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**The impact of Ca-rich diamond mining effluent on  
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1 The impact of Ca-rich diamond mining effluent on downstream cladoceran communities in  
2 softwater lakes of the Northwest Territories, Canada

3

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## 23 Abstract

24

25 Effluent from diamond mining operations rich in calcium (Ca) has transformed softwater  
26 tundra lakes in the Northwest Territories, Canada. Lakes downstream of the Dominion Diamond  
27 Corporation Ekati Mine have experienced marked changes in water chemistry and cladoceran  
28 community composition since establishment of the mine in 1998. The greatest changes have  
29 occurred at the sites closest to the effluent discharge, with [Ca] increasing from  $< 1 \text{ mg L}^{-1}$  to  $>$   
30  $30 \text{ mg L}^{-1}$ , and corresponding increases in pH from  $< 7$  to  $> 8$ . A split was identified in the  
31 cladoceran communities with *Holopedium glacialis* (a jelly-clad cladoceran tolerant of low [Ca])  
32 generally dominating the cladoceran community at  $[\text{Ca}] < 2.5 \text{ mg L}^{-1}$ , while in impacted lakes  
33 with  $[\text{Ca}] \geq 2.5 \text{ mg L}^{-1}$ , *Daphnia longiremis* and *D. middendorffiana* (taxa with higher [Ca]  
34 requirements) were often dominant. In contrast, the three study lakes that did not receive mining  
35 effluent maintained stable and low [Ca] (mean  $[\text{Ca}] = 0.66 \pm 0.06 \text{ mg L}^{-1}$  (stdev)) throughout the  
36 monitoring period, and have not experienced directional shifts in their cladoceran communities.

37

38

## 39 Introduction

40

41 The Barren Lands of the eastern Northwest Territories and Nunavut territory of Canada is  
42 mainly a rocky, undulating landscape that hosts numerous irregular-shaped lakes. The few  
43 available limnological surveys suggest that the lakes of the Barren Lands are generally  
44 oligotrophic and dilute (Pienitz et al. 1997; Rühland and Smol 1998) softwater systems, with  
45 calcium concentrations ( $[Ca] < 1.5 \text{ mg L}^{-1}$  and pH levels ranging from 5.5 to 8.6 (Pienitz et al.  
46 1997; Rühland and Smol 1998). This remote landscape is also the location of Canada's richest  
47 producing diamond mines.

48

49 Canada's first diamond mine, the Ekati Mine, is located in the eastern Barren Lands  
50 (Figure 1). Operations began at Ekati in 1998, under BHP Billiton Canada Inc., but since 2012 it  
51 has been owned and operated by Dominion Diamond Corporation (DDC Ekati). Diamond mines  
52 in the Northwest Territories conduct Aquatic Effects Monitoring Programs (AEMP) to identify  
53 effects of the mining process and waste deposition on downstream water bodies. The effluent  
54 from DDC Ekati mining activities is characterized by elevated concentrations of the major ions  
55  $\text{SO}_4^{2-}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{HCO}_3^-$  (Rollo and Jamieson 2006), and nitrogen (N) derived  
56 from N-based explosives used in mining activities (Bailey et al. 2013). The DDC Ekati  
57 monitoring program collects data on water quality, zooplankton communities and a variety of  
58 other parameters (e.g. metal concentrations, phytoplankton, lake benthos) from lakes  
59 downstream of the facility, as well as from several lakes both inside and outside the Koala  
60 watershed that are unaffected by diamond processing effluent. The Ca-rich nature of the effluent

61 is of particular interest, given its transformative potential for lakes in the surrounding low  
62 alkalinity landscape.

63  
64 Ca is an essential nutrient, and widespread Ca declines in other softwater regions of  
65 North America have been associated with ecologically significant changes in crustacean  
66 zooplankton communities, due to inter-specific differences in sensitivity to aqueous Ca among  
67 crustacean zooplankton (Jeziorski and Smol 2016; Jeziorski et al. 2008). For example, large-  
68 bodied species of *Daphnia* (with relatively high Ca content (Jeziorski and Yan 2006)) have  
69 decreased in regions experiencing marked Ca decline (falling below  $[Ca] < 1.5 \text{ mg L}^{-1}$ ) (Jeziorski  
70 et al. 2008) and at many of these sites, *Daphnia* are being replaced by jelly-clad *Holopedium*  
71 *glacialis* Rowe, Adamowicz & Hebert (Jeziorski et al. 2015), a similar-sized cladoceran that has  
72 a mucopolysaccharide jelly sheath instead of a heavily calcified carapace as its primary  
73 predatory defense, and thus lower Ca content (Jeziorski and Yan 2006). However, a recent study  
74 that explored the DDC Ekati monitoring dataset for potential effects of the mining effluent on the  
75 downstream lakes did not detect directional shifts in functional groups of crustacean zooplankton  
76 (the functional groups were defined based on trophic position, feeding type, and relative  
77 swimming speed) in response to the mining effluent (St-Gelais et al. 2017). Given the sensitivity  
78 of several cladoceran taxa to Ca availability, the lack of a detectable response to mining effluent  
79 among the downstream crustacean zooplankton functional groups was surprising. As St-Gelais et  
80 al. (2017) assessed biomass changes across all the crustacean zooplankton, and as copepods were  
81 by far the dominant taxonomic group in most of the monitored lakes (BHP 1994; ERM 2014;  
82 2015; Rescan 1996, 1997a, b, 1998, 1999, 2000, 2001, 2002, 2003, 2005, 2006, 2007, 2008,  
83 2009, 2010, 2011, 2012, 2013), an analysis of biomass fluctuations due to between-site

84 differences and inter-annual variation focused on changes within the crustacean zooplankton  
85 communities as a whole, and as such may have not identified some fundamental changes  
86 occurring among the cladoceran taxa. To explore the DDC Ekati AEMP dataset in greater detail,  
87 we used water chemistry and community species count data to calculate relative abundances  
88 (removing the effect of biomass fluctuations) of cladoceran taxa to examine the potential impacts  
89 of diamond mine effluent and to address the following questions: 1) Have the relative  
90 abundances of specific taxa within the cladoceran communities downstream of the mine changed  
91 in response to the Ca-rich effluent?; if so, 2) What are the spatial and/or temporal trends in the  
92 response?; and 3) How do the cladoceran community changes in these softwater tundra lakes  
93 compare with the impacts of [Ca] changes in other softwater lake regions?

94

#### 95 Site Description

96

97 The Barren Lands (or Barren Grounds) is an Arctic-tundra region extending north of the  
98 treeline from the eastern Northwest Territories into Nunavut, towards the Hudson Bay coastal  
99 plains (Figure 1). The low-lying topography of the Barren Lands is characterized by thin soils  
100 with irregular bedrock outcrops. The tundra vegetation includes shrub birch (*Betula glandulosa*),  
101 alder (*Alnus* spp.), willow (*Salix* spp.), grasses, forbs, mosses, lichens and peat deposits (Huang  
102 et al. 2004).

103

104 The DDC Ekati diamond mine facilities (Figure 1) are located in the Barren Lands within  
105 the Koala watershed and include the Koala Pit, Northern Koala Pit, Panda Pit, Fox Pit, and  
106 Beartooth Pit, where kimberlite pipes are processed in both underground and open pit mines.

107 Effluent from the kimberlite processing is released into the Long Lake containment facility,  
108 where, following treatment, it flows through a chain of lakes including Leslie Lake, Moose Lake,  
109 Nema Lake, and Slipper Lake (listed in increasing distance from the Long Lake containment  
110 facility), which are regularly sampled as part of the DDC Ekati AEMP. The drainage ultimately  
111 discharges into the northwest arm of Lac de Gras (LDG), where the AEMP sampling sites S2  
112 and S3 are located (Figure 1). The DDC Ekati AEMP also monitors three lakes that do not  
113 receive diamond mining effluent as reference sites: Vulture Lake (located upstream of the mine  
114 within the Koala watershed), and the more distant Nanuq Lake and Counts Lake (Figure 1). In  
115 addition to the DDC Ekati Mine operations described here, the nearby Diavik diamond mining  
116 operation is also located on Lac de Gras (Figure 1), although discharge from Diavik occurs more  
117 than 40 km east of the S2 and S3 sampling sites.

118  
119 The bedrock geology of the study region is primarily composed of volcanic and meta-  
120 sedimentary geological units. The northwest arm of LDG and Vulture Lake are located on a  
121 cordorite and andalusite meta-greywacke (Kjarsgaard et al. 1994a) and Leslie Lake, Moose  
122 Lake, Nema Lake, and Slipper Lake catchments are predominately comprised of quartz diorite, a  
123 resistant silicate bedrock (Kjarsgaard et al. 1994a). Meanwhile the more remote reference sites,  
124 Counts Lake and Nanuq Lake, are situated on a muscovite-biotite granite (Kjarsgaard et al.  
125 1994b, Kjarsgaard and Wyllie 1993). Based on the bedrock geology, it would be expected that  
126 the reference Vulture Lake and the Northwest Arm of LDG may receive a greater input of  $\text{Ca}^{2+}$ ,  
127  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{HCO}_3^-$  from the more-easily weathered calcareous bedrock, relative to the  
128 sites with catchments primarily comprised of silicate bedrock. The surficial till is generally less  
129 than 2 m thick with regular bedrock outcrops. The till is derived from granitoid rocks and the

130 Yellowknife Supergroup and, therefore, is not a likely source of Ca ions. The catchments of  
131 Slipper Lake and Vulture Lake contain areas of 5-30 m thick tills of the same composition (Ward  
132 et al. 1997). Reference sites Vulture, Nanuq, and Counts Lakes were oligotrophic and dilute in  
133 the most recent published AEMP data from the summer 2014 sampling season (Table 1). In  
134 contrast, the sites receiving discharge water generally had higher conductivity and nitrate  
135 concentrations than the reference sites (Table 1). Data from the DDC Ekati AEMPs were only  
136 accessible from the Wek'eezhii Land and Water Board public registry for the years 1994, 1996,  
137 1997 (water chemistry only), and 1998-2014.

