1 TITLE: Zooplankton biogeography across temperate to subarctic regions: taxonomic and

2 functional perspectives

5 Cindy Paquette[1,2,3], Irene Gregory-Eaves[2,3] and Beatrix E. Beisner[1,2]

8 [1]Department of Biological Sciences, University of Quebec at Montreal, Montreal, Quebec,
9 Canada;
10 [2]Group for Interuniversity Research in Limnology (Groupe de recherche interuniversitaire;
11 GRIL);
12 [3]Department of Biological Sciences, McGill University, Montreal, Quebec, Canada

16 ACKNOWLEDGENTS

33 BIOSKETCH: CINDY PAQUETTE is a PhD student part of the NSERC LakePulse
34 network undertaking her studies in the Department of Biology at UQAM, co- supervised
35 by I.G.-E. (McGill University) and B.E.B.

37 TITLE: Zooplankton biogeography across temperate to subarctic regions: taxonomic and

38 functional perspectives

39

40 RUNNING TITLE: Canadian zooplankton biogeography

41

42 ABSTRACT

43 **Aim:** We examined variation in crustacean zooplankton taxonomic and functional

44 composition and diversity across Canadian lakes. In addition to α-diversity patterns, we

45 also explore mechanisms behind β-diversity spatial variation, using taxonomic and

46 functional metrics.

47 **Location:** Canada.

48 **Time period:** 2017-2019.

49 **Major taxa studied:** Crustacean zooplankton.

50 **Methods:** We sampled and characterized pelagic zooplankton from 624 lakes across

51 Canada, spanning 12 ecozones (defined by climatic, vegetation and geological

52 differences) or 6 continental drainage basins as part of the NSERC Canadian LakePulse

53 Network project. We compared taxon and trait distributions, as well as spatial patterns of

54 community composition and diversity. We computed taxonomic and functional spatial β-

55 diversity indices, decomposing these into taxon replacement and richness differences.

56 Finally, species and local contributions to β-diversity (SCBD and LCBD) were estimated

57 by ecozone and continental basin.

58 **Results:** 90 crustacean zooplankton species were identified across the country.

59 Zooplankton taxonomic and functional composition differences were more distinct when

60 considered by continental basin than by ecozone. α-diversity varied greatly across space,

61 with greatest diversity in eastern Canada. β-diversity was greatest when based on

62 taxonomy and were driven by richness differences across all spatial and biodiversity

63 dimensions.

64 **Main conclusions:** Zooplankton taxon and trait compositions were best predicted for

65 continental drainage basins than ecozones. Our results point to the combined effects of

66 physical barriers to dispersal and climate change shaping zooplankton biogeography

67 across Canadian lakes. This research advances our understanding of large-scale pelagic

68 zooplankton distribution patterns and biogeographical processes in general. Furthermore,

69 our study highlights the strength of combining taxonomic and functional approaches in

70 biogeography research.

73

74 INTRODUCTION

75 Determining species distributions across regions and the factors affecting these are major

76 questions in ecology and biogeography (Lomolino *et al.*, 2010; Smith *et al.*, 2020). The

77 distribution of crustacean zooplankton in lakes is shaped by both local environmental

78 drivers and spatial factors limiting their dispersal capacity (Beisner *et al.*, 2006; De Bie *et

79 al.*, 2012; Padial *et al.*, 2014). Zooplankton in southern Canada appear to disperse equally

80 well by watercourses as they do overland (e.g. wind or animal vectors), likely primarily

81 via dormant stages (Beisner *et al.*, 2006). Across North American lakes, plankton have

82 also shown important large-scale patterns related to environmental gradients (Vyverman

83 *et al.*, 2007; Stomp *et al.*, 2011; Sodré *et al.*, 2020).

84 Distributions and structural considerations of crustacean zooplankton taxa and

85 traits can provide information about changes occurring within individual lakes, but are

86 also ideal for assessing ecological shifts in aquatic communities more generally at large

87 spatial scales (Pinel-Alloul *et al.*, 2013; Griffiths *et al.*, 2019). In the context of

88 increasing anthropogenic pressure, early-warning signals enabling the detection of

89 change in lakes are needed. The functional trait ensemble present in a zooplankton

90 community can be directly related to the environmental conditions of their habitat (Vogt

91 *et al.*, 2013; Hébert *et al.*, 2016) and thus may be more strongly linked to the mechanisms

92 driving some ecosystem functions than more traditional taxonomic methods (Lavorel &

93 Garnier, 2002; Messier *et al.*, 2010). Functional trait approaches can provide predictable

94 and generalizable answers to ecological questions (Barton *et al.*, 2013), and offer critical

95 tools to understanding diversity patterns (Spasojevic *et al.*, 2014; Liu *et al.*, 2016).

96   In addition to composition, diversity indicators such as α- and β-diversity are

97 commonly used to study biogeographical gradients (Whittaker, 1972; e.g. Norris, 2000;

98 Mittelbach *et al.*, 2007; Kraft *et al.*, 2011). Previous work with zooplankton has

99 demonstrated that at the continental scale, solar radiation was among the most important

100 predictors of richness across Canadian lakes (Pinel-Alloul *et al.*, 2013). β-diversity has

101 been increasingly useful as a conservation and biodiversity management tool as it can

102 identify key taxa (via species contribution to β-diversity; SCBD) or sites (local

103 contribution to β-diversity; LCBD) that substantially enrich regional diversity (Legendre

104 & De Cáceres, 2013). Because taxon compositional shifts are more common with

105 anthropogenic change than losses, (Vellend *et al.*, 2013; Dornelas *et al.*, 2014), β-

106 diversity can reveal biodiversity shifts that remain undetected with α-diversity

107 (Winegardner *et al.*, 2017). Furthermore, β-diversity estimated as total variance can be

108 decomposed into differences in composition (species substitution, turnover) and richness

109 gradients (gain or loss of individuals, species nestedness) (Legendre, 2014). Additional to

110 taxonomic β-diversity, it is also possible to analyze trait variation among communities

111 (Villéger *et al.*, 2013).

112   A large national sampling effort of > 600 lakes spanning many biogeoclimatic

113 zones via the NSERC Canadian Lake Pulse Network provided a unique opportunity to

114 advance knowledge on crustacean zooplankton biogeography and its drivers by

115 considering a suite of diversity and compositional metrics. Previously, biogeographical

116 studies in North American or European lakes have focused on spatial patterns in

117 zooplankton species composition (e.g. Patalas *et al.*, 1994; Pinel-Alloul *et al.*, 2013;

118 Marrone *et al.*, 2017; Stoch *et al.*, 2019; Dexter *et al.*, 2020), but such a conventional

119 approach does not consider functional redundancy among taxa (but see Sodré et al.

120 2020). Additionally, at the largest spatial extents, corresponding to ecozones or

121 continental basins, it remains an open question whether taxonomic and functional

122 zooplankton distribution and diversity variation, (α- or β-diversity) respond similarly.

123   Our study examined zooplankton taxonomic and functional composition

124   distribution patterns; the first pan-Canadian analysis of zooplankton functional trait

125   variation. We hypothesized that the compositional differentiation of zooplankton

126   communities would be stronger for continental basins than ecozone classifications.

127   Continental basins represent large-scale hydrodynamics and are thus expected to drive

128   more aquatic zooplankton dispersal across lakes than an ecozone classification that

129   characterizes mainly vegetation, geological and climate typologies. Second, we tested the

130   hypothesis that lakes in southern regions would harbour greater α-diversities, given that

131   solar radiation has been identified as an important predictor of Canadian zooplankton

132   richness (Pinel-Alloul *et al.*, 2013). Finally, we evaluated the degree of spatial

133   heterogeneity (β-diversity) and tested whether taxonomic or functional approaches better

134   explained spatial variation in diversity among ecozones and continental basins. Our last

135   hypothesis was that taxonomic β-diversity should demonstrate greater variation than traits

136   because of more limited species dispersal at this large spatial scale, whereas trait β-

137   diversity would show less variation owing to trait redundancy across taxa.

138   MATERIAL AND METHODS

139   **Data set**

140   Over three summers (2017-2019), 664 lakes were sampled in 12 different ecozones

141   across Canada following a standardized protocol developed by the NSERC Canadian

142   Lake Pulse Network (see Huot *et al.*, 2019). All lakes within 1 km of roads were selected

143   randomly with a factorial design according to lake size (three size classes: 0.1-1km^2, 1-

144   10km^2, 10-100km^2), human impact (HI; three classes of land-use in the watershed: low,

145   medium, high; defined in Appendix S1 in Supporting Information) and ecozone. "Core"

146   ecozones were those for which the most extensive sampling was done (Pacific Maritime,

147   Montane Cordillera, Semi-Arid Plateaux, Boreal Plains, Prairies, Boreal Shield,

148   Mixedwood Plains, Atlantic Highlands and Atlantic Maritime). In the remaining

149   ecozones (Boreal Cordillera, Taiga Cordillera, Taiga Plains), more restricted sampling

150   occurred due to limited road access. Study lakes spanned six continental drainage basins

151   separated by continental divides (Pacific Ocean, Arctic Ocean, Hudson Bay, Gulf of

152   Mexico, Great Lakes-St. Lawrence and Atlantic Ocean). These continental watersheds

153   were delimited by the National Hydrographic Network watershed data and represent

154   landscape surfaces where precipitation and river systems drain to distinct gulfs, seas or

155   oceans (Government of Canada, 2019).

156   Zooplankton sampling and identification

157   At the deepest point in each lake, integrated water column zooplankton samples were

158   collected using a 100μm mesh Wisconsin net. Samples were treated on site with an initial

159   CO_2 narcotization, and then preserved in 70% ethanol. Samples were stored at room

160   temperature and identified to species by BSA Environmental Services (Ohio, U.S.A.).

161   BSA also estimated biomasses using the average length of up to 10 measured individuals

162   per taxon per lake and pre-established size/weight relationships for each species (Dumont

163   *et al.*, 1975; McCauley, 1984; Lawrence *et al.*, 1987). (see Appendix S2 for details). The

164   final dataset consisted of 624 lakes across 12 ecozones and 6 continental basins (Figure

165   1), spanning a large range of lake size, depth and human impact (Appendix S3). Analysis

166   of Variance (ANOVA) was used to determine the effects of ecozones and continental

167   basins on mean total lake biomasses. All statistical analyses were performed in R v. 3.6.3

168   (R Core Team, 2020).

169   Taxa were classified functionally using species-specific traits from the literature

170   (Demott & Kerfoot, 1982; Barnett *et al.*, 2007; Griffiths *et al.*, 2019). The traits used

171   were characterized: resource acquisition (B(*Bosmina*)-filtration, C(*Chydorus*)-filtration,

172   D(*Daphia*)-filtration, S(*Sidae*)-filtration, stationary suspension or raptorial), habitat

173   (littoral, pelagic or intermediate) and trophic group (carnivore, herbivore, omnivore, or a

174   combination of these). Species mean size representsan integrative trait related to

175   predation, resource acquisition, productivity and competitive strength (Brooks & Dodson,

176   1965; Barnett *et al.*, 2007; Litchman *et al.*, 2013; Hébert *et al.*, 2017). Size was used as a

177   continuous trait in all diversity analyses. However, to estimate size-based distributions, a

178   cutoff of 0.5 mm was used to create two size classes (small and large) to have roughly

179   balanced group sizes (species numbers).

