser et al. (2001),

631 the authors demonstrated that different trophic levels (periphyton, benthic invertebrates and Brook
632 charr) exhibited different responses to metal contamination, which they associated with foraging
633 habits and food intake within each trophic level. The authors suggested that Cu concentrations
634 measured in benthic invertebrates and Brook charr were linked to the larger scale negative
635 impacts observed on fish populations and benthic invertebrate communities. Considering metal
636 concentrations measured in sediment from Lake Houston, our results could indicate a comparable
637 scenario. Furthermore, although Brook charr were longer, heavier and in a better condition in the
638 reference lake than in Lake Houston, they were older in Lake Houston, indicating faster growth in
639 fish from the reference site. As Lake Houston and the reference lake are experiencing a very
640 similar seasonality given their geographical proximity, the overall poorer condition of Lake
641 Houston *Salmoninæ* may be linked to indirect effects associated with reductions in food
642 availability. Nevertheless, our sampling only involves a limited number of fish (n = 20), all captured
643 in the same year. Therefore, inter-individual and inter-annual variability is expected given the
644 environment under study.

645 *Salmoninæ* sampled in the Schefferville area, particularly those captured in Lake Denault,
646 demonstrated high liver concentrations of Se compared to fish from the reference site, as well as
647 previous studies on *Salmoninæ* species (Arribère et al., 2008; Herrmann et al., 2016). Similar to
648 observations for liver THg, Se concentrations displayed a positive correlation with fish age,
649 indicating that *Salmoninæ* bioaccumulate this element, most likely through dietary sources, since
650 aqueous Se does not induce elemental bioaccumulation even in the most contaminated areas
651 (Stewart et al., 2010; Wood et al., 2011a). Liver Se concentration was strongly and positively
652 correlated to 8-OHdG concentrations, suggesting that Se is contributing to oxidative stress and
653 resulting DNA damage in our sampled individuals (Spallholz, 1994; Spallholz et al., 2001).
654 Although non-significant, the positive correlation of liver Se with SOD and GPx activities also
655 suggests that this oxidative stress induced an antioxidant response. A review published by Lemly

656 (1993a) recommended that 12 $\mu\text{g/g}$ should be considered as a toxic threshold for freshwater
657 fishes based upon the evaluation of numerous studies demonstrating negative impacts linked to
658 liver Se accumulation between 12 and 86 $\mu\text{g/g}$. *Salmoninæ* populations from Lake Denault, but
659 also those from lakes Houston and Cherny, exceeded that threshold (liver Se concentrations
660 ranging from 14 to 90 $\mu\text{g/g}$ for these lakes) indicating that fish populations at these locations may
661 be subjected to elemental Se-induced toxicity. The very fine boundary between essentiality and
662 toxicity of Se (Hamilton, 2004) represents a substantial environmental threat in our region of
663 study, especially for *Salmoninæ*, which are particularly sensitive (Lemly, 1993a). However, given
664 that the liver Se concentrations in our *Salmoninæ* were strongly and positively correlated with
665 liver Cd, Cu and Hg concentrations, this field study cannot exclude that the increase in oxidative
666 damage and antioxidant capacity that we observed in our system may be a response to the
667 contaminant mixture.

668 Bioaccumulation of Se through the aquatic food web can lead to deleterious impacts in
669 early-life stages of fish such as teratogenicity and ultimately lead to population level effects
670 through recruitment impairment (Janz et al., 2010; Lemly, 1993b, 2004). Palace et al. (2004)
671 suggested that oxidative stress in fish embryos could be a trigger for these effects. We could not
672 evaluate the toxicity of Se on early life-stages of *Salmoninæ* in our study. However, Chapman
673 (2007) demonstrated that Northern fish species could tolerate higher concentrations of Se than
674 more temperately located species and recommends the adoption of a “site and species-specific
675 studies” approach before drawing conclusions. Additionally, Se is known to have a protective
676 effect against MeHg (Ralston et al., 2008) when the molar ratio between Se and Hg approaches
677 or exceeds a 1:1 stoichiometry (Peterson et al., 2009; Ralston et al., 2007). Our results could
678 suggest a preventive action of Se against Hg toxicity (Se/THg molar ratios > 1). Since
679 demethylation takes place in the liver of fish (Wood et al., 2011b) and we only measured THg and
680 not MeHg, we do not know the proportion of THg that was in the methylated form in our samples.

681 Thus, it remains difficult to establish whether Se may be a protective or a toxic element given the
682 elevated Se concentrations measured in this study, even for Northern species. In other
683 circumstances (Se/Hg ratio < 1:1 stoichiometry), the very high affinity between Se and Hg induces
684 Se-Hg bonds, potentially leading to a decrease in Se availability for vital functions (Ralston and
685 Raymond, 2010).

686 ***Catostominæ***

687 *Catostominæ* in Lake Denault displayed significantly elevated concentrations of Mn
688 compared to those from the reference site. Manganese is an essential trace metal for fish and is
689 required for many biological functions (Goldhaber, 2003; Olmedo et al., 2013). However, more
690 recently it has become an emerging contaminant of interest in ecotoxicological studies (Pinsino
691 et al., 2012). The negative correlations determined between liver Mn concentration and biometric
692 variables in *Catostominæ* (as well as in *Coregoninæ*) indicate that Mn does not bioaccumulate
693 with age in fish liver. This is consistent with previous findings presented in Nussey (2000), that
694 suggested that Mn is well regulated through homeostatic mechanisms. As described in Bendell-
695 Young and Harvey (1986), at the water-sediment interface, Mn can be characterized as an
696 intermediate state between dissolved and particulate forms. White sucker and Longnose sucker
697 are both groundfish, feeding on benthic invertebrates. Their feeding behaviour induces a
698 resuspension of particles in the upper sedimentary layer where their prey is located, leading to
699 Mn contamination through both waterborne and dietary sources. Furthermore, several studies
700 have reported that Mn has the potential to disturb the abundance and the diversity of benthic
701 invertebrates (Dittman and Buchwalter, 2010; Wellnitz et al., 1994; Wilson et al., 2019). Therefore,
702 in our study, a decrease in prey availability due to Mn contamination could potentially explain the
703 lower condition observed in *Catostominæ* captured in Lake Denault, as these fish are directly
704 exposed to metal contamination from both contact with sediment and food consumption (Bendell-
705 Young and Harvey, 1986; Hamilton et al., 1990; Munkittrick and Dixon, 1988).

706 Similar to other essential and non-essential metals, excessive Mn accumulation can lead
707 to immune response disruptions (Aliko et al., 2018) and induce oxidative stress (Copat et al.,
708 2020; Hedayati et al., 2014) through the production of ROS or interference with antioxidant
709 capacities. Under laboratory conditions, Vieira et al. (2012) exposed goldfish to aqueous Mn at
710 0.1 and 1 mM (5.5 and 55 mg/L, respectively) for 96 h. They observed an increase of GPx activity
711 and an inhibition of CAT activity in liver tissue. The authors hypothesized that GPx represented
712 the first defense against waterborne Mn exposition. Their observation is supported by another
713 study from Gabriel et al. (2013) where the authors exposed juvenile tambaqui (*Colossoma*
714 *macropomum*) to aqueous Mn (3.9 mg/L Mn²⁺) for 96 hours. They reported a significant increase
715 in liver glutathione-S-transferase (GST) activity and GSH content along with unchanged CAT
716 activity. Their studies contrast strongly with the results of our study, in which wild fish were
717 chronically exposed to water Mn concentrations ranging from 0.49 to 3.18 µg/L (3 orders of
718 magnitude lower than the laboratory studies cited above), but also exposed to an unknown level
719 of dietary Mn, and where CAT activity was stimulated, but GPx activity inhibited, by Mn. Although
720 the comparison of results obtained from these studies with ours highlights the sharp contrast
721 between short-term responses of antioxidant mechanisms observed in acute laboratory
722 exposures and the long-term adaptations of animals chronically exposed to contaminants in the
723 field, both support that exposure to high Mn concentrations leads to a response of antioxidant
724 capacities. Our data allow to hypothesize that long-term (life-long) exposures to Mn like in our
725 study induce catalase, inhibit GPx and lead to oxidative damage to DNA (as indicated by 8-OHdG)
726 and metabolic costs. The negative correlations between liver Mn and Cd and THg concentrations
727 strongly support that in the case of the *Catostominae* from our study area, Mn, and not other
728 metals, is the metal of concern for the health of these fish.