138

## 139 Methods

140

141 Methods for water quality and zooplankton sampling and analyses conducted for DDC  
142 Ekati are detailed in the annual AEMP reports (BHP 1994; ERM 2014, 2015; Rescan 1996,  
143 1997a, b, 1998, 1999, 2000, 2001, 2002, 2003, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012,  
144 2013), and water chemistry and cladoceran taxonomic abundance data were transcribed from  
145 these reports (the exception being 1997, as zooplankton species counts were not available). In  
146 brief, water quality samples were obtained on two occasions during the open water period in  
147 1994 (July and August), once in 1996 (late July), once in 1997 (early Aug), on five occasions  
148 during the open water period in 1998 (once in late June, twice in July, and once in both August  
149 and September), on three occasions during the open water period from 1999 to 2009 (July,  
150 August, and September), and on one occasion from 2010 to 2014 (either late July or early  
151 August). Any modifications to sampling protocols are detailed in the annual AEMP reports (BHP  
152 1994; ERM 2014 2015; Rescan 1996, 1997a, b, 1998, 1999, 2000, 2001, 2002, 2003, 2005,

153 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013). On each sampling occasion, water was  
154 obtained from at least two depths, at the surface (0 m for 1994, 1996, 1997; or 1 m 1998-2014)  
155 and at a mid-depth. As the middle depths varied with lake morphometry, only water chemistry  
156 samples taken at 0-1 m depth were used in this analysis.

157  
158 Zooplankton samples were collected at least once a season, in mid-summer (July-  
159 August), using a conical 0.3 m diameter, 118  $\mu\text{m}$  mesh zooplankton net, pulled through the water  
160 column from approximately ~1-2 m above the lake bottom to the surface. Zooplankton samples  
161 were subsampled, enumerated, and identified to the lowest possible taxonomic level (for detailed  
162 methods see BHP 1994; ERM 2014, 2015; Rescan 1996, 1998, 1999, 2000, 2001, 2002, 2003,  
163 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013). Leslie Lake, Moose Lake, Slipper Lake,  
164 and Vulture Lake were sampled for zooplankton in 1994. Moose Lake, Nema Lake, Slipper  
165 Lake, and Vulture Lake were sampled regularly from 1996-2014, while zooplankton count data  
166 are available for the reference Counts Lake and Nanuq Lake from 1998-2014. Site S2 and Site  
167 S3 in Lac de Gras were not included in the monitoring program until 2000, and so no data are  
168 available for these sites in the early years of the mine's development. Leslie Lake was added to  
169 regular zooplankton sampling from 2003-2014.

170  
171 Cladoceran relative abundances were calculated from the DDC Ekati zooplankton  
172 taxonomic data for 1994, 1996, and from 1998-2014. The primary partitions in the relative  
173 abundances of the cladoceran taxa from all lakes, including the reference sites and for all years  
174 for which data were available, were assessed as a function of mid-summer (late July-August)  
175 [Ca] and pH (for all lakes and for all available sampling years) using multivariate regression

176 trees (MRT) and discriminant species analyses (De'Ath 2002) via the “mvpart” package  
177 (Therneau et al. 2014) and “MVPARTwrap” (Ouellette and Legendre 2013) for the R software  
178 environment (R Core Team 2013). The regression tree selected from the MRT analysis was the  
179 most parsimonious tree within one standard error of the tree with the lowest cross-validated  
180 relative error (Borcard et al. 2011). Unpaired, one-sided, 2 sample Wilcoxon tests were used to  
181 identify whether the mean relative abundances of *Holopedium* or *Daphnia* significantly differed  
182 between the groups identified in the MRT analyses.

183

## 184 Results

185

186 The monitoring data reveal a pronounced Ca gradient, with lakewater [Ca] from 2003-  
187 2014 decreasing as distance from the discharge location increases: from Leslie Lake (mean [Ca]  
188  $\sim 21.2 \text{ mg L}^{-1}$ ), through Moose Lake (mean [Ca]  $\sim 19.5 \text{ mg L}^{-1}$ ), Nema Lake (mean [Ca]  $\sim 9.9$   
189  $\text{mg L}^{-1}$ ), Slipper Lake (mean [Ca]  $\sim 4.4 \text{ mg L}^{-1}$ ), to the northwest arm of LDG (sites S2, mean  
190 [Ca]  $\sim 2.2 \text{ mg L}^{-1}$ ; and S3, mean [Ca]  $\sim 1.4 \text{ mg L}^{-1}$ ) (Figure 2). In contrast, the three reference  
191 lakes had stable and low [Ca] (mean [Ca] =  $0.66 \pm 0.06 \text{ mg L}^{-1}$  (stdev)) throughout the  
192 monitoring period. From 2003-2014, the mean [Ca] in the reference lakes were: Counts Lake,  
193 mean [Ca] =  $0.67 \pm 0.03 \text{ mg L}^{-1}$  (stdev); Nanuq Lake, mean [Ca] =  $0.69 \pm 0.05 \text{ mg L}^{-1}$  (stdev);  
194 and Vulture Lake, mean [Ca] =  $0.68 \text{ mg L}^{-1} \pm 0.02 \text{ mg L}^{-1}$  (stdev)) (Figure 3a). In addition to the  
195 spatial gradient, [Ca] have markedly increased over time in the lakes downstream of the effluent  
196 release (Figure 3), regularly reaching [Ca]  $> 25 \text{ mg L}^{-1}$  at the two most impacted sites (Leslie  
197 Lake and Moose Lake). In contrast, prior to mine development (1994, 1996-1998), the monitored  
198 lakes receiving discharge from the Long-Lake containment facility were similar in [Ca] to the

199 reference lakes (Figure 3a), with values typically below  $1 \text{ mg L}^{-1}$ . The pH at all sites was  $< 7.1$   
200 prior to 1999, and has generally increased at the impacted sites, with Leslie Lake reaching a pH  
201 of 8.05 in 2014, although there is considerable variability in the records (Figure 3b). At the  
202 reference sites pH has also increased, notably from 2012 to 2014, with pH increases from 6.4 to  
203 7.6 in Counts Lake, 6.6 to 6.7 in Nanuq Lake, and 6.5 to 7.1 pH units in Vulture Lake (Figure  
204 3b).

205  
206 Prior to 1999, when  $[\text{Ca}]$  at the monitored downstream sites (Leslie Lake, Moose Lake,  
207 Nema Lake, and Slipper Lake) were still low and the pH was below 7, the cladoceran  
208 communities contained or were dominated by either *Holopedium glacialis* (previously *H.*  
209 *gibberum* Zadach) or by small-bodied *Bosmina*, with *Daphnia* spp. in generally low abundances  
210 (Figure 4). However, from 1999 onwards, after the mine development, *Holopedium glacialis*  
211 largely disappears from the downstream lakes (with *Holopedium* falling to  $< 5\%$  relative  
212 abundance by 2003 (or before, as the lake was not sampled between the onset on mining in 1998  
213 and 2002) in Leslie Lake, 2002 in Moose Lake, 2003 in Nema Lake, and 2005 in Slipper Lake).  
214 The cladoceran communities of the lakes closest to the discharge location (Leslie Lake, Moose  
215 Lake, Nema Lake, and Slipper Lake) come to be largely dominated by *Daphnia* species (large-  
216 bodied *D. middendorffiana* Fisher, and intermediate-sized *D. longiremis* Sars (Peterson et al.  
217 1978)), while the more remote sites in LDG (Site S2, and Site S3) and reference lakes generally  
218 remain dominated by *H. glacialis* (Figure 4).

219  
220 The decline in *Holopedium* is also evident in the absolute counts of Cladocera in the  
221 zooplankton net tows, as *Holopedium* populations are largely undetected in Leslie Lake, Moose

222 Lake, Nema Lake, and Slipper Lake after 2004-2005 (Figure 5). The response of *Daphnia*  
223 absolute counts is more complex, with some variation in magnitude and timing of the daphniid  
224 increases relative to the *Holopedium* declines across lakes (Figure 5). The *Daphnia* increases in  
225 Leslie Lake, Moose Lake and Nema Lake are largely concurrent with the *Holopedium* decline,  
226 with the exception of a large bloom of *Daphnia* in 1998-1999 in Moose Lake that pre-dated the  
227 collapse of the *Holopedium*. Slipper Lake does not appear to show any marked response in the  
228 absolute counts of *Daphnia* (Figure 5).