180   Estimating distribution patterns

181   Maps for each taxon identified were produced to visualise distribution patterns

182   (Appendix S4). Species occurrences by lake were represented in a rank-frequency

183   diagram and relative biomass composition (by genus and by categorical trait) by ecozone

184   and continental basins were examined. Rare genera (< 2% relative biomass in all lakes)

185   were grouped in category "other". Based on similarity observed via the relative biomass

186   composition by taxa and traits, the Taiga Cordillera (n=3) and Taiga Plains (n=22) were

187   combined, as were the continental basins Gulf of Mexico (n=2) and Hudson Bay

188   (n=145); roughly balancing the number of lakes across groups (Appendix S3).

189   Multivariate analyses were used to reveal patterns in the compositional datasets.

190   We used canonical analysis of principal coordinates (CAP) to assess the distinctness of

191   zooplankton species or categorical functional traits across regions. These constrained

192   ordinations employ a non-parametric distance-based discriminant analysis to Hellinger-

193   transformed total biomass of common species or categorical functional traits by site.

194   Common taxa were defined as those with >2% relative biomass in at least one lake. Bray-

195   Curtis distances were used with the *CAPdiscrim()* function of the BiodiversityR package

196   (Kindt, 2020). For each analysis, we used the number of principal coordinate axes that

197   resulted in the best prediction of lake group identities (for ecozones or continental

198   basins), according to Anderson & Willis (2003). The *CAPdiscrim()* function returns the

199   percentage of correct predictions of the full model (i.e. percentage of lakes that were

200   classified in the correct region based on taxon or trait composition), as well as by region

201   (ecozone or continental basin). A multivariate analysis of variance (MANOVA) within

202   this function then assessed regional classification significance. To enable comparison of

203   CAP classifications at different regional scale groupings (differing in both the number

204   and sizes of groups by ecozone or continental basin), the adjusted % predicted was

205   estimated as the percentage of correct allocations minus the percentage of correct

206   allocations that could be attributed to a randomly correct prediction (adjusted % predicted

207   = % correct allocations - (100/N); where N is the number of groups).

208   To visualise spatial patterns in species composition and categorical functional

209   traits across Canada, the lake PCoA scores from the first axis of principal component

210   analyses (PCA) were used to produce heat-maps. The PCoA values were obtained with

211 *prcomp()* function applied on Hellinger-transformed biomass data of taxa or functional

212 traits.

Estimating diversity metrics

214 Using biomass and taxonomic composition data, we estimated, the most common

215 diversity indices (Hill, 1973): species richness, Shannon entropy, Simpson diversity and

216 Pielou's evenness (using *diversity()* in vegan package; Oksanen *et al.*, 2019). Rarefied

217 species richness was calculated using *rarefy()*, rounded to 109 individuals (lowest count

218 size). For functional diversity, we estimated functional evenness (*FEve*; Villéger *et al.*,

219 2008), functional dispersion (*FDis*; Laliberté & Legendre, 2010) and functional richness

220 (*FRic*; Villéger *et al.*, 2008). These were computed with all functional traits (resource

221 acquisition, habitat, trophic group and average species size) using the *dbFD()* function

222 with Gower distances, FD package (Laliberté *et al.*, 2014). This function was also used to

223 produce a cluster dendrogram of species based on functional traits. The number of

224 functional groups was determined after visual inspection of the dendrogram (as in

225 Borcard *et al.*, 2018; p.405).

226   To illustrate spatial patterns in α-diversity metrics, a PCA with lake longitude and

227 latitude values was computed. The taxonomic and functional diversity metrics were then

228 added passively to the plot to visualise correlations with the lake coordinates scores.

229   β-diversity was used to compare species composition and functional traits by site

230 across the country, within ecozones and continental basins. β-diversity ranges from 0 to

231 1, where 1 indicates maximum diversity between communities (Legendre & De Cáceres,

232 2013). We differentiated the total β-diversity (country-wide, within ecozones or

233 continental basins) into either the taxon replacement component (turnover; i.e. sites are

234 most different in terms of species composition) or the richness difference component

235 (nestedness; i.e. main differences between sites results from the number of species

236 present) (Baselga, 2012; Villéger *et al.*, 2013). β-diversity and its decomposition into

237 replacement and richness difference components were calculated using *beta()* function,

238 Biodiversity Assessment Tools (BAT) package (Cardoso *et al.*, 2020). We used the *beta()*

239 function to compute β-diversity because it allows inclusion of clustering objects (*hclust*

240 for functional diversity) in the dissimilarity matrices. For functional β-diversity, we used

241 a hierarchical clustering tree (function *quality_funct_space()*; Maire *et al.*, 2015). Species

242 abundances with the Sørensen family of measures (percentage difference index) were

243 used to obtain the dissimilarity matrices. Total variance across communities (all sites, by

244 ecozone or by continental basin), were computed by summing values in the lower

245 triangular dissimilarity matrices (total β-diversity, replacement and richness difference)

246 from *beta()* and dividing by (n*n-1) (as in Legendre, 2014).

247   Local contribution to β-diversity (LCBD) is a metric that is indicative of site

248 uniqueness (Legendre & De Cáceres, 2013). We estimated taxonomic LCBD using

249 species data with the function *beta.div()* in the adespatial package (Legendre & De

250 Cáceres, 2013; Legendre, 2014). The *beta.div()* function also estimates the species

251 contribution to β-diversity (SCBD) metric, identifying critical species for regional

252 diversity maintenance (Legendre & De Cáceres, 2013). Taxonomic LCBD was computed

253 with the percentage-difference Podani index-transformed species data, while SCBD was

254 computed on the Hellinger-transformed biomass species data (as described by Legendre

255 & De Cáceres, 2013). The significance of LCBD values for each lake was assessed using

256 the permutational *p*-value (permutations = 9,999). To test for significant LCBD sites after

257 correcting for multiple-testing, we used Holm's procedure (see Borcard *et al.*, 2018; p.

258 308). Critical species were identified as those having values larger than across-site mean

259 SCBD values (as in Borcard *et al.*, 2018; p. 386). Using an analogous approach,

260 functional LCBD values were obtained using *LCBD.comp()* from adespatial package

261 (Legendre & De Cáceres, 2013; Legendre, 2014) and the total β-diversity dissimilarity

262 matrix computed previously using *beta()*. The *LCBD.comp()* function produces the same

263 results as *beta.div()* when used on taxonomic data, but does not permit testing of LCBD

264 significance, nor does it calculate SCBD.

265   Although there is no equivalent to SCBD available for functional β-diversity, we

266 were still interested in examining which functional traits contributed most to β-diversity.

267 We related taxonomic SCBD to functional traits using beta regressions with a *logit* link

268 function. Functional traits were used to compute a matrix of species functional

269   dissimilarities between species, from which coordinates in the multidimensional

270   functional space (PCoA) were computed. PCoAs and the distance matrix were both

271   estimated using the *quality_funct_space()* function. The functional PCoA first two axes

272   were then used as predictor variables of SCBD in a beta regression model using *betareg()*

273   function from betareg package (Zeileis *et al.*, 2020).

274   Mean diversity indices (taxonomic and functional) across lakes were compared by

275   ecozone and continental basin using ANOVA. No interactions were detected between

276   ecozone and continental basin in all biomass and diversity ANOVA analyses. Significant

277   mean pair-wise differences among ecozones and among continental basins were

278   identified using a *TukeyHSD()* post-hoc test. Relative mean values of total biomass,

279   taxonomic and functional α-diversity, as well as taxonomic and functional β-diversity

280   were represented in radar charts by ecozone and continental basin.

281   RESULTS

282   **Biomass, distribution patterns and classification**

283   Zooplankton biomass varied greatly across Canada ($p<$2e-16, Figure 2a,

284   Appendix S5, S6, S7), with the largest totals observed in central Canada, in the Prairies

285   ecozone (mean biomass per lake = 1738.1 ± 341.89 µg d.w./L) and the Hudson Bay

286   continental basin (mean biomass per lake = 1091.0 ± 185. µg d.w./L).

287   Across all lakes, 90 crustacean zooplankton species were identified (distribution

288   maps in Appendix S4). *Bosmina longirostris* was the most frequently found crustancean

289   zooplankton (55% of the lakes, Appendix S8). The invasive cladoceran *Eubosmina*

290   *coregoni* occurred in 3% of lakes and only in eastern Canada. The functional trait

291   dendrogram identified six main functional groups of zooplankton (Appendix S9).

292   *Daphnia* was the dominant genus in 8 of the 12 ecozones, with a relative

293   contribution to total biomass generally decreasing from west to east (Figure 3a). In the

294   northernmost ecozones (Boreal Cordillera, Taiga Cordillera and Taiga Plains),

295   *Leptodiatomus* contributed most to total biomass. The Atlantic Highlands ecozone was

296   unique in Canada with *Eubosmina* dominating, although this pattern likely resulted from

297 extreme biomass values (87% of biomass) of *Eubosmina coregoni* in one hypereutrophic

298 lake. The relative biomass of functional trait groupings (Figure 3c,d) was more similar

299 across the landscape than for genera. The most common feeding types were stationary

300 suspension, D-filtration and raptorial. Pelagic zooplankton were uniformly dominant, as

301 would be expected from the sampling methodology. Herbivores were the most dominant

302 trophic type across ecozones as were small zooplankton (<0.5 mm). Similar results were

303 reflected among continental basins (Figure 3d).

304 A clear longitudinal pattern was observed in lake PCoA scores from the first PCA

305 axis of species biomass data (Figure 4a). Lakes plotting negatively were typically found

306 in the western and central Canada, while more lakes plotting positively were found in

307 eastern Canada. This longitudinally distinct pattern was weaker for functional trait

308 biomass PCoA scores, but not completely absent (Figure 4b).

309 **Spatial distinctiveness of taxonomic and functional trait composition**

310 Canonical analyses of principal components (CAP) were used to reveal spatial patterns in

311 zooplankton taxa or functional traits and ecozones or continental basins (Figure 5). All

312 four MANOVA models were significant (Table 1), indicating significant differences of

313 species and trait composition among regions. For both taxonomic and functional

314 analyses, the percentage of lakes correctly attributed to the appropriate region according

315 to their species or trait composition (% correct attribution) was greater using continental

316 basins (61.1% and 42.0% respectively) than ecozones (46.3% and 29.0% respectively),

317 even after correcting for the number of groups (Adjusted % predicted, Table 1). Among

318 ecozones, the Boreal Cordillera had the highest percentage of correct allocation with

319 83.3% of correct taxonomic and 76.7% of correct functional predictions (Table 1). These

320 trends were mainly attributable to responses by *Leptodiaptomus pribilofensis* and

321 *Heterocope septentrionalis*, and the carnivore functional trait that correlated strongly

322 with this ecozone (Figure 5 a,c). On the other hand, only 20% of Taiga Plains lakes were

323 correctly attributed according to species composition, while none of the lakes from the

324 Semi-Arid Plateaux had correct functional allocations, indicating that they have no

325 distinctive sets of species or traits. For continental basins, Pacific Ocean basin lakes had

326 the highest correct allocation percentage (taxonomic =77.1%; functional =59.6%), driven

327 by *Diacyclops thomasi*, *Daphnia pulicaria*, *Daphnia dentifera* as well as large size and

328 carnivore functional traits (Figure 5 b,d). The Arctic Ocean basin had the least distinct

329 species and traits (37.9% and 22.7% respective allocations).