729 Although liver Se concentration was higher in *Catostominae* from Lake Denault compared
730 to individuals from the reference location, our study did not reveal any significant relationships

731 with the biomarkers of effects examined. This suggests that Se accumulation did not reach toxic
732 levels in *Catostominae*. Additionally, liver Se concentrations in individuals from Lake Denault were
733 below the toxic threshold of 12 µg/g proposed by Lemly (1993a), which supports our observations.
734 Liver THg and Cd concentrations were higher in fish from the reference lake compared to Lake
735 Denault and were positively correlated to biometric variables, clearly demonstrating the
736 bioaccumulation potential of these two metals (Croteau et al., 2005; Kidd et al., 2012). As
737 observed in *Salmoninae*, an increase of GPx activity in *Catostominae* appeared to efficiently
738 prevent oxidative damage that may be induced by these elements, since the liver concentration
739 of both Cd and Hg was negatively correlated with 8-OHdg concentration, an indicator of DNA
740 oxidative damage.

741 ***Coregoninae***

742 In our study, the *Coregoninae* subfamily was represented by Lake whitefish and Round
743 whitefish, with both species being part of the *Salmonidae* family (Phillips and Rab, 2001).
744 *Coregoninae* also share similar ecological characteristics with *Catostominae*, in terms of habitat
745 and food preferences, even if *Coregoninae* can forage in deeper waters. These genetic and
746 ecological similarities with both *Salmoninae* and *Catostominae*, respectively, were reflected in the
747 observed patterns of liver element concentrations and their associated effects. As observed with
748 *Catostominae*, *Coregoninae* from Lake Denault exhibited higher liver concentrations of Mn when
749 compared to the reference site. Bioaccumulation of Mn also appeared to be regulated through
750 cellular homeostasis, as suggested by the negative correlations between Mn concentrations and
751 biometric biomarkers.

752 With *Coregoninae*, we reported negative correlations between HSI and liver concentrations
753 of Cd. Past laboratory studies have demonstrated that fish exposed to both dietary and
754 waterborne Cd displayed a decrease of HSI and disruptions to the antioxidant system (Drag-
755 Kozak et al., 2019; Jamwal et al., 2018; Pereira et al., 1993; Pretto et al., 2011). Lake Cherny

756 presented higher aqueous and sediment Cd concentrations compared to the reference lake and
757 Lake Denault. However, these elevated habitat concentrations appeared to not be reflected by
758 liver Cd accumulation, since *Coregoninæ* from Lake Cherny exhibited lower concentrations of
759 hepatic Cd compared to fish from the reference site and Lake Denault. Currently, our data does
760 not provide an explanation for this trend. We propose two alternative hypotheses to explain this
761 observation. An increased detoxification of intracellular Cd through GSH and metallothionein (MT)
762 mediation in *Coregoninæ* from Lake Cherny, induced by chronic exposure, could decrease Cd
763 accumulation (Chowdhury et al., 2005; Wood et al., 2011b). Alternately, an antagonistic action
764 with calcium could lead to a decrease in Cd bioavailability (Brzóska and Moniuszko-Jakoniuk,
765 1997), in agreement with our observation that Lake Cherny exhibited the highest conductivity
766 value among the lakes studied.

767 As observed in *Salmoninæ*, *Coregoninæ* from lakes Cherny and Denault exhibited higher
768 liver Se concentration than fish sampled in the reference site. Nevertheless, no correlation could
769 be established between Se concentrations and biomarkers of oxidative stress or oxidative
770 damage measured in this research. The absence of these associations provides further support
771 for the hypothesis that cold-water species can tolerate higher Se bioaccumulation, as liver Se
772 concentration in *Coregoninæ* from Lake Denault were above the potential toxic threshold of 12
773 µg/g. Additionally, compared to fish collected in the reference site and Lake Cherny, *Coregoninæ*
774 captured in Lake Denault displayed lower liver THg concentrations. In a study from Amundsen et
775 al. (2011), Whitefish (*Coregonus lavaretus*) feeding on plankton rather than on benthic
776 invertebrates exhibited higher Hg concentrations, suggesting that a pelagic feeding behaviour
777 leads to an increase of Hg bioaccumulation. Two additional studies (Kahilainen et al., 2017; Keva
778 et al., 2017) further supported this observation. This suggests that *Coregoninæ* from Lake Denault
779 may be primarily feeding on benthic invertebrates, since both *Catostominæ* and *Coregoninæ* from

780 this lake expressed low liver THg and high Mn concentrations compared to their counterparts
781 from the reference site.

782 **5 CONCLUSION**

783 Our study highlighted the interspecific variation of fish responses to contaminated
784 environments near past and current mining areas in Northern Québec. Fish sampled in Lake
785 Cherny (Fermont area) exhibited a generally better condition and appeared to be less impacted
786 by trace element contamination than fish captured in lakes Houston and Denault (Schefferville
787 area). Sediment Mn concentrations in Lake Denault and sediment concentrations of Cd, Cu, Fe,
788 Mn, Se and Zn, in addition to water concentrations of Mn and Pb in Lake Houston were higher
789 than those determined in the reference site. These observations can be attributed to the impacts
790 of both historical and current mining operations, as iron ore exploitation in the Schefferville area
791 has been more intense and precedes exploitation in the Fermont area. Trace element
792 accumulation in the liver of the fish studied differed among subfamilies, particularly for Mn, Se
793 and THg concentrations, and can most likely be related to trophic position and feeding behaviours.
794 In Lake Denault, benthic fish from the *Catostominæ* subfamily appeared to be negatively affected
795 by environmental Mn contamination, a legacy of past mining operations (IOC). This was evident
796 through the evaluation of relationships between Mn concentrations and biomarkers of oxidative
797 stress. *Salmoninæ* also exhibited indication of potential negative effects associated with
798 contamination from mining activity, as high values of Se measured in fish from contaminated lakes
799 compared to individuals from the reference lake were associated with an increase of 8-OHdG
800 concentrations. In Lake Houston, *Salmoninæ* also demonstrated higher liver concentrations of Al,
801 Cd, Co, Cr, Cu, Pb and lower condition than fish from the reference location. We could
802 hypothesize that at sites impacted by more recent iron exploitation as in Lake Houston (LIM closed
803 in 2014 and current TSMC activities), negative effects from sediment contamination are indirectly
804 contributing to deteriorate fish health. Yet, as we did not collect information on prey abundance,
805 food webs and since we have only one period of sampling, further research should be carried out
806 to examine the magnitude of contamination upon benthic fauna. Lastly, fish collected in Lake

807 Cherny appeared to be in a similar condition and health status as those captured from Lake
808 Perroche, the reference site. Only liver THg concentrations in fish from Lake Cherny were
809 concerning and could be possibly explained by a shift in foraging strategies to larger prey items.
810 It is important to consider that although strong correlations can be identified between liver trace
811 element concentrations and biochemical biomarkers, field studies do not allow for clear attribution
812 of an element's effect on the antioxidant system. The biomarkers under study are not entirely
813 element-specific and in a natural ecosystem where environmental variables cannot be controlled
814 as in laboratory studies, additive or antagonistic effects may occur. Further studies are required
815 to have a better understanding of the impacts associated with mining activities on Northern fish
816 species. Specifically, our study points to the relative importance of feeding behaviour on trace
817 element concentrations in fish and their associated effects on fish health. Therefore, stable
818 isotope analyses would be a valuable tool to estimate relationships between the parameters
819 evaluated here and ecological variables such as trophic level and feeding behaviour.