229

230 Multivariate regression tree analyses were applied to partition the cladoceran relative  
231 abundance data as a function of [Ca] (Figure 6a) and pH (Figure 6b). For Ca, the primary split  
232 occurred at [Ca] = 2.5 mg L<sup>-1</sup>, resulting in a model with a complexity parameter (R<sup>2</sup>) = 0.415, a  
233 cross-validated relative error = 0.62, and standard error = 0.057. *Holopedium* was identified by  
234 discriminant species analysis to be a significant (p = 0.001) indicator species (indicator value =  
235 0.9321) when [Ca] < 2.5 mg L<sup>-1</sup> and *Daphnia middendorffiana* was a significant (p = 0.001)  
236 indicator species (indicator value = 0.7069) when [Ca] ≥ 2.5 mg L<sup>-1</sup>. The mean relative  
237 abundances were significantly higher (p < 0.001) for *Holopedium* when [Ca] < 2.5 mg L<sup>-1</sup> than  
238 when [Ca] ≥ 2.5 mg L<sup>-1</sup> (Figure 7a, mean *Holopedium* relative abundance where [Ca] < 2.5 mg  
239 L<sup>-1</sup> is 70 ± 30 % (stdev), n = 101; mean *Holopedium* relative abundance where [Ca] ≥ 2.5 mg L<sup>-1</sup>  
240 is 3 ± 12 % (stdev), n = 51). The mean relative abundances were significantly lower (p < 0.001)  
241 for *Daphnia* when [Ca] < 2.5 mg L<sup>-1</sup> compared to when [Ca] ≥ 2.5 mg L<sup>-1</sup> (Figure 7c, mean  
242 *Daphnia* relative abundance where [Ca] < 2.5 mg L<sup>-1</sup> is 21 ± 26 % (stdev), n = 101; mean  
243 *Daphnia* relative abundance where [Ca] ≥ 2.5 mg L<sup>-1</sup> is 78 ± 34 % (stdev), n = 51).

244

245           The cladoceran relative abundance data was also partitioned into 2 groups as a function  
246 of pH by a MRT analysis (Figure 6b), with the primary split occurring at pH = 7.1, with a  
247 complexity parameter ( $R^2$ ) = 0.344, a cross-validated relative error = 0.73, and standard error =  
248 0.062. *Holopedium* was identified by discriminant species analysis to be a significant ( $p = 0.001$ )  
249 indicator species (indicator value = 0.8908) for the sites where the pH < 7.1 and *Daphnia*  
250 *middendorffiana* was a significant ( $p = 0.001$ ) indicator species (indicator value = 0.7007) in the  
251 sites where pH  $\geq$  7.1. The mean relative abundances were significantly higher ( $p < 0.001$ ) for  
252 *Holopedium* when pH < 7.1 than when pH  $\geq$  7.1 (Figure 7b, mean *Holopedium* relative  
253 abundance where pH < 7.1 is  $66 \pm 33$  % (stdev),  $n = 107$ ; mean *Holopedium* relative abundance  
254 where pH  $\geq$  7.1 is  $4 \pm 16$  % (stdev),  $n = 45$ ). The mean relative abundances were significantly  
255 lower ( $p < 0.001$ ) for *Daphnia* when pH < 7.1 compared to when pH  $\geq$  7.1 (Figure 7d, mean  
256 *Daphnia* relative abundance where pH < 7.1 is  $24 \pm 29$  % (stdev),  $n = 107$ ; mean *Daphnia*  
257 relative abundance where pH  $\geq$  7.1 is  $78 \pm 34$  % (stdev),  $n = 45$ ).

258  
259           Throughout the monitoring dataset, pH and Ca concentrations were significantly ( $p <$   
260 0.001) positively correlated ( $r = 0.89$ ), with *Holopedium* primarily found at a pH < 7.1 and at  
261  $[Ca] < 2.5$  mg L<sup>-1</sup> (Figure 8). Due to this strong correlation, only one datum point (Counts Lake  
262 in 2014), had both high pH and low Ca, where pH was 7.6 and  $[Ca] = 0.70$  mg L<sup>-1</sup>. *Holopedium*  
263 were present at a relative abundance of 61%, with *Daphnia* at a relative abundance of 28%.

264

265 Discussion

266

267 Effluent from the DDC Ekati mines has had a striking influence on the water quality of  
268 downstream lakes, evident in Ca and pH gradients positively related to proximity to the Long  
269 Lake containment facility (Figure 2), and the increase in Ca and pH since mine development at  
270 all the study sites receiving effluent (Figure 3). In contrast to the muted response to the diamond  
271 mining activity across the entire crustacean zooplankton community (St-Gelais et al. 2017), we  
272 identified a marked shift in cladoceran relative abundances within the crustacean zooplankton  
273 species assemblage. Prior to mining activities (1994-1998), downstream cladoceran communities  
274 closest to the containment facility were generally dominated by *Holopedium glacialis*, but have  
275 since experienced increases in the relative abundance of *Daphnia* spp. (Figure 4) concurrent with  
276 increasing pH and [Ca] (Figure 3) from the mining effluent.

277  
278 A strong spatial trend is evident in the composition of the cladoceran assemblages, with  
279 *Holopedium* virtually absent (Figure 4 and Figure 5) from those lakes closest to the effluent  
280 discharge, while the reference lakes and the monitoring sites within the northwest arm of Lac de  
281 Gras (sites S2 and S3) that have maintained relatively low and stable [Ca] (Figure 2, Figure 3),  
282 continue to be dominated by *Holopedium* (Figure 4). The monitoring data from prior to the  
283 development of the DDC Ekati diamond mine (in 1998) suggest that Leslie Lake, Moose Lake,  
284 Nema Lake, and Slipper Lake all had similar water chemistry to that of the reference sites (i.e.  
285  $[Ca] < 1 \text{ mg L}^{-1}$  and  $\text{pH} < 7$ ; Figure 3). The monitoring records also indicate that the cladoceran  
286 communities in these lakes have markedly changed, at the species level, since the early years of  
287 mine development when the communities were dominated by taxa with low Ca content  
288 (*Holopedium glacialis* and *Bosmina* spp.; Jeziorski and Yan 2006). *Holopedium glacialis* is now  
289 largely absent from recent monitoring surveys of the four lakes closest to the containment

290 facility, with corresponding increases in the proportions of *Daphnia longiremis* and *D.*  
291 *middendorffiana* (Figure 4), and with marked (Leslie Lake) or moderate (Moose Lake and Nema  
292 Lake) increases in *Daphnia* absolute abundances (Figure 5).

293  
294 The general replacement of *Holopedium* by *Daphnia* spp. is likely a response to the  
295 alteration of limnological conditions by mine effluent (particularly increases in Ca and pH).  
296 *Holopedium* and *Daphnia* spp. are both filter-feeders that graze on an overlapping range of  
297 particle sizes (Hessen et al. 1989), with *Daphnia* spp. generally being the more efficient grazers  
298 (Allan 1973; Tessier 1986). However, *Holopedium* can outcompete some *Daphnia* spp. at low-  
299 nutrient, low-Ca sites, when the pH is below 8 (Hessen et al. 1995), conditions present in the  
300 early years of mine development, as well as in the LDG sites and reference lakes. Under these  
301 conditions, *Holopedium* may have been better able than the *Daphnia* spp. to exploit the available  
302 resources, and dominate the assemblages.

303  
304 The dominance of *Holopedium* relative to *Daphnia* prior to mine development is likely  
305 due to the relatively low Ca requirements of *Holopedium* (Jeziorski and Yan 2006), and their  
306 ability thrive in the low Ca conditions (Jeziorski and Smol 2016; Jeziorski et al. 2015; Wærvågen  
307 et al. 2002). In contrast, at the start of the monitoring record, Ca levels were generally  $< 1 \text{ mg L}^{-1}$   
308 <sup>1</sup>, below the requirements of many daphniid taxa including *D. longiremis* (that has a field-  
309 identified lower Ca threshold of  $1.26 \text{ mg L}^{-1}$ ; (Cairns 2010). The high Ca requirements of the  
310 daphniids are largely due to their Ca-rich carapace, and low aqueous [Ca] can limit their growth,  
311 reproduction, and fecundity (Ashforth and Yan 2008). In contrast, *Holopedium* has a much lower  
312 Ca burden than some larger daphniid species (Jeziorski and Yan 2006), as rather than a carapace,

313 its principal defense from predators is an enveloping mucopolysaccharide jelly sheath. Low Ca  
314 demands and high fecundity relative to daphniids (supplementary material; Jeziorski et al. 2015),  
315 allows *Holopedium* to rapidly exploit food resources when daphniid populations are low.

316

317         Increased production at the DDC Ekati mines after 1999 resulted in elevated Ca and  
318 higher pH levels in the downstream lakes (Figure 3), with a corresponding increase in the  
319 proportion of *Daphnia* spp. (primarily *D. longiremis* and *D. middendorffiana* (Figure 4)) in the  
320 lakes closest to the discharge location. The marked, negative relationship between [Ca] and  
321 *Holopedium* relative abundances (Figure 7a) is most likely an indirect relationship, resulting  
322 from increased competition between *Holopedium* and *Daphnia*, with *Daphnia* species able to  
323 exploit the new high-Ca environment due to the input of Ca-rich effluent. A structural equation  
324 model analyzing long-term monitoring data from lakes in south-central Ontario spanning a  
325 similar range of [Ca] indicated that *Holopedium* did not directly respond to changes in lakewater  
326 Ca (Jeziorski et al. 2015). Instead, members of *Daphnia* spp. were highly sensitive to falling Ca,  
327 and as concentrations fell below threshold values their populations declined, freeing up food  
328 resources for *Holopedium* (Jeziorski et al. 2015). The Barren Lands' lakes downstream of the  
329 diamond mines have experienced similar changes, but in reverse, with *Daphnia* spp. increasing  
330 in relative abundances (Figure 4) and in many cases in absolute abundances (Figure 5), as [Ca]  
331 increased, with a corresponding decline in *Holopedium*. In Moose Lake, the disappearance of  
332 *Holopedium* was preceded by a large spike in *Daphnia* concentrations in 1998-1999 (Figure 5),  
333 which, given their competition for food resources, may have accelerated the virtual elimination  
334 of *Holopedium* from the lake. In addition to the increasing [Ca], concurrent changes in the  
335 phytoplankton community may have also contributed to the cladoceran community shift in the

336 impacted lakes. Recent increases in the biomass of edible planktonic diatoms within the effluent-  
337 receiving lakes (St-Gelais et al. 2017) may also have favoured daphniids over *Holopedium*, as  
338 they are generally more efficient algal grazers (Allan 1973, Tessier 1986).