Spatial patterns in total biomass and α-diversity

331 Taxonomic and functional α-diversity also varied considerably across space (Figure 2a,

332 Appendix S5, S10, S11), with overall greater diversity in the east. All taxonomic

333 diversity indices varied significantly across ecozones ($p<0.05$), and all but evenness

334 varied across continental basins ($p<0.001$). Overall, total and rarefied species richness

335 varied the most among ecozones ($p <2e\text{-}16$). Mean richness across all lakes was 7 taxa

336 (varying from 2 to 16 species) and 6 functional traits (varying from 1 to 12 unique trait

337 combinations; excluding species size, data not shown). Taxonomic richness, Simpson,

338 and Shannon indices were all significantly greater in the Boreal Shield, Mixedwood

339 Plains and Atlantic Highlands ecozones and in the Great lakes-St. Lawrence basin, while

340 taxonomic evenness was largest in the Boreal Cordillera. *FDis* was significantly larger in

341 the Boreal Cordillera, Boreal Shield, Mixedwood Plains and Atlantic Highlands ecozones

342 and in the Great lakes-St. Lawrence basin. *FRic* was greatest in the Boreal Plains,

343 Prairies, Mixedwood Plains and Atlantic Highlands ecozones, and in Hudson Bay and

344 Great lakes-St. Lawrence basins. No significant difference was found for *FEve* by

345 ecozone or continental basin. The PCA ordination with lake coordinates revealed strong

346 associations between most of the α-diversity (Shannon and Simpson diversity, richness,

347 rarefied richness and *FDis*) indices and longitude (Appendix S12).

Spatial β-diversity

349 Overall β-diversity indices varied little. Comparing across all sites, taxonomic and

350 functional β-diversity were 0.47 and 0.37 respectively (Appendix S13, S14). Both types

351 of β-diversity estimate were greatest in the Pacific Maritimes ecozone and in the Hudson

352 Bay basin, and lowest in the Semi-Arid Plateaux (Figure 2b). Richness difference

353 (nestedness) was always the dominant mechanism of compositional change among lakes,

354 for all ecozones and continental basins. The relative contribution of richness differences

355 to among-lake β-diversity was 0.63 for taxonomic and 0.77 for functional trait estimates

356 (Appendix S13).

357   Taxonomic and functional and LCBD values varied significantly among ecozones

358   (p <2e-16) and continental basins (p<0.001). The northern-most ecozones (Boreal

359   Cordillera and Taiga Plains) as well as the Prairies had the largest taxonomic LCBD

360   values; for continental basins it was the Arctic Ocean and Hudson Bay (Figure 2b,

361   Appendix S15). At an individual site level, 62 lakes were identified as having significant

362   LCBD (without correction for multiple comparison, Figure 6). There was a clear pattern

363   of more sites in western and northern Canada with significant LCBD. All ecozones and

364   continental basins contained lakes with significant taxonomic LBCD values although

365   after correcting for multiple testing, no site had a significant value. Functional LCBD

366   showed similar patterns among ecozones and continental basins, with Taiga Plains,

367   Pacific Maritimes and Prairies ecozones, as well as the Hudson Bay basin having lakes

368   with the greatest contribution to local diversity on average (Figure 2b, Appendix S15).

369   Twenty-seven species were identified as key species, with SCBD values larger

370   than the national SCBD mean; Appendix S16). *Daphnia pulicaria* contributed the most

371   of any species to β-diversity with a value of 0.15. Functional traits were also compared

372   to SCBD using beta regressions and PCoA axes as predictors. The two first trait vectors

373   were significant in the trait model (p<0.001, pseudo R^2= 0.42, Table 2). The first axis

374   separated C-filtration herbivores (group 2, Appendix S9) from pelagic stationary-

375   suspension species (group 3). The second PCoA trait-axis separated raptorial species

376   (group 5) from D-filtration species (group 4) (results not shown).

377   DISCUSSION

378   Our study provides one of the most intensive and extensive analyses of biogeographical

379   patterns of crustacean zooplankton by considering both taxonomic and functional

380   biodiversity dimensions across lakes covering the east-west axis of the second largest

381   country in the world. This approach clearly identified trends in zooplankton composition

382   among continental basins and ecozones, as well as differences across a suite of diversity

383   metrics. The β-diversity analyses pointed to richness differences driving variation among

384   regions. Overall, we found support for most of our hypotheses, and were able to build

385   upon previous studies of zooplankton biogeography, bringing novel insights into the role

386   of large-scale patterns and drivers.

The role of spatial extent

We were first interested in investigating at which scale, the regional ecozone level or larger scale continental basin, would zooplankton community composition and functional traits be most differentiated. Both taxonomic composition and functional trait regional distinctness (CAP analyses) were ~15% stronger for continental basins than for ecozones, in agreement with our original hypothesis. Based on watercourse distributions and hydrological flows, we had hypothesized that the continental basin framework would be a stronger structuring determinant than ecozones. Our results also concord with previous work demonstrating that factors influencing zooplankton community structure vary across spatial scales, and that at the broadest scale, continental hydrodynamics become increasingly important for zooplankton biomass or diversity, while habitat variables are more relevant at smaller scales (Mackas *et al.*, 1985; Borcard *et al.*, 2004; Declerck *et al.*, 2011; Anas *et al.*, 2017). These previous studies were conducted at smaller spatial scales than ours, and distinctness detected among ecozones and continental basins with our results extend these to continental scales.

Latitudinal and longitudinal patterns

The most evident pattern in diversity and composition that we observed in the country-wide data was a longitudinal one, with more diverse, but homogeneous zooplankton communities across eastern Canada. Indeed, all α-diversity indices were greater in the eastern regions: Boreal Shield, Mixedwood Plains and in the Atlantic Highlands ecozones, as well as in the Great lakes-St. Lawrence continental basin. Significant LCBD sites were also relatively more scarce in eastern Canada, indicating that while being more species-rich, the communities are relatively homogeneous. One explanation for this apparent longitudinal trend, would invoke the relative lack of physical barriers to dispersal in these regions compared to the more mountainous west. Important physical or environmental barriers could limit species dispersal such that some central and eastern species might not be able to reach or survive in northern and western lakes. For example, the Rocky Mountains likely isolate lakes from the Pacific Ocean divide, and colder temperatures in the north might not provide a suitable habitat for southern species. Zooplankton dispersal limitation by mountain chain barriers was

417   similarly suggested to influence copepod diversity in the Western Palearctic (Marrone *et*

418   *al.*, 2017). Moreover, the prevailing westerlies (winds moving air from west to east) in

419   Canada could lead to this pattern, with lakes in the eastern part of the country being

420   exposed to greater immigration of freshwater zooplankton from potentially the majority

421   of the country. This hypothesis is further supported by the canonical analysis of principal

422   coordinates (CAP) results, indicating that lakes from the Pacific Ocean divide and from

423   the Boreal Cordillera ecozone have very distinct species and traits (species and traits

424   CAPs Pacific Ocean = 77.1 and 59.6%; Boreal Cordillera = 83.3 and 76.7%

425   respectively), while eastern regions are less distinctly defined (Table 1). A distinct

426   longitudinal pattern was also observed across lake PCoA scores based on species biomass

427   data, where eastern Canadian lakes were very distinct from western ones. On the other

428   hand, greater diversities could be expected in regions with deeper and more strongly

429   stratified lakes because such lakes can host more vertical habitat niches (Jeppesen *et al.*,

430   2000; Barnett & Beisner, 2007; Longhi & Beisner, 2010). However, both western and

431   eastern lakes are often deep and stratified, whereas shallow lakes dominate in the flatter

432   prairies of central Canada (see Appendix S3). Yet, we did not observe greater diversities

433   in the western-most regions of Canada, which would provide support for this explanation.

434   We noted a trend toward greater (but not significant) mean taxonomic and trait

435   (*FRic*) richness in the Mixedwood Plains, Canada's southernmost ecozone. This result

436   partially supports our second hypothesis, predicting greater diversity in southern

437   Canadian regions. This hypothesis was informed by earlier analyses of taxonomic species

438   richness across 1665 Canadian lakes, where Pinel-Alloul *et al.* (2013) attributed these

439   patterns to the richness-energy hypothesis (Brown, 1981; Wright, 1983) that stipulates

440   co-existing species richness is driven by available energy (e.g., solar radiation). However,

441   Pinel-Alloul *et al.* (2013) did not include diversity indicators based on abundances,

442   biomasses and functional traits. Furthermore, their sampling was done over three

443   decades, which in itself could introduce artefacts such as varying responses to

444   acidification or climate change. Determining whether our observations are a consequence

445   of the richness-energy relationship, or an indirect effect of higher productivity related to

446   greater nutrient loading in highly populated regions of the Mixedwood Plains (Gibbs *et*

447   *al.*, 2009; Chambers *et al.*, 2012) would necessitate further investigation into local habitat

448   factors. However, it is important to note that the mean species richness of the Mixedwood

449   Plains was not statistically different from other ecozones (the Boreal Plains, Boreal

450   Shield and Atlantic Highlands). Likewise, *FRic* in the Mixedwood Plains was not

451   statistically different from the other ecozones in *post hoc* tests. Moreover, the association

452   between α-diversity indices and lake coordinates visualized with a PCA ordination

453   revealed strong congruence between numerous α-diversity indices and longitude rather

454   than latitude, implying that an energetic explanation is not likely.

455   Zooplankton diversity did not appear to be reduced by the more intensive human

456   habitat perturbation (mainly via agriculture and urbanization) in the southern regions of

457   the Prairies, Mixedwood Plains and Atlantic Maritimes ecozones (Gibbs *et al.*, 2009;

458   Huot *et al.*, 2019). Several studies have demonstrated negative zooplankton diversity

459   responses to increases in: eutrophication from macronutrients (e.g., nitrogen and

460   phosphorus) (Dodson *et al.*, 2000; Yan *et al.*, 2008; Jeppesen *et al.*, 2011), salinity

461   (Brucet *et al.*, 2009), toxic substances (Cattaneo *et al.*, 1998), and reductions in: water

462   levels (Nevalainen *et al.*, 2011), pH (Yan & Strus, 1980) and calcium (Jeziorski *et al.*,

463   2008). Instead, we observed greater diversities in these more human-impacted regions,

464   although it should be noted that our sampling design explicitly targeted a range of

465   different land use intensities in each ecozone, which could have masked anthropogenic

466   effects without explicit use of local environmental variables. Overall, at the continental

467   extent, spatial determinants appear to be important for zooplankton diversity and

468   distribution (Stoch *et al.*, 2019).