820

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828

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838 **Data availability statement**

839 All untreated data for this study is available here:
840 [https://borealisdata.ca/privateurl.xhtml?token=13b5b017-30f5-4cf3-8de4-](https://borealisdata.ca/privateurl.xhtml?token=13b5b017-30f5-4cf3-8de4-44ef8ae00c69)
841 [44ef8ae00c69](https://borealisdata.ca/privateurl.xhtml?token=13b5b017-30f5-4cf3-8de4-44ef8ae00c69)

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TABLES

1234

1235 Table 1: Water (temperature, pH and conductivity) and lake (maximal length, maximal width and mining inputs) parameters and fishing usage of
 1236 sampled sites in the summer 2019.

Lake	Water parameters			Lake parameters			Fishing usage
	Temperature (°C)	pH	Conductivity (µS/cm)	Maximal length (m)	Maximal width (m)	Mining inputs	
Perroche	15.3	6.6	5.7	2090	364	None	Recreational fishing outfitter
Cherny	15.7	7.0	52.0	3300	866	Upstream sources (6 km distance) of metal contamination (from 2011 to 2014) including: (i) 200 000 m ³ of iron mine tailing released in the environment after a dam break, (ii) 14 500 L of iron sulfate discharged and (iii) several incidents of mine tailings discharged after heavy rainfall	None known
Houston	15.1	6.8	19.0	2690	388	Atmospheric contamination from current mining exploitation (TSMC) in addition to historical atmospheric contamination (LIM, from 2011 to 2013)	Subsistence fishing
Denault	15.8	7.7	34.3	2035	505	Immediate contamination from mine tailings (IOC, from 1954 to 1982) with residuals being in direct contact with the lake	Subsistence fishing

1237

1238 Abbreviations: TSMC (Tata Steel Minerals Canada), LIM (Labrador Iron Mine Limited) and IOC (Iron Ore Company of Canada)

1239 Table 2: Capture data for fish species collected in summer 2019 at the reference site (Lake
 1240 Perroche), as well as in the Fermont (Lake Cherny) and Schefferville (lakes Houston, Denault
 1241 and Star) areas.

1242

Lake	Perroche	Cherny	Houston	Denault	Star	TOTAL
Lake charr (<i>Salvelinus namaycush</i>)	3	2		7		12
Brook charr (<i>Salvelinus fontinalis</i>)	20		20	1		41
White sucker (<i>Catostomus commersonii</i>)	7			14		21
Longnose sucker (<i>Catostomus catostomus</i>)				20		20
Round whitefish (<i>Prosopium cylindraceum</i>)		2		6		8
Lake whitefish (<i>Coregonus clupeaformis</i>)	20	20		3		43
Northern pike (<i>Esox lucius</i>)	11	1				12
Lake chub (<i>Couesius plumbeus</i>)	11	20		20		51
Slimy sculpin (<i>Cottus cognatus</i>)	1			2	1	4
Burbot (<i>Lota lota</i>)				3		3
TOTAL	73	45	20	76	1	215

1244 Table 3: Fork length, total weight, FCF, HSI, sex ratio and age of *Salmoninæ*, *Catostominae* and *Coregoninae* captured in the summer of 2019.
 1245 Values are expressed as mean \pm standard deviation with ranges (minimum; maximum); n.a., not available. Different letters indicate significant
 1246 differences for the given species between sampled lakes and sample number is provided for HSI and age in brackets when different from total
 1247 sample number.

Lake	Subfamily	Species	N	Fork length (mm)	Whole weight (g)	FCF	HSI	Sex	Age (years)
Perroche	<i>Salmoninæ</i>	Brook charr	20	319 \pm 100 ^a 127; 493	500 \pm 486 ^a 21; 1997	1.14 \pm 0.17 ^a 0.79; 1.67	0.94 \pm 0.36 0.57; 2.25	55.0% Male 40.0% Female 5.0% n.d.	3 \pm 1 ^b 1; 6 (18)
		Lake charr	3	568 \pm 43 518; 598	2133 \pm 497 1562; 2467	1.15 \pm 0.02 1.12; 1.17	1.28 \pm 0.46 0.79; 1.70	66.7% Male 33.3% Female	9 \pm 2 8; 11
	<i>Catostominae</i>	White sucker	7	502 \pm 37 ^a 434; 548	1883 \pm 386 ^a 1198; 2326	1.47 \pm 0.06 ^a 1.41; 1.59	1.99 \pm 2.37 ^a 0.97; 7.35	57.1% Male 42.9% Female	11 \pm 3 ^a 8; 18
	<i>Coregoninae</i>	Lake whitefish	20	280 \pm 118 108; 487	440 \pm 530 14; 1692	1.19 \pm 0.13 1.01; 1.47	0.67 \pm 0.16 ^b 0.43; 0.94	65.0% Male 25.0% Female 10.0% n.d.	4 \pm 2 1; 8
Cherny	<i>Salmoninæ</i>	Lake charr	2	570 \pm 50 534; 605	1958 \pm 663 1489; 2426	1.04 \pm 0.08 0.98; 1.10	1.02 \pm 0.51 0.66; 1.38	50.0% Male 50.0% Female	9 (1)
		Lake whitefish	20	281 \pm 74 152; 454	355 \pm 319 39; 1450	1.26 \pm 0.13 1.05; 1.55	0.89 \pm 0.18 ^a 0.57; 1.12 (19)	45.0% Female 45.0% Male 10.0% n.d.	5 \pm 2 2; 11
	<i>Coregoninae</i>	Round whitefish	2	222 \pm 23 205; 238	109 \pm 42 79; 138	0.97 \pm 0.08 0.92; 1.02	1.17 \pm 0.30 0.96; 1.38	100.0% Male	4 \pm 1 3; 4