339

340 The split identified in the regression tree analysis at  $[Ca] \sim 2.5 \text{ mg L}^{-1}$ , between samples  
341 with high relative abundances of *Holopedium* and those with little to no *Holopedium* (Figure 6a,  
342 Figure 7a), is slightly above the Ca requirements of *D. longiremis* ( $1.26 \text{ mg L}^{-1}$ ; Cairns 2010).  
343 Although we are unaware whether the Ca optimum of *D. middendorffiana* has been identified,  
344 the general increase in daphniid relative abundances as  $[Ca]$  increased above  $\sim 2.5 \text{ mg L}^{-1}$   
345 (Figure 7c) suggests that conditions have become more favorable for both *D. longiremis* and *D.*  
346 *middendorffiana*, allowing these efficient grazers (Allan 1973, Tessier 1986) to outcompete  
347 *Holopedium* for resources.

348

349 The decrease in *Holopedium* in the impacted lakes was often, but not always  
350 accompanied by an increase in the absolute counts of *Daphnia* (e.g. Slipper Lake; Figure 5)  
351 suggesting that not only competition from daphniids, but physiological stress from the novel  
352 limnological conditions created by the diamond mining effluent may be impacting the  
353 *Holopedium*. *Holopedium* populations may be stressed as a result of pH increases concurrent  
354 with the  $[Ca]$  increases. High pH ( $\text{pH} > 8$ ) conditions can result in increased mortality and  
355 reduced fecundity in *Holopedium* (Hessen et al. 1995), and at the DDC Ekati sites *Holopedium*  
356 were found at significantly lower relative abundances when  $\text{pH} \geq 7.1$  (Figure 7b). Given the  
357 significant correlation (Figure 8) between increasing  $[Ca]$  and lakewater pH (Figure 3), it is  
358 difficult to distinguish the relative impact of each factor in the study lakes, as the diamond

359 mining effluent has contributed  $\text{Ca}^{2+}$ , other major ions, and alkalinity to the softwater lakes  
360 (Rollo and Jamieson 2006). However, the pH of the lakes is unlikely having direct effects on the  
361 *Daphnia* spp., as daphniids are commonly found in circumneutral pH waters similar to that of the  
362 downstream lakes prior to the onset of mining. Increasing relative abundance and absolute  
363 abundances of daphniids over the monitoring record is most likely due to [Ca] increasing above  
364 threshold concentrations. However, the decline in *Holopedium* in these lakes is likely due to a  
365 combination of increased competition for food resources with daphniids and increased  
366 physiological stress from higher pH and altered limnological conditions.

367

368         The changes in [Ca] experienced by the Barren Lands' lakes downstream of DDC Ekati  
369 mines are particularly interesting, as they are the opposite to those occurring in many previously-  
370 acidified softwater lakes in eastern Canada and the United States (e.g. Jeziorski et al. 2008; Leys  
371 et al. 2016) and Scandinavia (e.g. Hessen et al. 2017). During acidification, lakes can lose acid-  
372 sensitive *Daphnia* taxa (Malley and Chang 1986; Walton et al. 1982), but even after recovery,  
373 the long-term decline in exchangeable cations may still hinder biological recovery (e.g. Keller et  
374 al. 2001). Many softwater lakes are experiencing substantial decreases in lakewater [Ca], as a  
375 legacy of acid deposition with corresponding decreases in Ca-sensitive daphniids (Jeziorski and  
376 Smol 2016) and their replacement by *Holopedium* (Jeziorski et al. 2015; Korosi and Smol 2012).  
377 In contrast, the lakes in the Northwest Territories receiving diamond mine effluent are exhibiting  
378 the reverse, in a process that mirrors trends associated with liming (Labaj et al. 2014) or an  
379 influx of Ca-rich road dust suppressant (Shapiera et al. 2012). The sites downstream of the DDC  
380 Ekati mines offer an opportunity to examine the impacts of Ca additions in otherwise undamaged

381 ecosystems (i.e. elimination of fish communities etc. as a result of acidification is not a factor  
382 affecting the cladoceran community changes at these sites).

383

384         The potential impacts of the cladoceran community shift on other trophic levels are not  
385 clear. *Daphnia* typically contain more phosphorus (as percent dry weight) than *Holopedium*  
386 (Andersen and Hessen 1991), and furthermore, *Holopedium* and *Daphnia* differ in their  
387 composition of polyunsaturated fatty acids (Persson and Vrede 2006). Thus, changes in their  
388 relative dominance may alter nutrient transfer throughout food webs. However, selective  
389 predation (Arts and Sprules 1988), the vulnerability of *Daphnia* relative to *Holopedium* to  
390 invertebrate predation (Vinyard and Menger 1980), and gap-limited predation (Tessier 1986) will  
391 also affect how the cladoceran community may influence higher trophic levels. To assess the  
392 effects on upper trophic levels within the effluent receiving lakes, continued monitoring of the  
393 fish and invertebrate predators will be required. The diamond mining AEMPs in the Barren  
394 Lands provide a multi-decadal monitoring record of remote northern lakes, and are a valuable  
395 dataset for further work examining lake ecosystems in the Canadian North. The unique  
396 circumstances of the naturally dilute and softwater lakes of the eastern Barren Lands receiving  
397 Ca-rich effluent, coupled with the regular monitoring conducted by the DDC Ekati Mine, create  
398 important records of whole lake manipulation of Ca (and other major ion) inputs, which have  
399 implications for the restoration of acidified lakes, and the impacts of cladoceran community  
400 changes on foodweb structure and function.

401

402         The changes in the cladoceran species composition of lakes downstream of Ekati mines  
403 highlight the importance of considering high-level taxonomic data in aquatic monitoring

404 programs. The monitoring record of Lac de Gras (sites S2 and S3, as well as others in the main  
405 basin monitored by the Diavik Diamond Mining AEMP (Golder 2016), suggest that, at least  
406 within its embayments, this large lake may be approaching [Ca] of 2.5 mg L<sup>-1</sup> identified in this  
407 study as the threshold concentration for *Holopedium* in the upstream lakes closer to the mine.  
408 Continued robust monitoring may capture a transition from *Holopedium* to daphniid dominance  
409 of the cladoceran community within Lac de Gras, and on a regional level will be important for  
410 providing insights on the zooplankton community response to an unusual environmental stress,  
411 and provide the data necessary for the development of informed mitigation plans.

412

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414

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423

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Table 1: Lake parameters and water chemistry data from August 2014, collected by the DDC Ekati AEMP for lakes downstream of mining operations (Leslie, Moose, Nema, Slipper, and both Site S2 and Site S3 in Lac de Gras), as well as the reference lakes (Counts Nanuq, and Vulture).

Site	Latitude (°N)	Longitude (°W)	Surface area (ha)	Max depth (m)	Conductivity ( $\mu\text{S cm}^{-1}$ )	pH	Nitrate ( $\text{mg L}^{-1}$ )	Total Phosphate ( $\text{mg L}^{-1}$ )	Total Calcium ( $\text{mg L}^{-1}$ )
Leslie Lake	64.6820	110.6969	61	12	830	8.05	2.14	0.0040	22.25
Moose Lake	64.6799	110.6512	43	9.5	526	7.92	0.996	0.0058	14.70
Nema Lake	64.6651	110.7127	78	9.5	408	7.70	0.130	0.0070	12.20
Slipper Lake	64.6121	110.8547	183	14.7	194	7.51	0.0083	0.0056	6.78
Site S2	64.6044	110.8419	56900	5.1	56.4	7.05	0.0117	0.0023	4.45
Site S3	64.6028	110.8731	56900	13.2	49.3	7.05	<0.0050	0.0024	1.90
Counts Lake	64.6510	110.2891	114	10.8	14.1	7.65	<0.0050	0.0090	0.699
Nanuq Lake	64.9146	110.2751	305	28.4	12.1	6.73	<0.0050	0.0053	0.749
Vulture Lake	64.7525	110.5519	174	38.9	13.1	7.18	<0.0050	0.0027	0.718

Figure 1: Map of the study region in the southern Northwest Territories (red box) with yellow stars representing towns and cities around Great Slave Lake, NWT. Inset A shows the Lac De Gras region of the Barren Lands with the location of reference lakes Counts and Nanuq. Inset B shows the primary DDC Ekati diamond mine operation with the study lakes in the Koala watershed, showing the generalized path of flow from the Long Lake Containment Facility (LLCF) marked with an orange line and arrows. Basemap data from Natural Resources Canada Canvec database. Satellite underlay for Inset B from Google Earth imagery.