469   **Trophic cascades and biomass patterns**

470   Zooplankton community variation could also be shaped by adjacent trophic levels,

471   primarily phytoplankton or fish communities. Fish community variation is commonly

472   attributed to lake morphometry (O'Brien *et al.*, 2004) and depth (Jackson & Harvey,

473   1989), with larger, deeper lakes hosting more piscivorous fish (Post *et al.*, 2000). We

474   observed greatest total zooplankton biomass in the Prairies (average biomass = 1738.1 μg

475   d.w./L), which may be an indirect product of reduced fish predation. Prairies lakes are

476   typically shallow and susceptible to winter fishkills, reducing fish planktivory (Barica &

477   Mathias, 1979; Carpenter *et al.*, 1985; Meding, 2000). Prairie lakes also support high

478   phytoplankton growth, which can benefit zooplankton biomass production (Dupuis &

479   Hann, 2009). Indeed, our functional trait results point to the importance of feeding type,

480   which varied the most across ecozones and continental divides. When investigating the

481   distinctiveness of functional traits (CAP analysis) across ecozones, trophic group and

482   feeding type were also both important predictors of composition. Both are known to be

483   related to how, and which type of phytoplankton prey zooplankton will feed on (Barnett

484   *et al.*, 2007), pointing to the key importance of trophic interactions in shaping

485   zooplankton distribution patterns.

486   In our dataset, Prairies lakes were also unique in having high taxonomic and

487   functional LCBD values, suggesting at first glance that there was high heterogeneity

488   across lakes of this region. This was unexpected, because typically LCBD and spatial

489   heterogeneity correlate negatively with human development and nutrient loading (Olden

490   *et al.*, 2004; Donohue *et al.*, 2009; Winegardner *et al.*, 2017). These lake are among the

491   most nutrient-rich, with low N:P ratios and susceptible to large toxic cyanobacteria

492   blooms (Quinlan *et al.*, 2002; Taranu *et al.*, 2010), because of intense agriculture and the

493   natural geology in the region. A possible explanation for this paradox is the fact that

494   LCBD estimates consider not only variation in species composition, but also species

495   abundances. Thus, these high LCBD values are likely related to the large variation in

496   species biomasses observed across Prairies lakes. Further supporting the lack of

497   compositional variation, these lakes were not especially unique in terms of species or trait

498   distinctiveness (% correct attribution = 49.3% and 25.4%) relative to other ecozones.

499   **Mechanisms shaping β-diversity**

500   We observed little variation in β-diversity across regions. The overall taxonomic β-

501   diversity value found in this study (0.47) is slightly higher than the one reported in

502   Winegardner's *et al.* (2017) study of U.S. diatom (0.36); however, their analyses were

503   conducted at the genus level. On the other hand, Soininen *et al.* (2018) found higher

504   average β-diversity (0.62) in their global meta-analysis of species beta-diversity studies

505   covering taxa from bacteria to mammals.

506   The mechanism shaping total β-diversity was dominated by the richness-

507   difference component (nestedness), regardless of the diversity dimension (taxonomic and

508   functional). This result could be linked to the α-diversity patterns that we observed and to

509   the dispersal barriers and westerlies as discussed previously. According to Leprieur *et al.*

510   (2011) a higher richness-difference component of β-diversity can indicate that there has

511   been a large-scale uniform pressure on the territory, such as climate change. Canada is

512   indeed facing intense climate change, at twice global rates, especially in the Prairies,

513   British Columbia and in the North (Warren & Lemmen, 2014; Bush *et al.*, 2019;

514   Environment and Climate Change Canada, 2019). A strong effect of climate change on

515   zooplankton has previously been detected in western Canada, whereby important

516   association between climate variables and taxonomic or functional composition were

517   detected (Loewen *et al.*, 2019). On the other hand, Winegardner *et al.* (2017) studied

518   diatom β-diversity at the genus level across the United Stated and observed the opposite

519   pattern, with variation dominated by the taxon-replacement component. When taxon-

520   replacement is the dominant mechanism generating β-diversity, variation likely results

521   from strong environmental gradients (Leprieur *et al.*, 2011). Thus, while the Canadian

522   landscape is clearly heterogeneous in terms of landforms, soils, water features,

523   vegetation, climate and human activities (Wiken, 1986; CCEA, 2016), our results point to

524   climate change and important physical barriers to dispersal as important factors shaping

525   zooplankton biogeography. Further exploration of these ideas will come from future

526   LakePulse analyses, wherein we have the opportunity to explore the relative role of

527   spatial vs. environmental factors, consider food web interactions, as well as quantify

528   changes in sub-fossil assemblages between pre-industrial vs contemporary times through

529   sediment core analyses.

530   **Multidimensional approaches to biodiversity**

531   Accounting for taxonomic and functional approaches may be essential in disentangling

532   large scale biogeographical patterns. As expected, functional overall β-diversity values

533   (0.37) were slightly reduced relative to taxonomic β-diversity (0.47). Greater taxonomic

534   variation, as observed here, is expected when there is high functional redundancy among

535   sites (Gianuca *et al.*, 2018).

536   While we did observe the well-documented negative relationship between species

537   (or trait) richness and LCBD (Legendre & De Cáceres, 2013; Heino & Grönroos, 2017;

538   Heino & Alahuhta, 2019; da Silva *et al.*, 2020) where taxonomic LCBD values were

539   greatest and taxonomic richness lowest, this pattern did not always hold for functional

540   diversity in our study. We found instead that the relationship between functional

541   ecological uniqueness and diversity was region-dependent, and trait-poor sites did not

542   necessarily contribute the most to local functional β-diversity as would be expected.

543   Instead, in the Hudson Bay basin, high *FRic* was also associated with more specialized

544   traits, while in the Great lakes-St. Lawrence, lakes with high *FRic* had more common

545   traits. In a conservation context, sites characterize by high richness, but also high

546   functional and taxonomic uniqueness (LCBD) such as in the Hudson Bay basin could be

547   prioritized in management plans (see Figure 2). Indeed, Devictor *et al*. (2010) proposed

548   targeting sites having both high α- and β-diversity values, as a compromise between

549   preserving species-rich sites and ecologically unique sites.

550   Conservation goals are often focused on individual taxa (e.g., endangered,

551   threatened or keystone species), and in this context, the species contribution to β-diversity

552   (SCBD) metric can be particularly informative. However, most of the zooplankton

553   species in our study that contributed more SCBD than average (e.g. *Daphnia pulicaria,*

554   *Bosmina longirostris, Mesocyclops edax*) were also broadly distributed and were not

555   typically found in lakes with significant LCBD. Thus, SCBD was not useful to identify

556   rare zooplankton taxa across the country. In a functional trait context, feeding mode

557   variation contributed the most to the overall SCBD-trait relationships (r^2=0.42),

558   corroborating the importance of feeding traits found in our relative biomass and CAP

559   analyses. In contrast, Heino et Grönroos (2017) detected weaker (0.29) predictive power

560   in SCBD for stream insect functional traits. Correlation between SCBD and species

561   functional traits might thus depend on the predominant taxonomic group or ecosystem

562   type studied.

563   Although this is the first pan-Canadian sampling of zooplankton that adopted a

564   standardized approach over three years and covered 6765 km (longitude) by 2807 km

565   (latitude), some regions of Canada remain poorly represented. In particular, Canada's

566   northernmost ecozones could be more extensively sampled in the future. Eventual

567   inclusion of these northern freshwater zooplankton communities is essential to make

568   further advancements.

Conclusions

Overall, our study demonstrates the importance of incorporating different spatial scales and diversity dimensions in biogeographical studies. Zooplankton community biomass, composition, α- and β-diversity all varied significantly across ecozones and continental divides, highlighting important heterogeneity in Canadian lakes. By incorporating taxonomic and functional dimensions of β-diversity, we revealed spatial patterns that have not previously been observed. Future work exploring zooplankton biogeography across Canada including a variety of environmental variables will be essential to disentangle the effects of space from those of land use and climate. Moreover, considering zooplankton longer-term temporal β-diversity will be key to understand how communities have changed during the Anthropocene, and to predict how they will continue to change in the future.

584   REFERENCES

585   Anas, M.U.M., Meegahage, B.J., Evans, M.S., Jeffries, D.S. & Wissel, B. (2017) Scale-
586   dependent effects of natural environmental gradients, industrial emissions and
587   dispersal processes on zooplankton metacommunity structure: Implications for the
588   bioassessment of boreal lakes. *Ecological Indicators*, **82**, 484–494.
589   Anderson, M.J. & Willis, T.J. (2003) Canonical analysis of principal coordinates: A
590   useful method of constrained ordination for ecology. *Ecology*, **84**, 511–525.
591   Barica, J. & Mathias, J.A. (1979) Oxygen Depletion and Winterkill Risk in Small Prairie
592   Lakes Under Extended Ice Cover. *Journal of the Fisheries Research Board of*
593   *Canada*, **36**, 980–986.
594   Barnett, A. & Beisner, B.E. (2007) Zooplankton biodiversity and lake trophic state:
595   explanations invoking resource abundance and distribution. *Ecology*, **88**, 1675–
596   1686.
597   Barnett, A.J., Finlay, K. & Beisner, B. (2007) Functional diversity of crustacean
598   zooplankton communities: Towards a trait-based classification. *Wiley Online*
599   *Library*, **52**, 796–813.
600   Barton, A.D., Pershing, A.J., Litchman, E., Record, N.R., Edwards, K.F., Finkel, Z. V,
601   Kiørboe, T. & Ward, B.A. (2013) The biogeography of marine plankton traits.
602   *Ecology Letters*, **16**, 522–534.
603   Baselga, A. (2012) The relationship between species replacement, dissimilarity derived
604   from nestedness, and nestedness. *Global Ecology and Biogeography*, **21**, 1223–
605   1232.
606   Beisner, B.E., Peres-Neto, P.R., Lindström, E.S., Barnett, A. & Longhi, M.L. (2006) The
607   role of environmental and spatial processes in structuring lake communities from
608   bacteria to fish. *Ecology*, **87**, 2985–2991.
609   De Bie, T., De Meester, L., Brendonck, L., Martens, K., Goddeeris, B., Ercken, D.,
610   Hampel, H., Denys, L., Vanhecke, L., Gucht, K., Wichelen, J., Vyverman, W. &
611   Declerck, S.A.J. (2012) Body size and dispersal mode as key traits determining
612   metacommunity structure of aquatic organisms. *Ecology Letters*, **15**, 740–747.
613   Borcard, D., Gillet, F. & Legendre, P. (2018) *Numerical ecology with R*, second edition.
614   Springer.
615   Borcard, D., Legendre, P., Avois-Jacquet, C. & Tuomisto, H. (2004) Dissecting the
616   spatial structure of ecological data at multiple scales. *Ecology*, **85**, 1826–1832.
617   Brooks, J.L. & Dodson, S.I. (1965) Predation, Body Size, and Composition of Plankton.
618   *Science*, **150**, 28–35.
619   Brown, J.H. (1981) Two Decades of Homage to Santa Rosalia: Toward a General Theory
620   of Diversity. *American Zoologist*, **21**, 877–888.
621   Brucet, S., Boix, D., Gascón, S., Sala, J., Quintana, X.D., Badosa, A., Søndergaard, M.,
622   Lauridsen, T.L. & Jeppesen, E. (2009) Species richness of crustacean zooplankton
623   and trophic structure of brackish lagoons in contrasting climate zones: north
624   temperate Denmark and Mediterranean Catalonia (Spain). *Ecography*, **32**, 692–702.
625   Bush, E., Gillett, N., Bonsal, B., Cohen, S., Derksen, C., Flato, G., Greenan, B., Sheperd,
626   M. & Zhang, X. (2019) *Canada's changing climate report*,.
627   Cardoso, P., Mammola, S., Rigal, F. & Carvalho, J.C. (2020) BAT: Biodiversity
628   Assessment Tools. R package version 2.0.1.