Houston	<i>Salmoninae</i>	Brook charr	20	186 ± 30 ^b 153; 270	66 ± 38 ^b 36; 190	0.97 ± 0.10 ^b 0.74; 1.25	0.97 ± 0.42 0.36; 1.89 (18)	70.0% Male 30.0% Female	4 ± 1 ^a 3; 5
Denault	<i>Salmoninae</i>	Brook charr	1	133	23	0.98	n.a.	100.0% Female	2
		Lake charr	7	534 ± 104 398; 645	1809 ± 935 634; 3165	1.09 ± 0.14 0.90; 1.26	1.13 ± 0.27 0.87; 1.60	57.1% Male 42.9% Female	8 ± 2 5; 11
	<i>Catostominae</i>	White sucker	14	230 ± 94 ^b 171; 456	257 ± 426 ^b 53; 1362	1.17 ± 0.14 ^b 1.01; 1.49	0.64 ± 0.47 ^b 0.28; 1.33 (7)	28.6% Female 14.3% Male 57.1% n.d.	6 ± 3 ^b 4; 13
		Longnose sucker	20	220 ± 52 165; 384	145 ± 147 50; 720	1.12 ± 0.10 0.99; 1.28	0.79 ± 0.25 0.42; 1.08 (8)	15.8% Male 5.3% Female 78.9% n.d.	5 ± 2 3; 12
	<i>Coregoninae</i>	Lake whitefish	3	184 ± 44 155; 234	82 ± 66 42; 159	1.15 ± 0.08 1.08; 1.24	0.50 ± 0.14 ^b 0.38; 0.65	66.7% Female 33.3% n.d.	2 ± 0 2; 2
		Round whitefish	6	231 ± 23 213; 273	117 ± 35 84; 179	0.93 ± 0.09 0.83; 1.06	0.59 ± 0.21 0.44; 0.74 (2)	83.3% Female 16.7% Male	4 ± 1 3; 5

1248
1249

Abbreviations: Fulton's condition factor (FCF), hepatosomatic index (HSI)

1250 Table 4: Trace element concentrations of Al, Cd, Co, Cr, Cu, Fe, Hg, Mn, Pb, Se, Zn in the liver of *Salmoninae*, *Catostominae* and *Coregoninae*
 1251 captured in the summer 2019. Values are expressed in µg/g dw as mean ± standard deviation with ranges (minimum; maximum) and sample
 1252 number in brackets. THg concentrations are expressed in wet weight (µg/g). Different letters indicate significant differences of the given subfamily
 1253 among lakes.
 1254

Lake	Perroche		Cherry		Houston		Denault		
Subfamily	<i>Salmoninae</i>	<i>Catostominae</i>	<i>Coregoninae</i>	<i>Salmoninae</i>	<i>Coregoninae</i>	<i>Salmoninae</i>	<i>Catostominae</i>	<i>Coregoninae</i>	
Al	13 ± 14 ^b 1; 72 (23)	13 ± 12 ^b 4; 36 (7)	23 ± 38 ^a 4; 172 (18)	3 ± 2 ^b 2; 5 (2)	7 ± 5 ^b 1; 17 (21)	37 ± 33 ^a 8; 150 (16)	5 ± 5 ^b 1; 16 (7)	183 ± 332 ^a 8; 1853 (31)	27 ± 17 ^a 7; 56 (6)
Cd	0.8 ± 0.5 ^b 0.3; 2.2 (23)	3.6 ± 1.7 ^a 1.2; 5.7 (7)	1.2 ± 0.6 ^a 0.3; 2.6 (18)	0.5 ± 0.1 ^b 0.4; 0.5 (2)	0.5 ± 0.3 ^b 0.1; 1.4 (22)	3.2 ± 2.1 ^a 0.6; 8.7 (16)	1.3 ± 0.6 ^b 0.5; 2.4 (7)	0.7 ± 0.6 ^b 0.1; 3.1 (31)	1.1 ± 0.3 ^a 0.6; 1.6 (6)
Co	0.54 ± 0.29 ^{ab} 0.25; 1.48 (23)	0.72 ± 0.34 ^a 0.40; 1.39 (7)	0.31 ± 0.11 ^a 0.15; 0.63 (18)	0.32 ± 0.11 ^{ab} 0.25; 0.40 (2)	0.21 ± 0.07 ^b 0.10; 0.38 (22)	1.09 ± 0.99 ^a 0.29; 3.43 (16)	0.39 ± 0.21 ^b 0.16; 0.82 (7)	0.30 ± 0.13 ^b 0.12; 0.73 (31)	0.48 ± 0.24 ^a 0.22; 0.90 (6)
Cr	0.56 ± 0.16 ^b 0.35; 0.94 (23)	0.57 ± 0.14 0.39; 0.77 (7)	0.95 ± 0.41 ^a 0.46; 2.17 (18)	0.44 ± 0.10 ^b 0.37; 0.51 (2)	0.40 ± 0.26 ^b 0.19; 1.40 (22)	1.78 ± 0.99 ^a 0.66; 4.09 (16)	0.47 ± 0.20 ^b 0.30; 0.82 (7)	0.68 ± 0.84 0.05; 4.69 (30)	0.50 ± 0.20 ^b 0.21; 0.71 (6)
Cu	48 ± 20 ^b 7; 93 (23)	54 ± 26 ^a 28; 103 (7)	21 ± 11 ^a 9; 57 (18)	107 ± 47 ^{ab} 74; 140 (2)	15 ± 5 ^a 9; 25 (22)	94 ± 47 ^a 21; 205 (16)	83 ± 30 ^{ab} 25; 115 (7)	35 ± 33 ^b 7; 192 (31)	7 ± 1 ^b 5; 8 (5)
Fe	624 ± 368 158; 1661 (23)	969 ± 525 ^a 346; 1606 (7)	277 ± 116 ^{ab} 136; 603 (18)	642 ± 327 411; 873 (2)	217 ± 92 ^b 131; 511 (22)	971 ± 555 52; 1978 (17)	632 ± 301 308; 1044 (7)	397 ± 661 ^b 56; 3702 (31)	340 ± 122 ^a 183; 480 (6)

Hg	0.30 ± 0.25 ^b 0.11; 1.29 (22)	0.26 ± 0.12 ^a 0.12; 0.45 (7)	0.28 ± 0.16 ^a 0.12; 0.66 (17)	1.18 ± 0.32 ^a 0.95; 1.40 (2)	0.22 ± 0.10 ^a 0.11; 0.54 (22)	0.15 ± 0.04 ^b 0.10; 0.21 (7)	0.37 ± 0.27 ^a 0.14; 0.86 (7)	0.04 ± 0.01 ^b 0.01; 0.07 (27)	0.05 ± 0.01 ^b 0.04; 0.07 (5)
Mn	3.9 ± 1.3 1.5; 6.3 (23)	4.9 ± 1.1 ^b 3.8; 7.0 (7)	5.2 ± 1.9 ^a 3.0; 9.2 (18)	3.3 ± 0.5 2.9; 3.7 (2)	3.6 ± 0.8 ^b 2.2; 5.4 (22)	4.3 ± 1.8 2.7; 8.9 (17)	3.3 ± 1.4 1.6; 5.8 (7)	39.1 ± 30.2 ^a 7.1; 109.9 (31)	7.0 ± 2.5 ^a 4.4; 10.1 (6)
Pb	0.22 ± 0.49 ^a 0.00; 2.22 (23)	0.14 ± 0.10 0.04; 0.29 (7)	0.25 ± 0.28 ^a 0.01; 0.98 (16)	0.02 ± 0.01 ^{ab} 0.01; 0.02 (2)	0.05 ± 0.06 ^b 0.00; 0.25 (17)	0.13 ± 0.14 ^a 0.02; 0.61 (16)	0.01 ± 0.01 ^b 0.00; 0.03 (7)	0.29 ± 0.40 0.01; 1.56 (31)	0.64 ± 1.47 ^{ab} 0.00; 3.63 (6)
Se	5.4 ± 2.4 ^c 3.4; 15.0 (23)	7.6 ± 2.5 ^b 4.8; 11.6 (7)	8.8 ± 2.0 ^c 6.1; 13.1 (18)	20.1 ± 2.5 ^b 18.3; 21.9 (2)	11.3 ± 2.7 ^b 6.4; 17.3 (22)	14.0 ± 7.7 ^b 2.8; 27.5 (16)	90.0 ± 46.2 ^a 52.0; 174.2 (7)	11.4 ± 5.9 ^a 4.2; 30.4 (31)	20.1 ± 4.3 ^a 17.2; 28.6 (6)
Zn	124 ± 35 ^b 58; 178 (23)	64 ± 17 40; 89 (7)	158 ± 44 ^a 104; 266 (15)	85 ± 17 ^{bc} 73; 97 (2)	88 ± 24 ^b 50; 132 (22)	206 ± 96 ^a 87; 481 (17)	47 ± 5 ^c 43; 50 (7)	63 ± 14 45; 92 (31)	76 ± 27 ^b 50; 126 (6)