Figure 2: Open water (June, July and August) Ca concentrations (A) and pH (B) from 2003 (when all sites were regularly monitored) to 2014 for the effluent-receiving lakes Leslie, Moose, Nema, Slipper lakes, Site S2 and Site S3 in Lac de Gras, and reference Counts Lake, Nanuq Lake, and Vulture Lake, from the DDC Ekati AEMP. The whiskers represent 1.5 x the interquartile range (from the 25<sup>th</sup> to 75<sup>th</sup> percentiles), while data beyond the end of the whiskers are outliers and plotted as points.

Figure 3: Mid-summer (late July / early August) Ca concentrations (A) and pH (B) over the sampling record for which we have data (1994, 1996-2014) for potentially impacted Leslie, Moose, Nema, Slipper lakes, Site S2 in and Site S3 in Lac de Gras, and reference Counts Lake, Nanuq Lake, and Vulture Lake, from the DDC Ekati AEMP. The markers for Vulture Lake and Nanuq Lake [Ca] are obscured by those for Counts Lake due to the similarity of the three lake's calcium records.

Figure 4: Mean relative abundance of genera and species of Cladocera for Leslie (closest to the mine discharge), Moose, Nema, Slipper lakes, Lac de Gras sites S2 and S3 (farthest from the mine discharge), and reference Counts Lake, Nanuq Lake, and Vulture Lake, from date of first sampling (1994, 1996, 1998 or 2000 depending on the site) to 2014. Samples from 1994 in Leslie Lake, 1994 and 1996 from Moose Lake and Slipper Lake, and 1996 from Nema Lake represent the pre-impact communities as the DDC Ekati mine was established in 1998.

Figure 5: Species counts from water column sampling of *Daphnia* (sum of *Daphnia middendorffiana*, *D. longispina* and *Daphnia* spp.) and *Holopedium glacialis* for all monitored lakes receiving mining effluent (Leslie, Moose, Nema, Slipper lakes, Lac de Gras sites S2 and S3 - from closest to farthest from the mine discharge) from the date of first sampling (1994, 1996, 1998 or 2000 depending on the site) to 2014. Moose Lake is plotted on a log+1 axis to account for a large peak in *Daphnia* abundance in 1998.

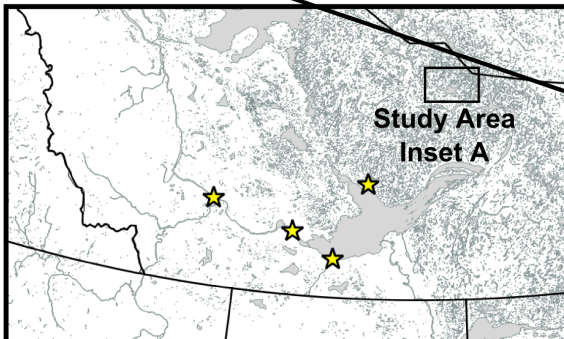
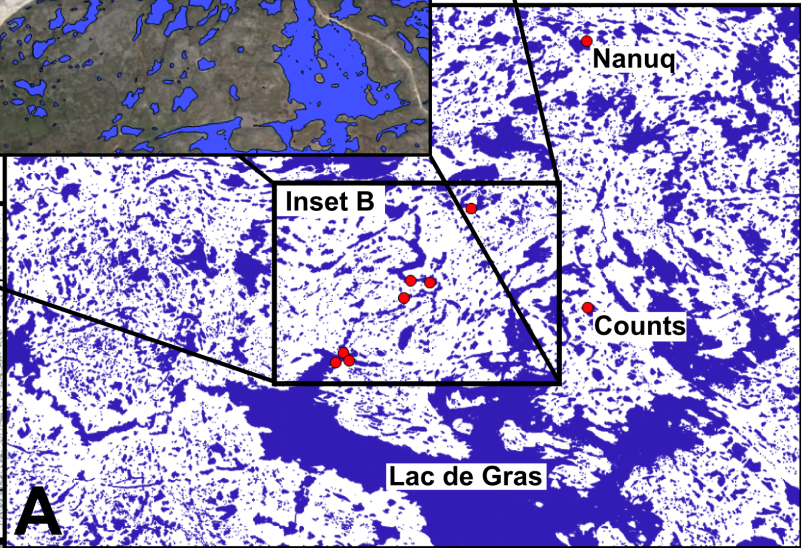
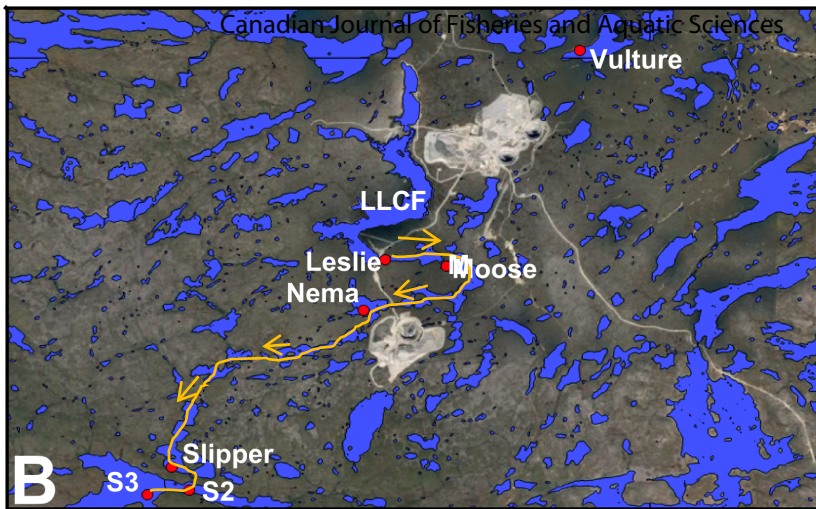
Figure 6: Multivariate regression trees (MRT) partitioning the full species dataset by mid-summer Ca concentrations (A) and pH (B). The MRTs each have one division splitting the sites into two leaves. Box plots show the *Daphnia* and *Holopedium* relative abundances for the sites that fall under each leaf of the MRT. The complexity parameter ( $R^2$ ), cross-validated error (CV Error) and standard error (SE) are shown for each MRT.

Figure 7: *Holopedium* (A and B) and *Daphnia* (C and D) relative abundance data for all sampling occasions for which we have data (1994, 1996, 1998-2014) as a function of the

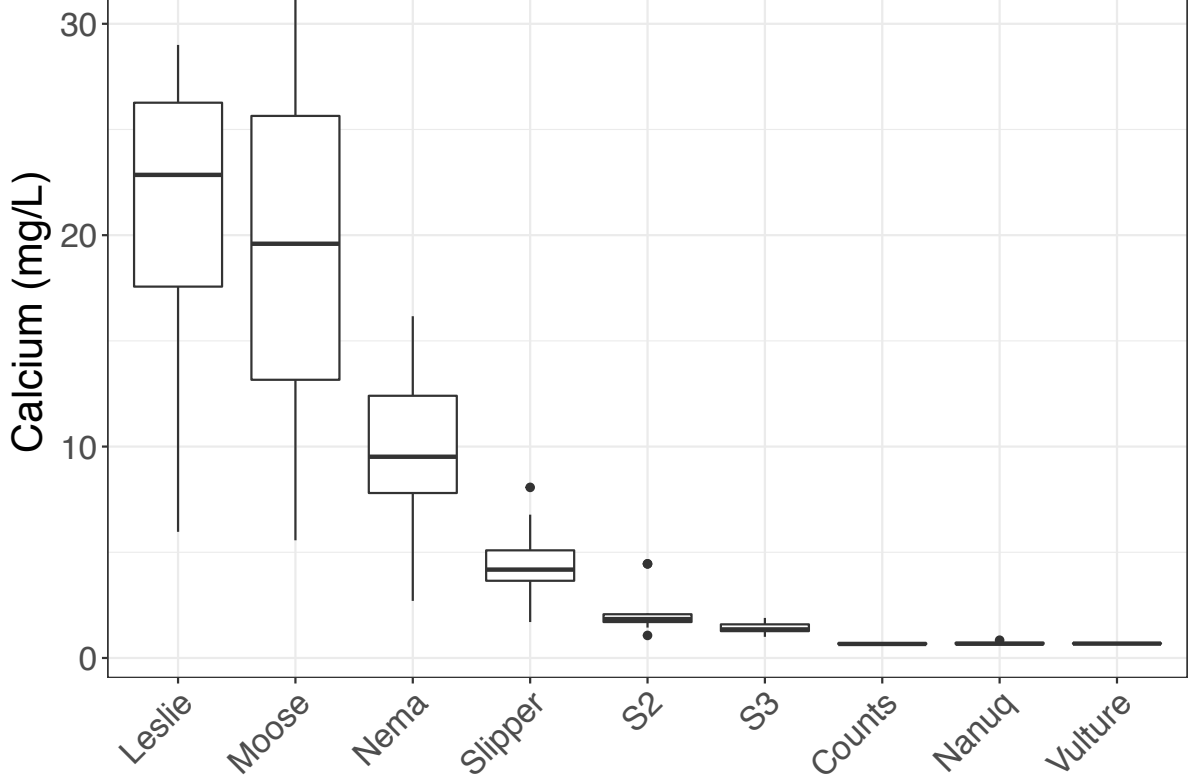
measured mid-summer Ca concentrations (A and C) and pH (B and D). The dashed line represents the first significant split identified by MRT analyses.