629 Carpenter, S.R., Kitchell, J.F. & Hodgson, J.R. (1985) Cascading Trophic Interactions
630   and Lake Productivity . *BioSciences*, **35**.
631 Cattaneo, A., Asioli, A., Comoli, P. & Manca, M. (1998) Organisms' response in a
632   chronically polluted lake supports hypothesized link between stress and size.
633   *Limnology and Oceanography*, **43**, 1938–1943.
634 CCEA (2016) Ecozone introduction.
635 Chambers, P.A., McGoldrick, D.J., Brua, R.B., Vis, C., Culp, J.M. & Benoy, G.A. (2012)
636   Development of Environmental Thresholds for Nitrogen and Phosphorus in Streams.
637   *Journal of Environmental Quality*, **41**, 7–20.
638 Declerck, S.A.J., Coronel, J.S., Legendre, P. & Brendonck, L. (2011) Scale dependency
639   of processes structuring metacommunities of cladocerans in temporary pools of
640   High-Andes wetlands. *Ecography*, **34**, 296–305.
641 Demott, W.R. & Kerfoot, W.C. (1982) Competition among cladocerans: nature of the
642   interaction between Bosmina and Daphnia. *Ecology*, **63**, 1949–1966.
643 Devictor, V., Mouillot, D., Meynard, C., Jiguet, F., Thuiller, W. & Mouquet, N. (2010)
644   Spatial mismatch and congruence between taxonomic, phylogenetic and functional
645   diversity: The need for integrative conservation strategies in a changing world.
646   *Ecology Letters*, **13**, 1030–1040.
647 Dexter, E., Bollens, S.M., Cordell, J. & Rollwagen-Bollens, G. (2020) Zooplankton
648   invasion on a grand scale: insights from a 20-yr time series across 38 Northeast
649   Pacific estuaries. *Ecosphere*, **11**.
650 Dodson, S.I., Arnott, S.E. & Cottingham, K.L. (2000) The relationship in lake
651   communities between primary productivity and species richness. *Ecology*, **81**, 2662–
652   2679.
653 Donohue, I., Jackson, A.L., Pusch, M.T. & Irvine, K. (2009) Nutrient enrichment
654   homogenizes lake benthic assemblages at local and regional scales. *Ecology*, **90**,
655   3470–3477.
656 Dornelas, M., Gotelli, N.J., McGill, B., Shimadzu, H., Moyes, F., Sievers, C. &
657   Magurran, A.E. (2014) Assemblage time series reveal biodiver- sity change but not
658   systematic loss. *Science*, **344**, 296–299.
659 Dumont, H.J., Van De Velde, I. & Dumont, S. (1975) The Dry Weight Estimate of
660   Biomass in a Selection of Cladocera, Copepoda and Rotifera from the Plankton,
661   Periphyton and Benthos of Continental Waters. *Oecologia*, **19**, 75–97.
662 Dupuis, A.P. & Hann, B.J. (2009) Warm spring and summer water temperatures in small
663   eutrophic lakes of the Canadian prairies: Potential implications for phytoplankton
664   and zooplankton. *Journal of Plankton Research*, **31**, 489–502.
665 Environment and Climate Change Canada (2019) *Canadian Environmental Sustainability*
666   *Indicators: Temperature change in Canada*,.
667 Gianuca, A.T., Engelen, J., Brans, K.I., Hanashiro, F.T.T., Vanhamel, M., van den Berg,
668   E.M., Souffreau, C. & Meester, L. De (2018) Taxonomic, functional and
669   phylogenetic metacommunity ecology of cladoceran zooplankton along urbanization
670   gradients. *Ecography*, **41**.
671 Gibbs, K.E., Mackey, R.L. & Currie, D.J. (2009) Human land use, agriculture, pesticides
672   and losses of imperiled species. *Diversity and Distributions*, **15**, 242–253.
673 Government of Canada (2019) National Hydrographic Network .
674 Griffiths, K., Winegardner, A.K., Beisner, B.E. & Gregory-Eaves, I. (2019) Cladoceran

675 assemblage changes across the Eastern United States as recorded in the sediments
676 from the 2007 National Lakes Assessment, USA. *Ecological Indicators*, **96**, 368–
677 382.
678 Hébert, M.P., Beisner, B.E. & Maranger, R. (2016) A meta-analysis of zooplankton
679 functional traits influencing ecosystem function. *Ecology*, **97**, 1069–1080.
680 Hébert, M.P., Beisner, B.E. & Maranger, R. (2017) Linking zooplankton communities to
681 ecosystem functioning: Toward an effect-Trait framework. *Journal of Plankton*
682 *Research*, **39**, 3–12.
683 Heino, J. & Alahuhta, J. (2019) Knitting patterns of biodiversity, range size and body size
684 in aquatic beetle faunas: significant relationships but slightly divergent drivers.
685 *Ecological Entomology*, **44**, 413–424.
686 Heino, J. & Grönroos, M. (2017) Exploring species and site contributions to beta
687 diversity in stream insect assemblages. *Oecologia*, **183**, 151–160.
688 Hill, M.O. (1973) Diversity and Evenness: A Unifying Notation and Its Consequences.
689 *Ecology*, **54**, 427–432.
690 Huot, Y., Brown, C.A., Potvin, G., Antoniades, D., Baulch, H.M., Beisner, B.E.,
691 Bélanger, S., Brazeau, S., Cabana, H., Cardille, J.A., del Giorgio, P.A., Gregory-
692 Eaves, I., Fortin, M.J., Lang, A.S., Laurion, I., Maranger, R., Prairie, Y.T., Rusak,
693 J.A., Segura, P.A., Siron, R., Smol, J.P., Vinebrooke, R.D. & Walsh, D.A. (2019)
694 The NSERC Canadian Lake Pulse Network: A national assessment of lake health
695 providing science for water management in a changing climate. *Science of the Total*
696 *Environment*, **695**.
697 Jackson, D.A. & Harvey, H.H. (1989) Biogeographic Associations in Fish Assemblages:
698 Local vs. Regional Processes. *Ecology*, **70**, 1472–1484.
699 Jeppesen, E., Nõges, P., Davidson, T.A., Haberman, J., Nõges, T., Blank, K., Lauridsen,
700 T.L., Søndergaard, M., Sayer, C., Laugaste, R., Johansson, L.S., Bjerring, R. &
701 Amsinck, S.L. (2011) Zooplankton as indicators in lakes: a scientific-based plea for
702 including zooplankton in the ecological quality assessment of lakes according to the
703 European Water Framework Directive (WFD). *Hydrobiologia*, **676**, 279–297.
704 Jeppesen, E., Peder Jensen, J., Søndergaard, M., Lauridsen, T. & Landkildehus, F. (2000)
705 Trophic structure, species richness and biodiversity in Danish lakes: changes along a
706 phosphorus gradient. *Freshwater Biology*, **45**, 201–218.
707 Jeziorski, A., Yan, N.D., Paterson, A.M., Desellas, A.M., Turner, M.A., Jeffries, D.S.,
708 Keller, B., Weeber, R.C., McNicol, D.K., Palmer, M.E., McIver, K., Arseneau, K.,
709 Ginn, B.K., Cumming, B.F. & Smol, J.P. (2008) The widespread threat of calcium
710 decline in fresh waters. *Science (New York, N.Y.)*, **322**, 1374–7.
711 Kindt, R. (2020) BiodiversityR: Package for Community Ecology and Suitability
712 Analysis. R package version 2.12.1.
713 Kraft, N.J., Comita, L.S., Chase, J.M., Sanders, N.J., Swenson, N.G., Crist, T.O., Stegen,
714 J.C., Vellend, M., Boyle, B., Anderson, M.J., Cornell, H. V, Davies, K.F., Freestone,
715 A.L., Inouye, B.D., Harrison, S.P. & Myers, J.A. (2011) Disentangling the Drivers
716 of b Diversity Along Latitudinal and Elevational Gradients. *Science*, **333**, 1755–
717 1758.
718 Laliberté, E. & Legendre, P. (2010) A distance-based framework for measuring
719 functional diversity from multiple traits. *Ecology*, **91**, 299–305.
720 Laliberté, E., Legendre, P. & Shipley, B. (2014) Measuring functional diversity (FD)