1255

1256

Abbreviations: Aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), lead (Pb), selenium (Se), zinc (Zn)

1257 Table 5: Spearman correlations (rs) among biometric biomarkers (fork length, whole weight, FCF, HSI and age), biochemical biomarkers (8-
 1258 OHdG, MDA, CAT, SOD and GPx) and trace element concentrations (Cd, Cu, Hg, Mn and Se) in the liver of *Salmoninæ* (n = 53) from sampled
 1259 lakes in the summer 2019. Bolded values indicate significant correlations and the significance level of the correlation is denoted as follows: p <
 1260 0.05 *; p < 0.001 **; p < 0.0001***.

	Fork length	Whole weight	FCF	HSI	Age	8-OHdG	MDA	CAT	SOD	GPx	Cd	Cu	Hg	Mn
Whole weight	0.99***	1												
FCF	0.42	0.49*	1											
HSI	0.17	0.19	0.12	1										
Age	0.57*	0.57*	0.03	0.16	1									
8-OHdG	-0.12	-0.24	0.24	-0.13	-0.44	1								
MDA	-0.09	-0.09	0.03	-0.03	-0.23	0.69*	1							
CAT	-0.25	-0.34	-0.22	-0.50	-0.41	0.31	0.38	1						
SOD	0.41	0.17	-0.01	-0.43	0.21	0.56	0.38	0.07	1					
GPx	0.46	0.54	0.07	-0.14	0.33	0.18	0.00	-0.25	0.43	1				
Cd	-0.27	-0.29	-0.51*	-0.03	0.36	0.15	0.41	0.24	0.30	-0.50	1			
Cu	-0.22	-0.25	-0.43	-0.01	0.18	0.42	-0.10	0.08	0.33	0.46	0.40	1		
Hg	0.89***	0.88***	0.21	0.15	0.91***	-0.47	-0.33	-0.49	-0.03	0.46	0.24	0.07	1	
Mn	-0.27	-0.25	-0.09	-0.02	-0.33	-0.36	0.10	0.20	-0.34	-0.43	0.22	-0.09	-0.34	1
Se	0.18	0.15	-0.26	0.03	0.59*	0.74*	0.53	0.11	0.69	0.46	0.61**	0.56*	0.60*	-0.02

1261

1262 Abbreviations: Fulton's condition factor (FCF), hepatosomatic index (HSI), 8-hydroxy-2'deoxyguanosine (8-OHdG), malondialdehyde (MDA), catalase (CAT), superoxide
 1263 dismutase (SOD), glutathione peroxidase (GPx), cadmium (Cd), copper (Cu), mercury (Hg), manganese (Mn), selenium (Se)

1264 Table 6: Spearman correlations (rs) among biometric biomarkers (fork length, whole weight, FCF, HSI and age), biochemical biomarkers (8-
 1265 OHdG, MDA, CAT, SOD and GPx) and trace element concentrations (Cd, Cu, Hg, Mn and Se) in the liver of *Catostomineæ* (n = 41) from sampled
 1266 lakes in the summer 2019. Bolded values indicate significant correlations and the significance level of the correlation is denoted as follows: p <
 1267 0.05 *; p < 0.001 **; p < 0.0001***.

	Fork length	Whole weight	FCF	HSI	Age	8-OHdG	MDA	CAT	SOD	GPx	Cd	Cu	Hg	Mn
Whole weight	0.98***	1												
FCF	0.64*	0.74***	1											
HSI	0.60	0.59	0.58	1										
Age	0.82***	0.78***	0.58*	0.44	1									
8-OHdG	-0.43	-0.45	-0.49	-0.23	-0.48	1								
MDA	0.24	0.25	0.42	0.28	0.16	0.07	1							
CAT	-0.36	-0.33	-0.11	-0.64	-0.36	0.25	0.12	1						
SOD	0.28	0.26	0.27	0.20	0.24	-0.06	0.49	-0.07	1					
GPx	0.40	0.39	0.46	0.51	0.29	-0.22	0.55*	0.11	0.33	1				
Cd	0.80***	0.78***	0.66*	0.68	0.83***	-0.53	0.27	-0.35	0.31	0.47	1			
Cu	0.19	0.14	-0.02	0.12	0.19	-0.10	-0.20	-0.36	0.05	-0.12	0.28	1		
Hg	0.95***	0.94***	0.74**	0.58	0.77***	-0.56	0.22	-0.34	0.22	0.56	0.76***	0.16	1	
Mn	-0.71**	-0.70**	-0.50	-0.65	-0.56	0.53	-0.17	0.49	-0.10	-0.39	-0.60*	-0.34	-0.66*	1
Se	0.13	0.12	0.09	0.01	0.30	0.14	-0.09	-0.03	-0.08	-0.15	0.27	0.06	0.00	0.24

1268

1269 Abbreviations: Fulton's condition factor (FCF), hepatosomatic index (HSI), 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), catalase (CAT), superoxide
 1270 dismutase (SOD), glutathione peroxidase (GPx), cadmium (Cd), copper (Cu), mercury (Hg), manganese (Mn), selenium (Se)

1271 Table 7: Spearman correlations (rs) among biometric biomarkers (fork length, whole weight, FCF, HSI and age), biochemical biomarkers (8-
 1272 OHdG, MDA, CAT, SOD and GPx) and trace element concentrations (Cd, Cu, Hg, Mn and Se) in the liver of *Coregoninæ* (n = 51) from sampled
 1273 lakes in the summer 2019. Bolded values indicate significant correlations and the significance level of the correlation is denoted as follows: p <
 1274 0.05 *; p < 0.001 **; p < 0.0001***.

	Fork length	Whole weight	FCF	HSI	Age	8-OHdG	MDA	CAT	SOD	GPx	Cd	Cu	Hg	Mn
Whole weight	0.99***	1												
FCF	0.69***	0.75***	1											
HSI	-0.05	-0.03	0.14	1										
Age	0.94***	0.92***	0.61**	0.01	1									
8-OHdG	0.00	0.00	-0.15	-0.42	0.00	1								
MDA	-0.13	-0.12	-0.16	-0.16	-0.24	0.45	1							
CAT	-0.38	-0.38	-0.40	-0.34	-0.44	0.42	0.62*	1						
SOD	-0.30	-0.28	-0.26	-0.44	-0.33	0.38	0.23	0.36	1					
GPx	0.28	0.28	0.13	-0.26	0.22	0.14	0.08	0.21	0.26	1				
Cd	0.40	0.39	0.05	-0.54*	0.44	0.46	-0.05	0.01	0.31	0.27	1			
Cu	0.56*	0.54*	0.41	-0.32	0.52*	0.27	-0.09	-0.29	-0.01	0.07	0.40	1		
Hg	0.90***	0.90***	0.75***	-0.16	0.82***	0.28	-0.18	-0.36	-0.19	0.23	0.38	0.61*	1	
Mn	-0.55*	-0.57*	-0.64**	-0.20	-0.48	0.20	-0.15	0.21	0.44	-0.02	0.24	-0.38	-0.52	1
Se	0.24	0.21	0.06	0.14	0.40	0.10	-0.09	-0.28	-0.08	0.15	0.19	-0.05	0.01	-0.08