Figure 8: Mid-summer pH as a function of the measured Ca concentrations for all sampling occasions for which we have data (1998, 1999, 2004-2014) with points coloured according to the *Holopedium* relative abundance, with darker points reflecting higher relative abundances and light grey points reflecting sites without *Holopedium*. A logarithmic regression line (blue) with the equation  $\text{pH} = 0.7393 \log([\text{Ca}]) + 6.7671$  indicates a significant ( $p < 0.001$ ) positive relationship between pH and Ca concentrations with an adjusted  $R^2_{\text{adj}} = 0.7975$ .

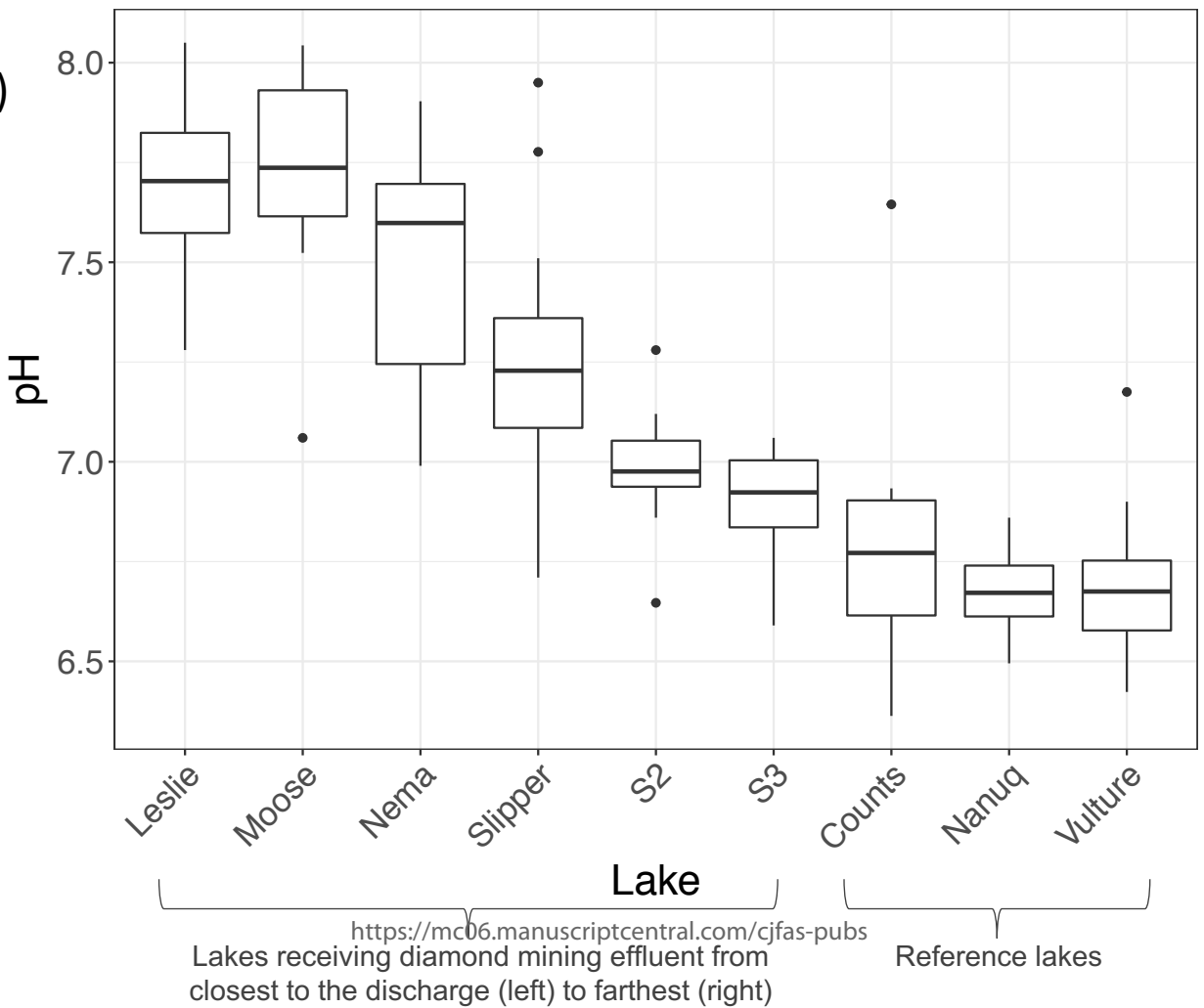
Draft



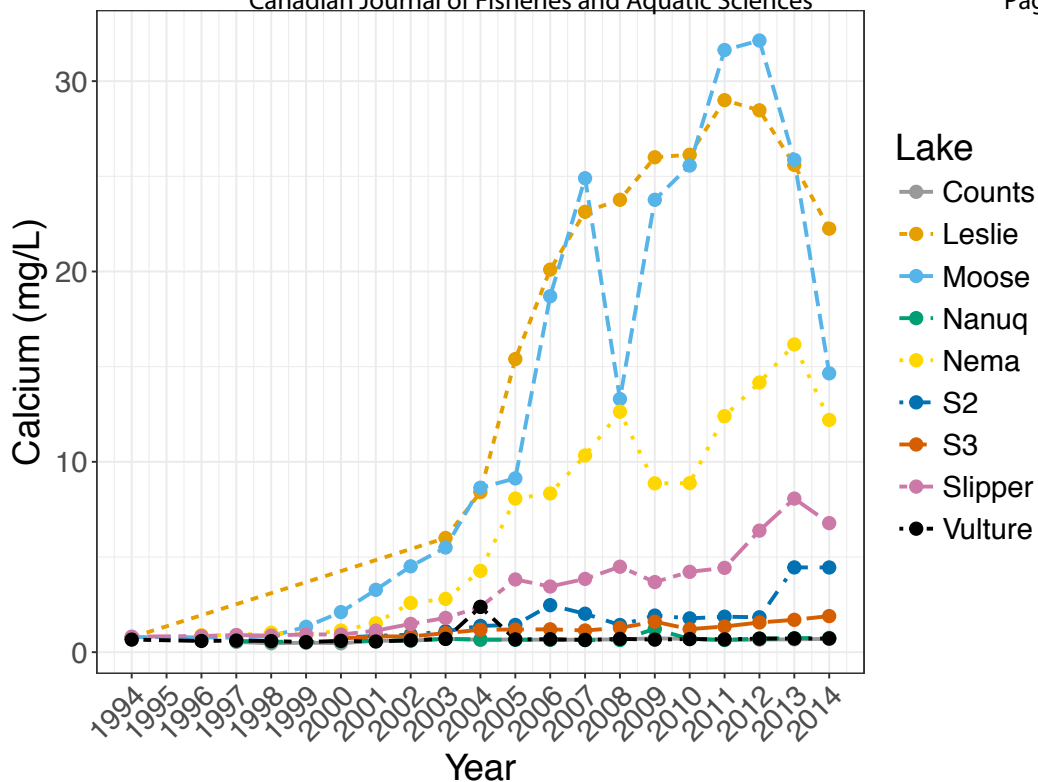
A)