721   from multiple traits, and other tools for functional ecology. R package version 1.0-
722   12.
723 Lavorel, S. & Garnier, E. (2002) Predicting changes in community composition and
724   ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional*
725   *Ecology*, **16**, 545–556.
726 Lawrence, S.G., Malley, D.F., Findlay, W.J., Maclver, M.A. & Delbaere, I.L. (1987)
727   Method for Estimating Dry Weight of Freshwater Planktonic Crustaceans from
728   Measures of Length and Shape. *Canadian Journal of Fisheries and Aquatic*
729   *Sciences*, **44**, 264.
730 Legendre, P. (2014) Interpreting the replacement and richness difference components of
731   beta diversity. *Global Ecology and Biogeography*, **23**, 1324–1334.
732 Legendre, P. & De Cáceres, M. (2013) Beta diversity as the variance of community data:
733   Dissimilarity coefficients and partitioning. *Ecology Letters*, **16**, 951–963.
734 Leprieur, F., Tedesco, P.A., Hugueny, B., Beauchard, O., Dürr, H.H., Brosse, S. &
735   Oberdorff, T. (2011) Partitioning global patterns of freshwater fish beta diversity
736   reveals contrasting signatures of past climate changes. *Ecology Letters*, **14**, 325–334.
737 Litchman, E., Ohman, M.D. & Kiørboe, T. (2013) Trait-based approaches to zooplankton
738   communities. *J. Plankton Res*, **35**, 473–484.
739 Liu, C., Guénard, B., Blanchard, B., Peng, Y.Q. & Economo, E.P. (2016) Reorganization
740   of taxonomic, functional, and phylogenetic ant biodiversity after conversion to
741   rubber plantation. *Ecological Monographs*, **86**, 215–227.
742 Loewen, C.J.G., Strecker, A.L., Larson, G.L., Vogel, A., Fischer, J.M. & Vinebrooke,
743   R.D. (2019) Macroecological drivers of zooplankton communities across the
744   mountains of western North America. *Ecography*, **42**, 791–803.
745 Lomolino, M.V., Riddle, B.R., Whittaker, R.J. & Brown, J.H. (2010) *Biogeography*,
746   Fourth edn. Sinauer Associates, Inc. Publishers, Sunderland, MA.
747 Longhi, M.L. & Beisner, B.E. (2010) Patterns in taxonomic and functional diversity of
748   lake phytoplankton. *Freshwater Biology*, **55**, 1349–1366.
749 Mackas, D.L., Denman, K.L. & Abbott, M.R. (1985) Plankton patchiness: biology in the
750   physical vernacular. *Bulletin of Marine Science*, **37**, 652–674.
751 Maire, E., Grenouillet, G., Brosse, S. & Villéger, S. (2015) How many dimensions are
752   needed to accurately assess functional diversity? A pragmatic approach for assessing
753   the quality of functional spaces. *Global Ecology and Biogeography*, **24**, 728–740.
754 Marrone, F., Alfonso, G., Naselli-Flores, L. & Stoch, F. (2017) Diversity patterns and
755   biogeography of Diaptomidae (Copepoda, Calanoida) in the Western Palearctic.
756   *Hydrobiologia*, **800**, 45–60.
757 Mccauley, E. (1984) *The estimation of the abundance and biomass of zooplankton in*
758   *samples*. *A Manual for the Assessment of Secondary Productivity in Fresh Waters*
759   (ed. by J.A. Downing) and F.H. Rigler), pp. 228–265. Blackwell Scientific
760   Publishers.
761 Meding, M.E. (2000) Structure and function in shallow prairie lakes: macrophytes and
762   winter anoxia .
763 Messier, J., McGill, B.J. & Lechowicz, M.J. (2010) How do traits vary across ecological
764   scales? A case for trait-based ecology. *Ecology Letters*, **13**, 838–848.
765 Mittelbach, G.G., Schemske, D.W., Cornell, H. V., Allen, A.P., Brown, J.M., Bush,
766   M.B., Harrison, S.P., Hurlbert, A.H., Knowlton, N., Lessios, H.A., McCain, C.M.,

McCune, A.R., McDade, L.A., McPeek, M.A., Near, T.J., Price, T.D., Ricklefs, R.E., Roy, K., Sax, D.F., Schluter, D., Sobel, J.M. & Turelli, M. (2007) Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. *Ecology Letters*, **10**, 315–331.

Nevalainen, L., Sarmaja-Korjonen, K. & Luoto, T.P. (2011) Sedimentary Cladocera as indicators of past water-level changes in shallow northern lakes. *Quaternary Research*, **75**, 430–437.

Norris, R.D. (2000) Pelagic species diversity, biogeography, and evolution. *Paleobiology*, **26**, 236–258.

O'Brien, W.J., Barfield, M., Bettez, N.D., Gettel, G.M., Hershey, A.E., McDonald, M.E., Miller, M.C., Mooers, H., Pastor, J., Richards, C. & Schuldt, J. (2004) Physical, chemical, and biotic effects on arctic zooplankton communities and diversity. *Limnology and Oceanography*, **49**, 1250–1261.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E. & Wagner, H. (2019) vegan: Community ecology package R package version 2.5-6.

Olden, J.D., LeRoy Poff, N., Douglas, M.R., Douglas, M.E. & Fausch, K.D. (2004) Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology & Evolution*, **19**, 18–24.

Padial, A.A., Ceschin, F., Declerck, S.A.J., De Meester, L., Bonecker, C.C., Lansac-Tôha, F.A., Rodrigues, L., Rodrigues, L.C., Train, S., Velho, L.F.M. & Bini, L.M. (2014) Dispersal Ability Determines the Role of Environmental, Spatial and Temporal Drivers of Metacommunity Structure. *PLoS ONE*, **9**, 1–8.

Patalas, K., Patalas, J. & Salki, A.G. (1994) Planktonic crustaceans in lakes of Canada (Distribution of species, bibliography). *Canadian Technical Report of Fisheries and Aquatic Sciences*, **1954**, 222.

Pinel-Alloul, B., André, A., Legendre, P., Cardille, J.A., Patalas, K. & Salki, A. (2013) Large-scale geographic patterns of diversity and community structure of pelagic crustacean zooplankton in Canadian lakes. *Global Ecology and Biogeography*, **22**, 784–795.

Post, D., Pace, M. & Hairston, N. (2000) Ecosystem size determines food-chain length in lakes. *nature.com*, **405**, 1047–1049.

Quinlan, R., Leavitt, P.R., Dixit, A.S., Hall, R.I. & Smol, J.P. (2002) Landscape effects of climate, agriculture, and urbanization on benthic invertebrate communities of Canadian prairie lakes. *Limnol. Oceanogr*, **47**, 378–391.

RCoreTeam (2020) R: A language and environment for statistical computing.

da Silva, P.G., Bogoni, J.A. & Heino, J. (2020) *Can taxonomic and functional metrics explain variation in the ecological uniqueness of ecologically-associated animal groups in a modified rainforest?*, Elsevier B.V.

Smith, J.R., Hendershot, J.N., Nova, N. & Daily, G.C. (2020) The biogeography of ecoregions: Descriptive power across regions and taxa. *Journal of Biogeography*, **47**, 1413–1426.

Sodré, E.D.O., Langlais-Bourassa, A., Pollard, A.I. & Beisner, B.E. (2020) Functional and taxonomic biogeography of phytoplankton and zooplankton communities in relation to environmental variation across the contiguous USA. *Journal of Plankton Research*, **42**, 141–157.

813 Spasojevic, M.J., Copeland, S. & Suding, K.N. (2014) Using functional diversity patterns
814   to explore metacommunity dynamics: A framework for understanding local and
815   regional influences on community structure. *Ecography*, **37**, 939–949.
816 Stoch, F., Vagaggini, D. & Margaritora, F.G. (2019) Macroecological and spatial patterns
817   in the distribution of cladocerans in Alpine lakes. *Limnetica*, **38**, 119–136.
818 Stomp, M., Huisman, J., Mittelbach, G.G., Litchman, E. & Klausmeier, C.A. (2011)
819   Large-scale biodiversity patterns in freshwater phytoplankton. *Ecology*, **92**, 2096–
820   2107.
821 Taranu, Z.E., Köster, D., Hall, R.I., Charette, T., Forrest, F., Cwynar, L.C. & Gregory-
822   Eaves, I. (2010) Contrasting responses of dimictic and polymictic lakes to
823   environmental change: A spatial and temporal study. *Aquatic Sciences*, **72**, 97–115.
824 Vellend, M., Baeten, L., Myers-Smith, I.H., Elmendorf, S.C., Beauséjour, R., Brown,
825   C.D., De Frenne, P., Verheyen, K. & Wipf, S. (2013) Global meta-analysis reveals
826   no net change in local-scale plant biodiversity over time. *Proceedings of the*
827   *National Academy of Sciences of the United States of America*, **110**, 19456–9.
828 Villéger, S., Grenouillet, G. & Brosse, S. (2013) Decomposing functional β-diversity
829   reveals that low functional β-diversity is driven by low functional turnover in
830   European fish assemblages. *Global Ecology and Biogeography*, **22**, 671–681.
831 Villéger, S., Mason, N.W.H. & Mouillot, D. (2008) New multidimensional functional
832   diversity indices for a multifaceted framework in functional ecology. *Ecology*, **89**,
833   2290–2301.
834 Vogt, R.J., Peres-Neto, P.R. & Beisner, B. (2013) Using functional traits to investigate
835   the determinants of crustacean zooplankton community structure Trait-base
836   approaches View project Functional species diversity and ecosystem function View
837   project.
838 Vyverman, W., Verleyen, E., Sabbe, K., Vanhoutte, K., Sterken, M., Hodgson, D.A.,
839   Mann, D.G., Juggins, S., Vijver, B. Van de, Jones, V., Flower, R., Roberts, D.,
840   Chepurnov, V.A., Kilroy, C., Vanormelingen, P. & Wever, A. De (2007) Historical
841   processes constrain patterns in global diatom diversity. *Ecology*, **88**, 1924–1931.
842 Warren, F. & Lemmen, D. (2014) *Canada in a Changing Climate: Sector Perspectives*
843   *on Impacts and Adaptation*,.
844 Whittaker, R.H. (1972) Evolution and Measurement of Species Diversity. *Taxon*, **21**,
845   213–251.
846 Wiken, E.B. (1986) *Terrestrial EcoZones of Canada*, Hull.
847 Winegardner, A.K., Legendre, P., Beisner, B.E. & Gregory-Eaves, I. (2017) Diatom
848   diversity patterns over the past c. 150 years across the conterminous United States of
849   America: Identifying mechanisms behind beta diversity. *Global Ecology and*
850   *Biogeography*, **26**, 1303–1315.
851 Wright, D.H. (1983) Species-energy theory: an extension of species-area theory. *Oikos*,
852   **41**, 496–506.
853 Yan, N.D., Somers, K.M., Girard, R.E., Paterson, A.M., Keller, W., Ramcharan, C.W.,
854   Rusak, J.A., Ingram, R., Morgan, G.E. & Gunn, J.M. (2008) Long-term trends in
855   zooplankton of Dorset, Ontario, lakes: the probable interactive effects of changes in
856   pH, total phosphorus, dissolved organic carbon, and predators. *Canadian Journal of*
857   *Fisheries and Aquatic Sciences*, **65**, 862–877.
858 Yan, N.D. & Strus, R. (1980) Crustacean Zooplankton Communities of Acidic, Metal-

859   Contaminated Lakes Near Sudbury, Ontario. *Canadian Journal of Fisheries and*
860   *Aquatic Sciences*, **37**, 2282–2293.
861 Zeileis, A., Cribari-Neto, F., Gruen, B., Kosmidis, I., Simas, A.B. & Rocha, A. V. (2020)
862   betareg: Beta Regression. R package version 3.1-3.
863
864

865   DATA ACCESSIBILITY

866   The public LakePulse database will contain data collected over three summers (2017-

867   2019) in 664 lakes. Over 100 variables (including zooplankton communities) were

868   sampled in each lake. All the data will eventually be publicly available:

869   https://lakepulse.ca/national-lake-pulse-database/. Zooplankton data will be made

870   publicly available once CP's thesis final publications are published.

871 Table 1. Percentage of correct allocation, p-value and random-corrected percentage from
872   the species and trait CAP analyses by ecozone (a) and continental basin (b) of
873   crustacean zooplankton in Canadian lakes. Regions are listed from west to east. The
874   largest values in each set are highlighted in bold.