1275

1276 Abbreviations: Fulton's condition factor (FCF), hepatosomatic index (HSI), 8-hydroxy-2-deoxyguanosine (8-OHdG), malondialdehyde (MDA), catalase (CAT), superoxide
 1277 dismutase (SOD), glutathione peroxidase (GPx), cadmium (Cd), copper (Cu), mercury (Hg), manganese (Mn), selenium (Se)

1 **ASSESSING THE IMPACTS OF MINING ACTIVITIES ON FISH**
2 **HEALTH IN NORTHERN QUÉBEC**

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5

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10

FIGURE CAPTIONS

11 Figure 1: Map of the sampled lakes in the region of Schefferville (left) and Fermont (right)
12 during summer 2019. The orange triangle on the bottom map indicates the relative distance of
13 Lake Perroche (the reference site) from Fermont and Schefferville.

14

15 Figure 2: Trace element concentrations of aluminum (Al), cadmium (Cd), cobalt (Co),
16 chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), lead (Pb), selenium (Se) and zinc
17 (Zn) for water (panel A) and sediment (panel B) from lakes sampled in the summer 2019. Water
18 (n = 2) and sediment (n = 2) trace element concentrations are expressed in $\mu\text{g/L}$ and $\mu\text{g/g dw}$
19 respectively. Values below the detection limits were excluded from the figure. A threshold value
20 corresponding to the Provincial Water Quality Objectives from Québec (Ministère de
21 l'Environnement et de la Lutte contre les changements climatiques, 2021) is also displayed on
22 panel A.

23

24 Figure 3: Principal component analysis (PCA) of biometric biomarkers (fork length, whole
25 weight, age, Fulton's condition factor (FCF) and hepatosomatic index (HSI)), biochemical
26 biomarkers (8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA), catalase
27 (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)) and trace element
28 concentrations (aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron
29 (Fe), manganese (Mn), mercury (Hg), lead (Pb), selenium (Se) and zinc (Zn)) of *Salmoninae*
30 (n = 53) among the sampled lakes. Each point indicates an individual fish and ellipses
31 represent individuals from the same site.

32

33 Figure 4: Principal component analysis (PCA) of biometric biomarkers (fork length, whole
34 weight, age, Fulton's condition factor (FCF) and hepatosomatic index (HSI)), biochemical

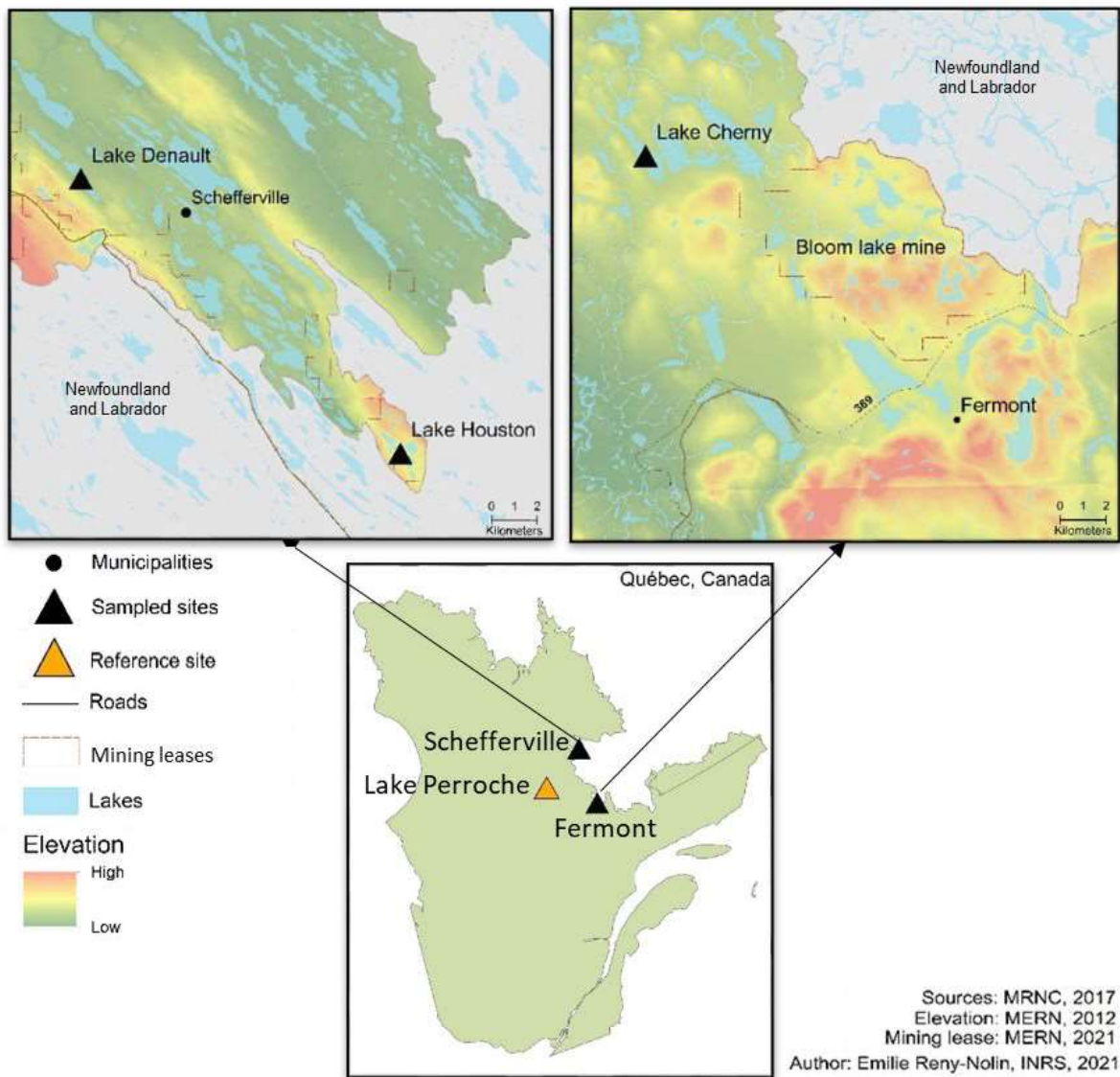
35 biomarkers (8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA), catalase
36 (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)) and trace element
37 concentrations (aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron
38 (Fe), manganese (Mn), mercury (Hg), lead (Pb), selenium (Se) and zinc (Zn)) of *Catostominae*
39 (n = 41) among the sampled sites. Each point indicates an individual fish and ellipses represent
40 individuals from the same site.

41

42 Figure 5: Principal component analysis (PCA) of biometric biomarkers (fork length, whole
43 weight, age, Fulton's condition factor (FCF) and hepatosomatic index (HSI)), biochemical
44 biomarkers (8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA), catalase
45 (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)) and trace element
46 concentrations (aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron
47 (Fe), manganese (Mn), mercury (Hg), lead (Pb), selenium (Se) and zinc (Zn)) of *Coregoninae*
48 (n = 51) among the sampled sites. Each point indicates an individual fish and ellipses represent
49 individuals from the same site.