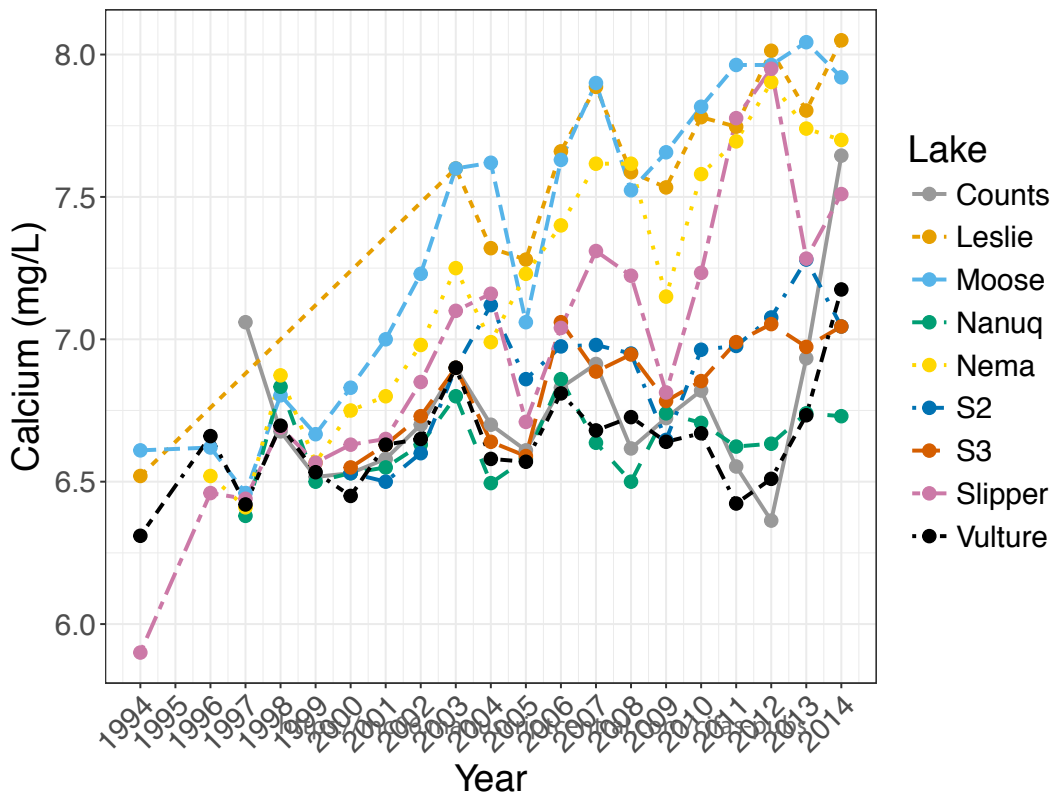
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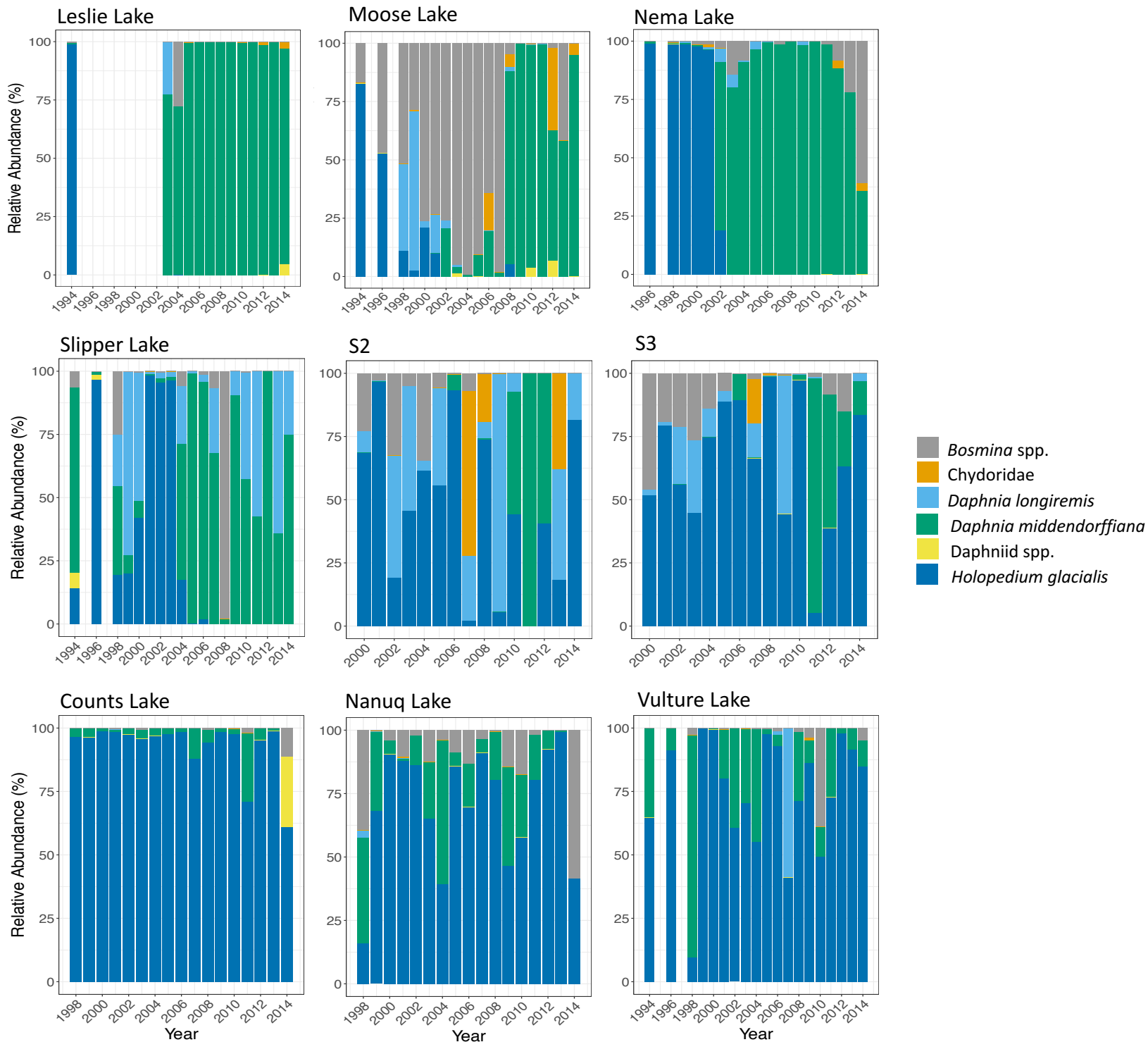


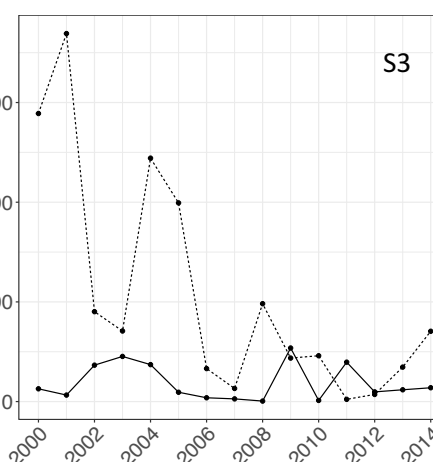
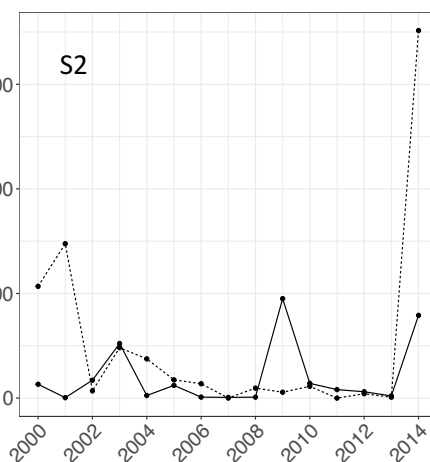
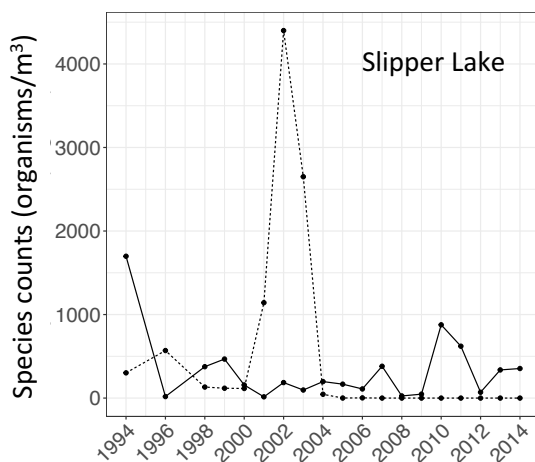
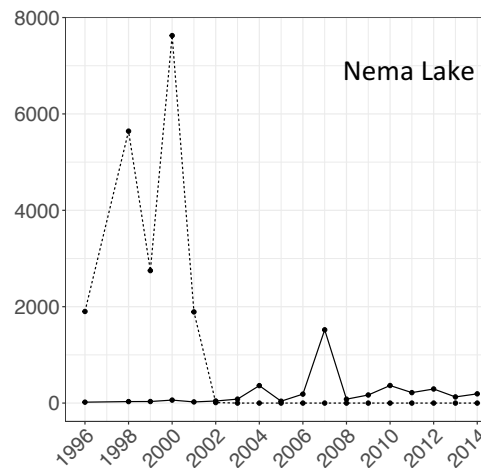
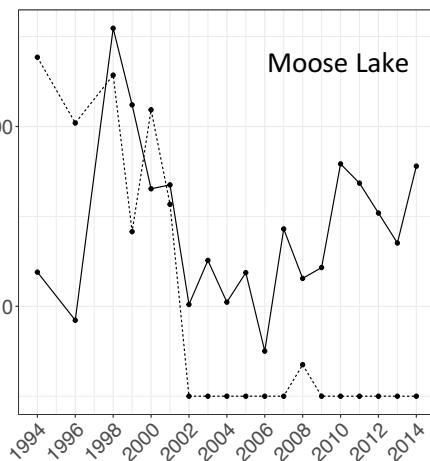
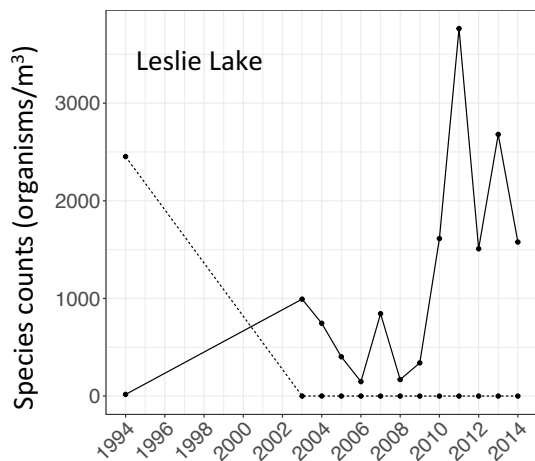
A)