875

a) CAP by ecozone	Taxonomic % correct attribution	Functional % correct attribution
All	46.3	29.0
Boreal Cordillera	**83.3**	**76.7**
Taiga Plains	20.0	8.0
Pacific Maritime	52.2	33.3
Montane Cordillera	62.7	50.8
Semi-Arid Plateaux	36.4	0.00
Boreal Plains	43.5	11.6
Prairies	49.3	25.4
Boreal Shield	25.9	40.0
Mixedwood Plains	54.7	22.6
Atlantic Highlands	23.8	7.9
Atlantic Maritime	63.5	38.1
p-value	<2.2e-16	<2.2e-16
Adjusted % predicted	36.9	19.9

876

b) CAP by continental basin	taxonomic% correct attribution	Functional % correct attribution
All	61.1	42.0
Pacific Ocean	**77.1**	**59.6**
Arctic Ocean	37.9	22.7
Hudson Bay	60.6	23.2
Great Lakes-St. Lawrence	56.6	52.0
Atlantic Ocean	53.1	32.1
p-value	<2.2e-16	<2.2e-16
Adjusted %	41.1	22.0

878
879
880
881
882
883   Table 2. Results of beta regression analyses using the two first PCoA axis of functional
884   traits as predictors of SCBD of crustacean zooplankton in Canadian lakes.
885
886