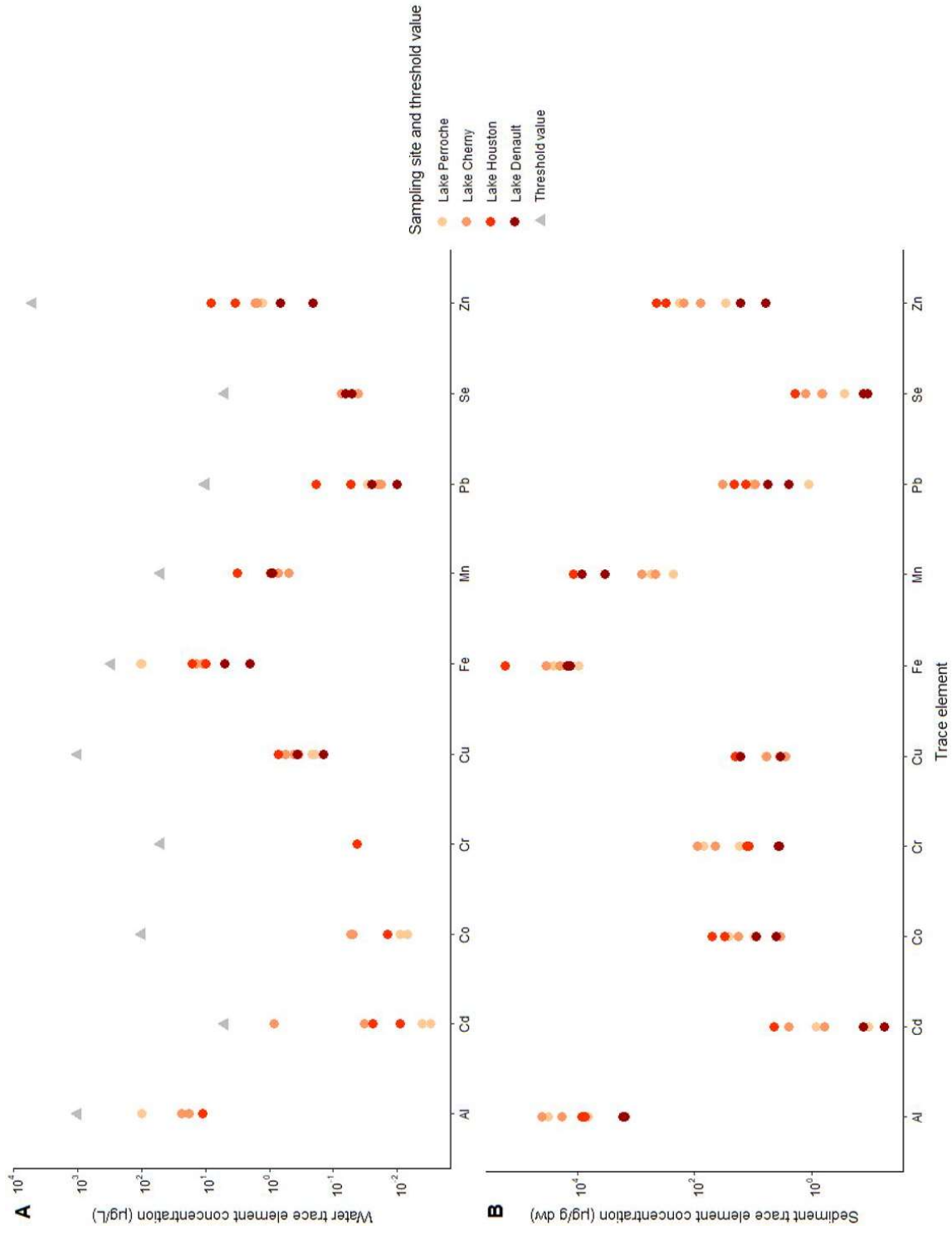
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FIGURES



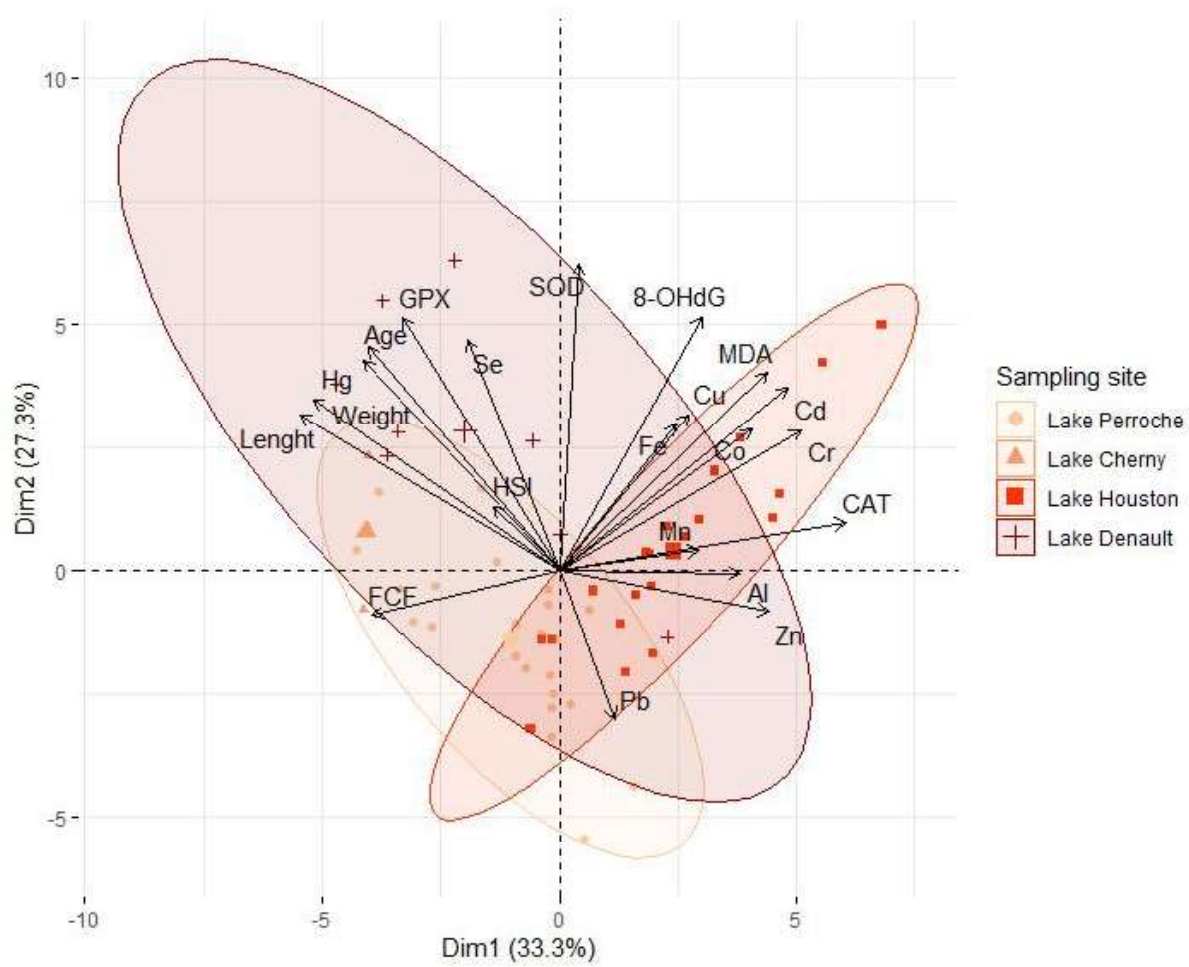
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52 Figure 1