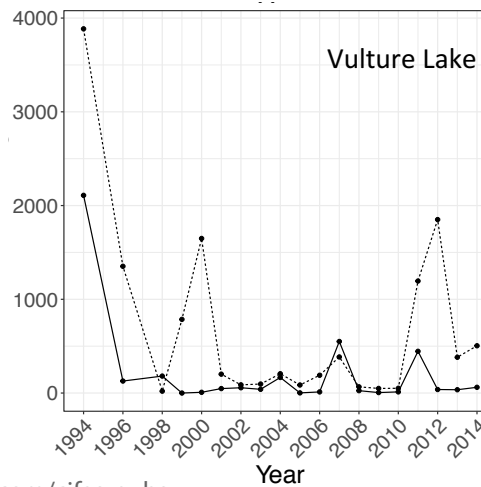
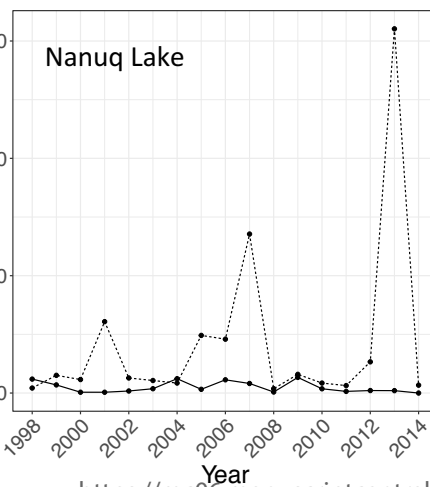
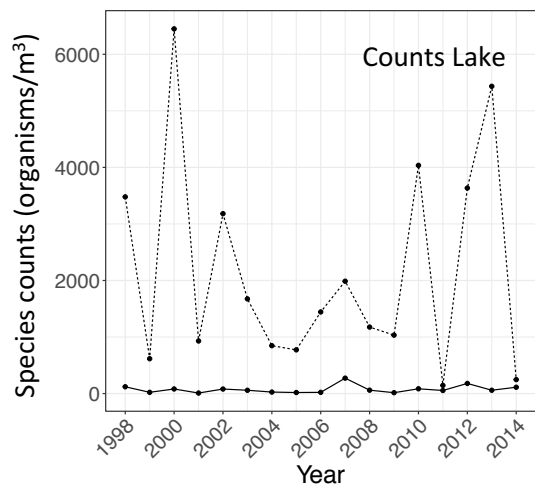
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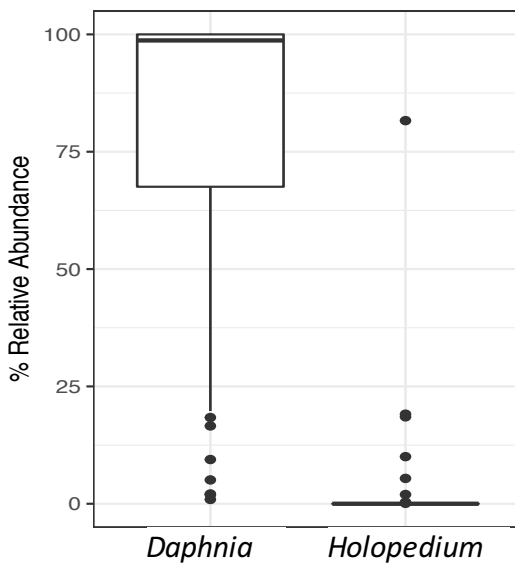
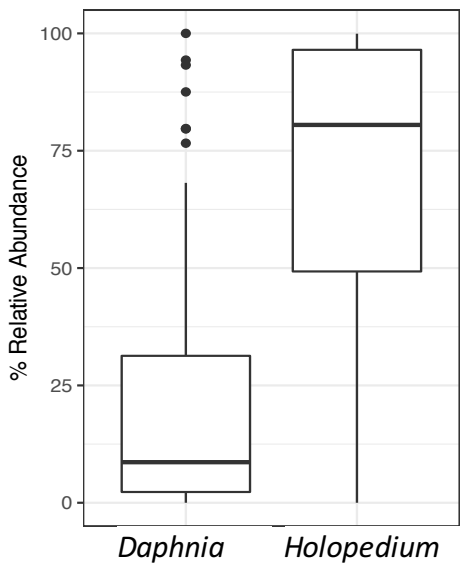


— *Daphnia*  
 ... *Holopedium*



A)

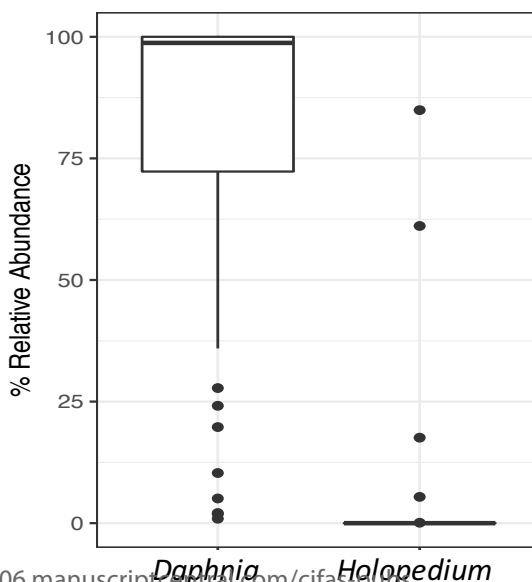
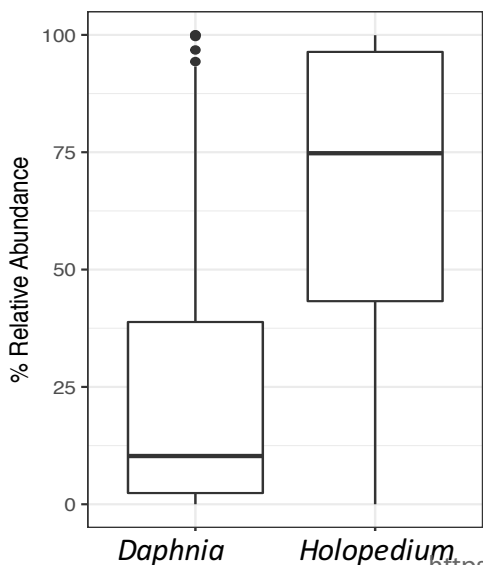
< 2.5 Ca ≥ 2.5



$R^2 = 0.415$   
CV Error = 0.62  
SE = 0.057

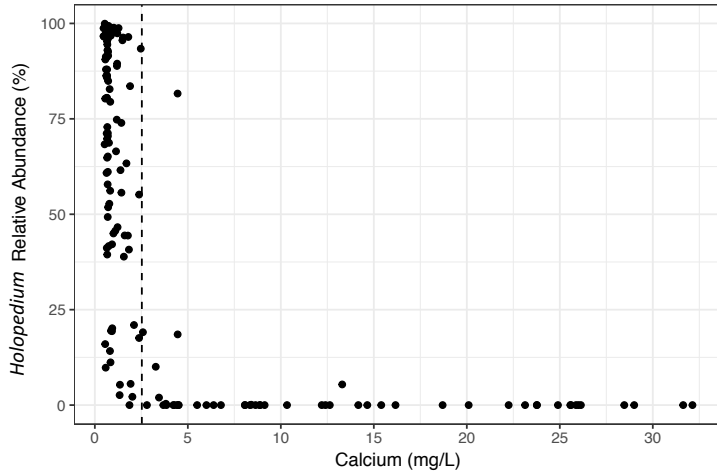
B)

< 7.1 pH ≥ 7.1

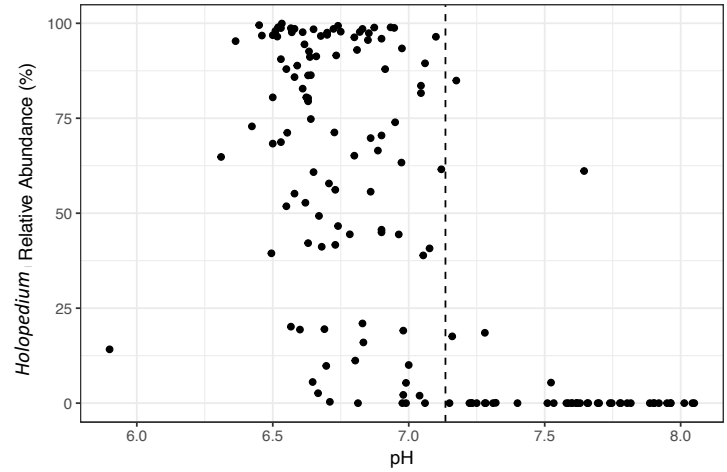


$R^2 = 0.344$   
CV Error = 0.73  
SE = 0.062

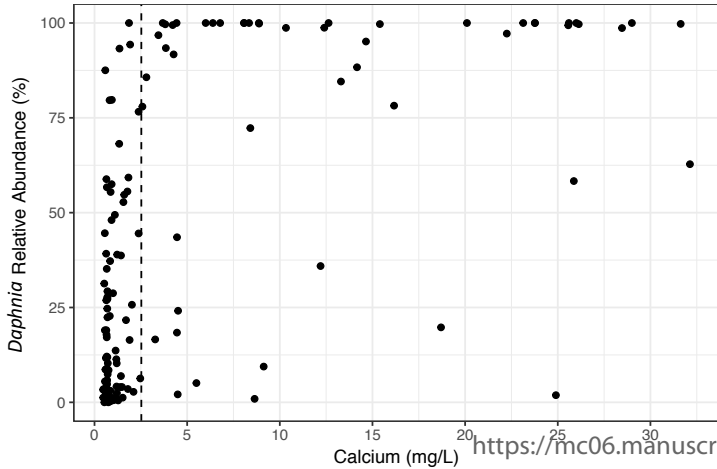
A)



B)



C)



D)