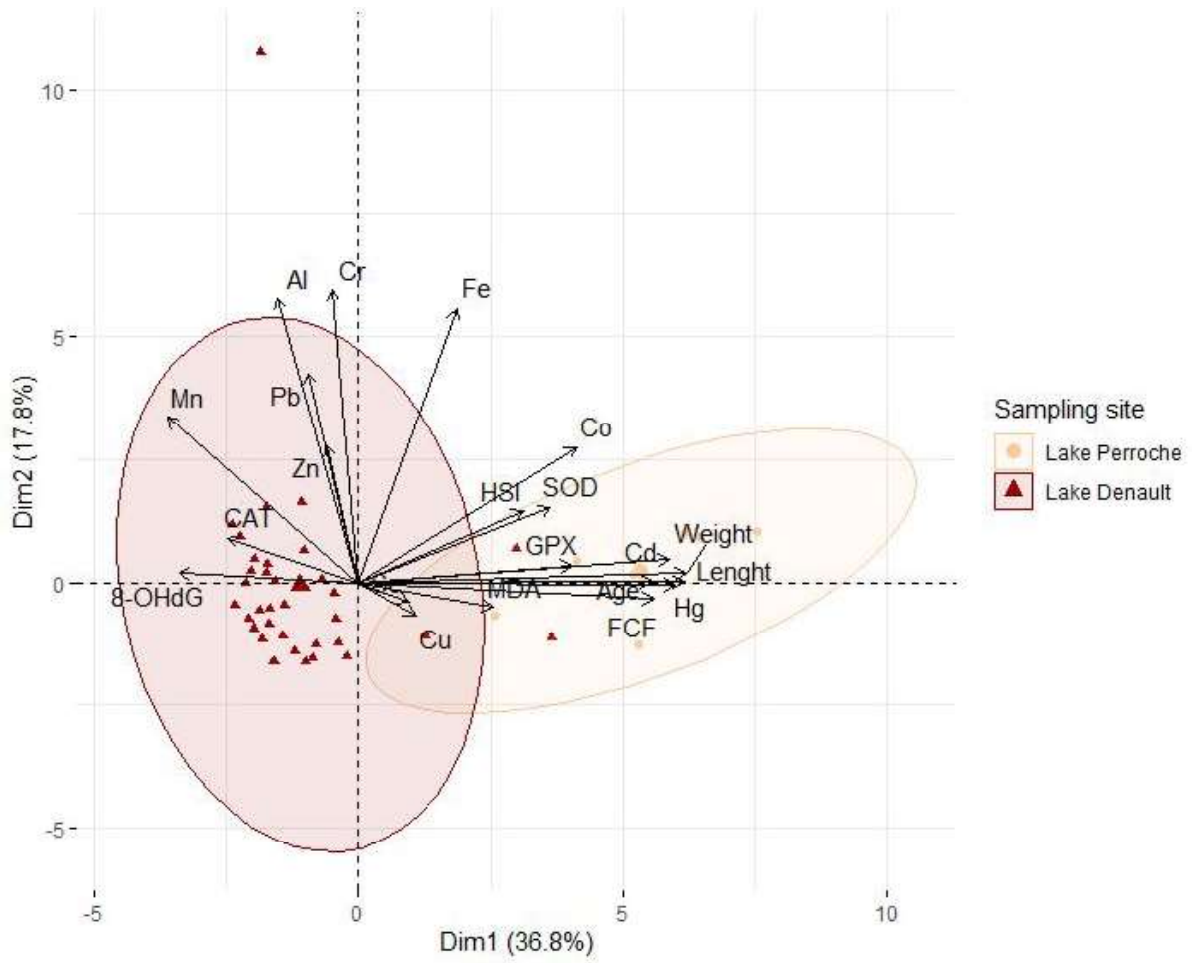
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54 Figure 2



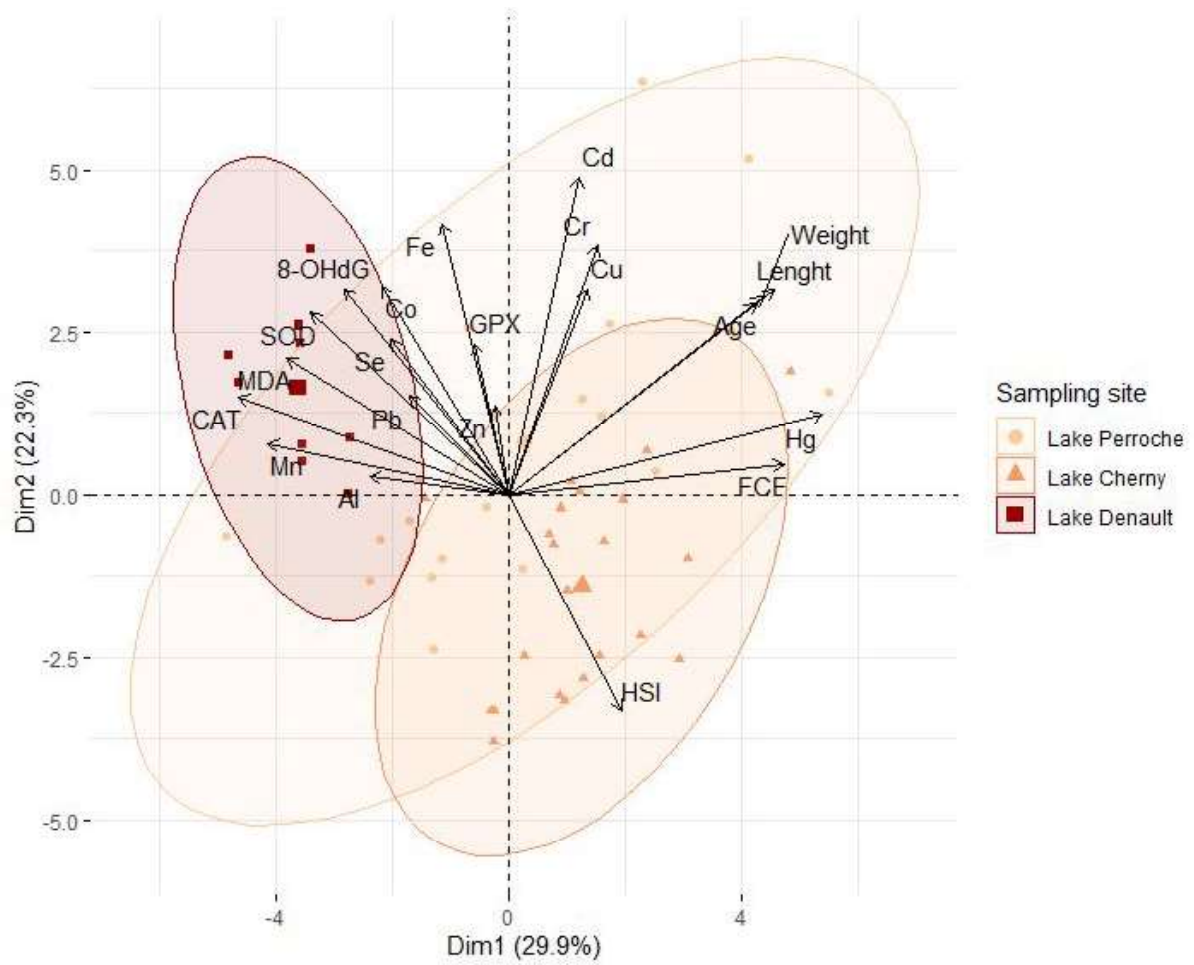
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56 Figure 3



57

58 Figure 4



59

60 Figure 5