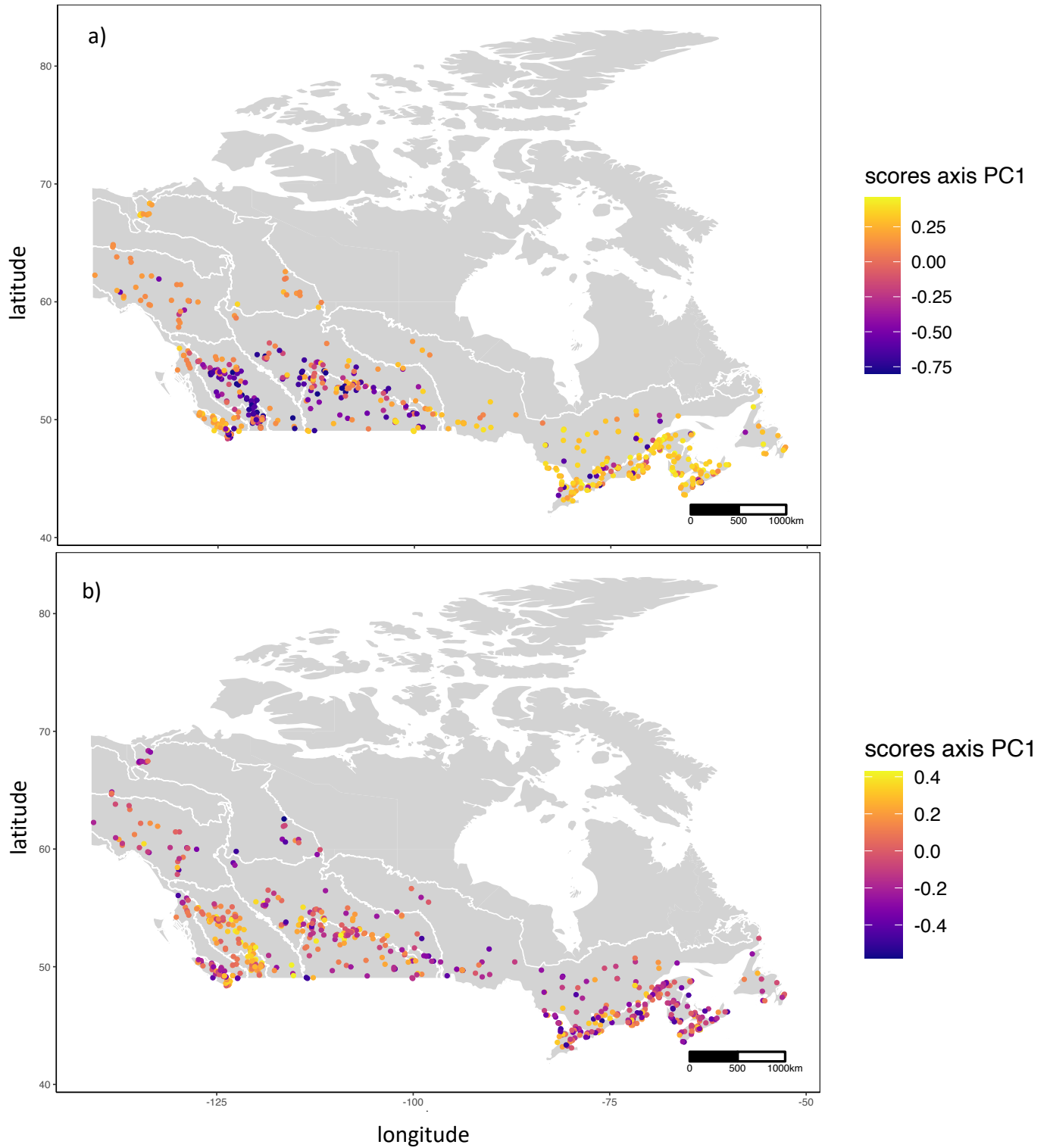|	Estimate	SE	z	p	*	Model Pseudo-R2
(Intercept)	-4.71	0.16	-30.19	< 2e-16	***	-
PCoA 1	1.87	0.39	4.75	2.03e-06	***	-
PCoA 2	0.98	0.49	1.98	0.05	*	0.42
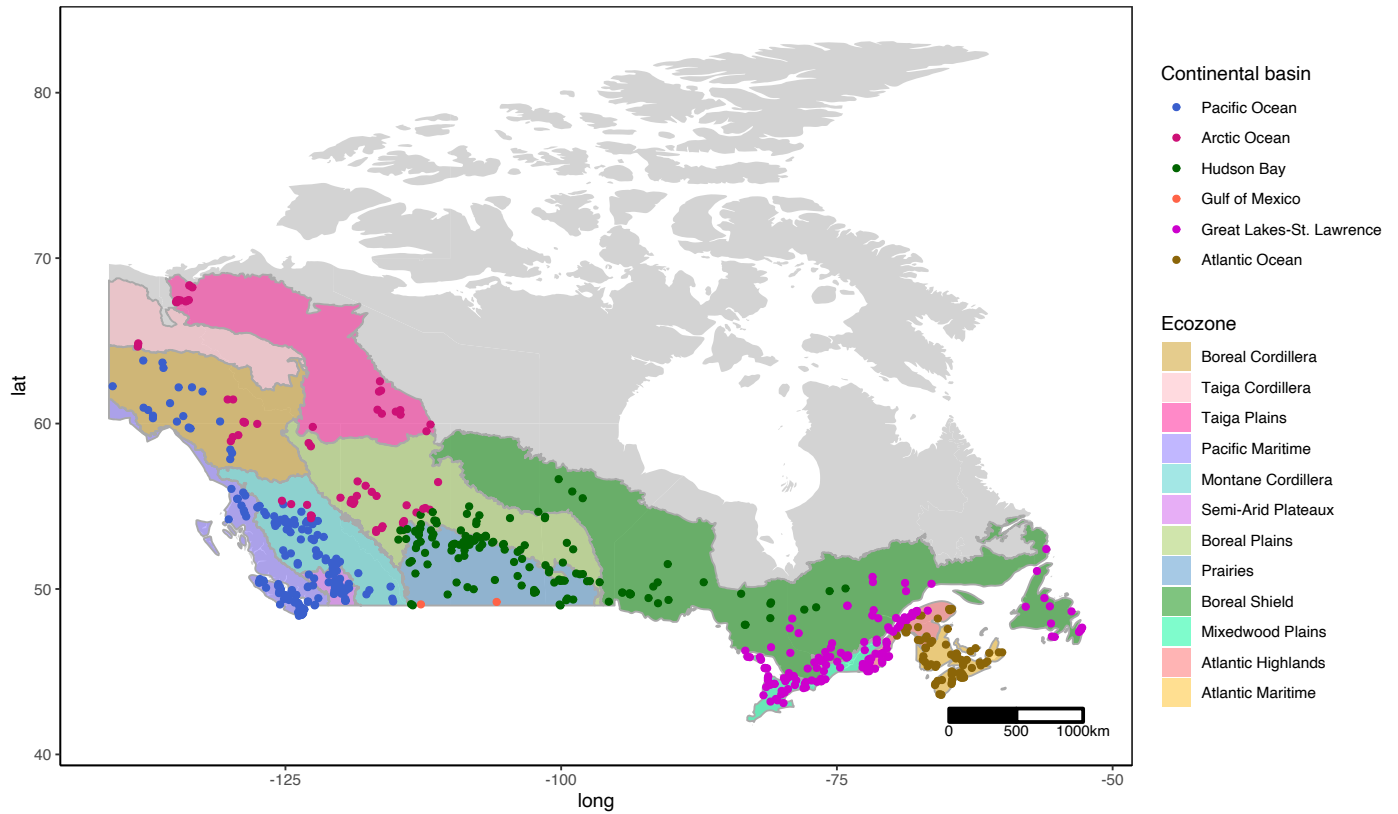
887   **Figure 1**. Map of lakes sampled as part of the NSERC Canadian LakePulse Network for
888   pelagic zooplankton (n=624). Lakes are contoured by ecozone and colored by
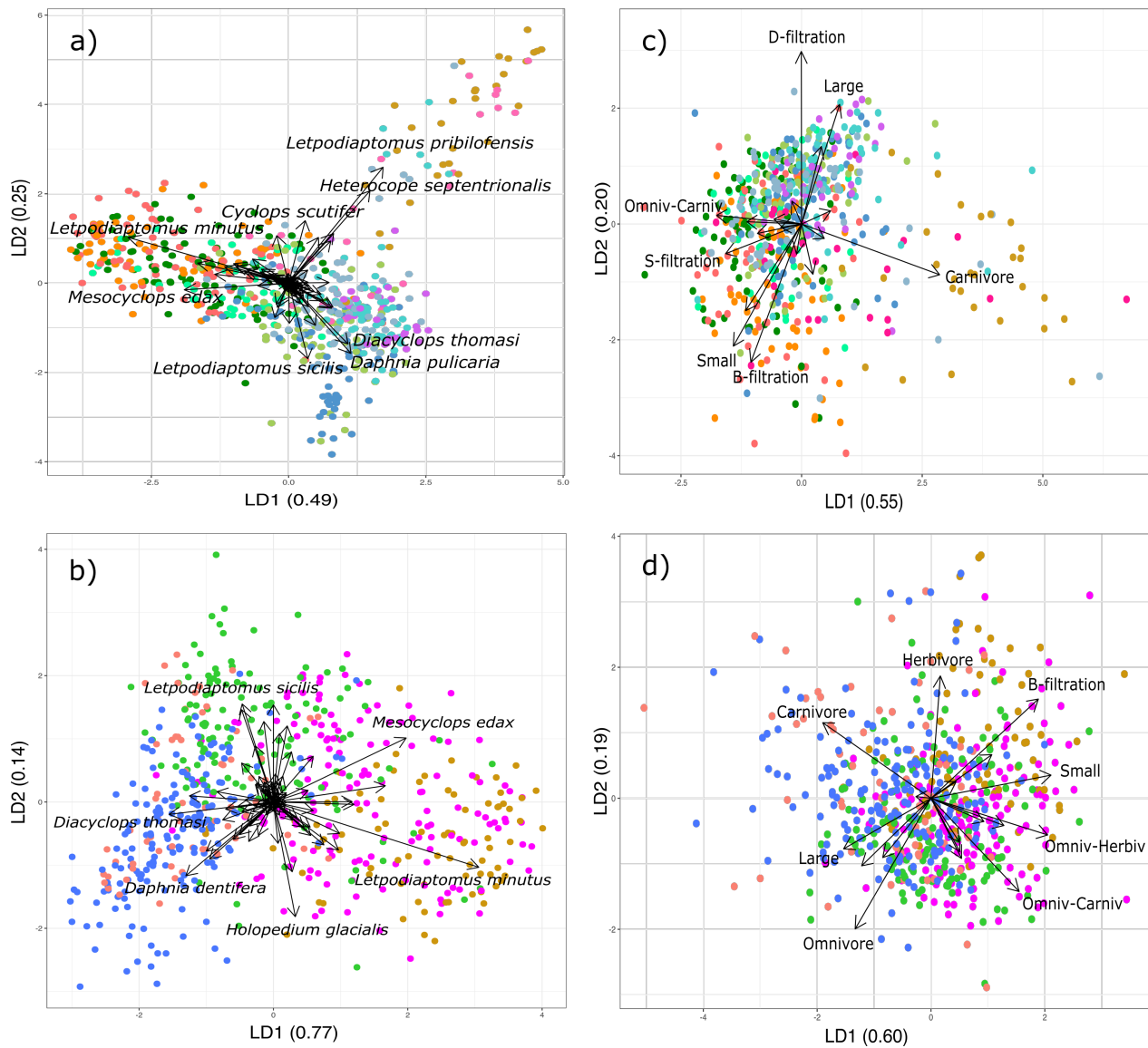889   continental basin. Regions are listed from west to east.
890
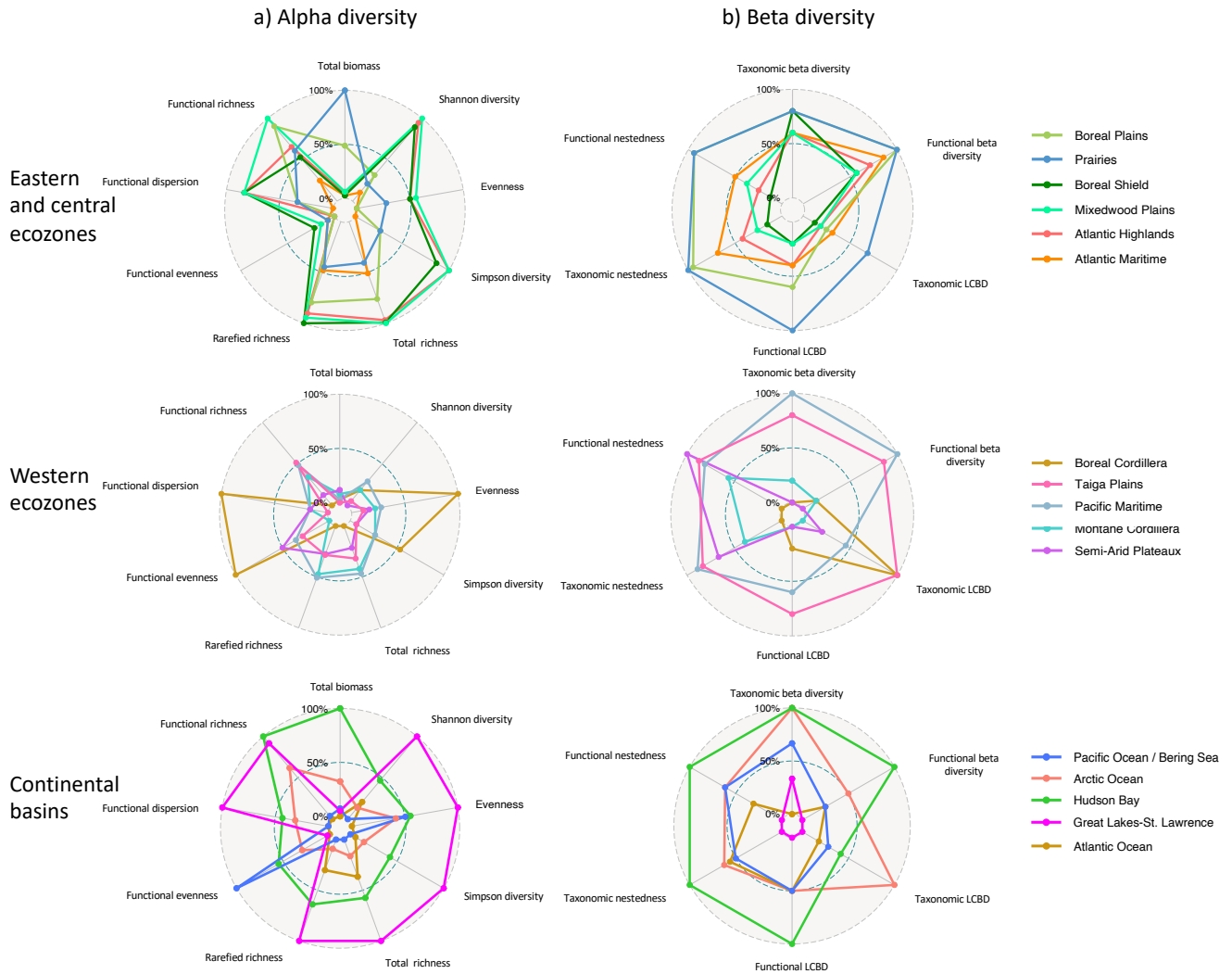
891
892 **Figure 2.** Radar charts showing relative values (rescaled from 0 to 100%) of total
893 biomass and mean values of alpha (a) and beta (b) diversity by ecozone of crustacean
894 zooplankton from 624 Canadian lakes (western and central/eastern ecozones illustrated
895 separately; although the rescaling was done on the full ecozone dataset) and continental
896 basin. LCBD refers to local contribution to beta diversity. Nestedness values are
897 relativized to total beta diversity (nestedness + replacement components).
898
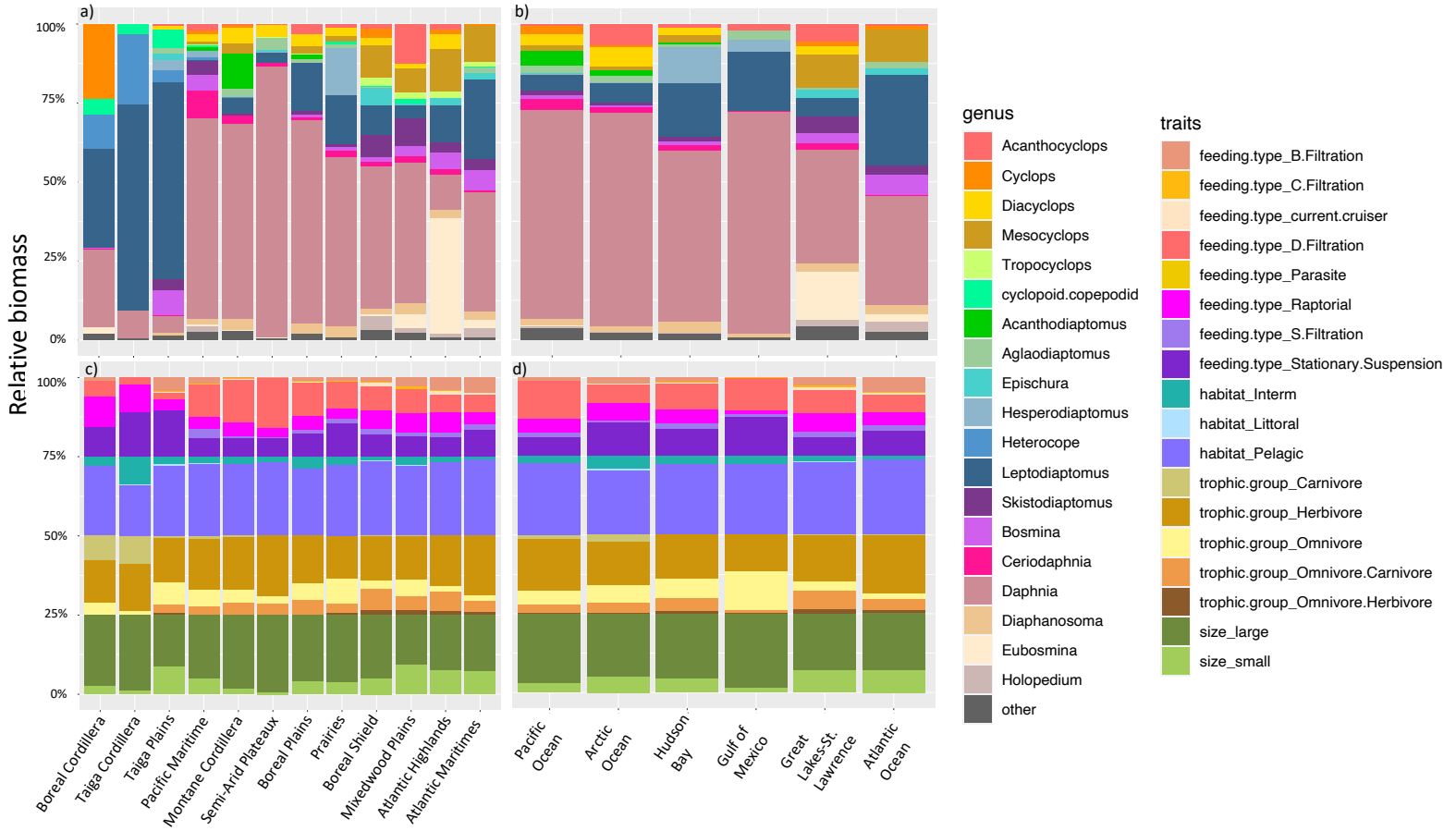
899 **Figure 3**. Canadian zooplankton genera (a,b) and functional traits (c,d) relative biomass
900 by ecozone and continental basin. Rare genera (relative biomass <2% in at least one
901 ecozone or continental basin) are grouped in "other".
902

scores axis PC1

0.25
0.00
-0.25
-0.50
-0.75

latitude

scores axis PC1

0.4
0.2
0.0
-0.2
-0.4

latitude

longitude

is (PCoA)
l traits (b)

0   500   1000km

Figure 5. Canonical Analysis of Principal Coordinates based on Discriminant Analysis (CAP, Scaling = 1) assessing the distinctness of Canadian zooplankton composition (a,b) and functional traits (c,d) in the different ecozones and continental basins. The proportion of the among-group variance explained is displayed by each of the linear discriminant axes.
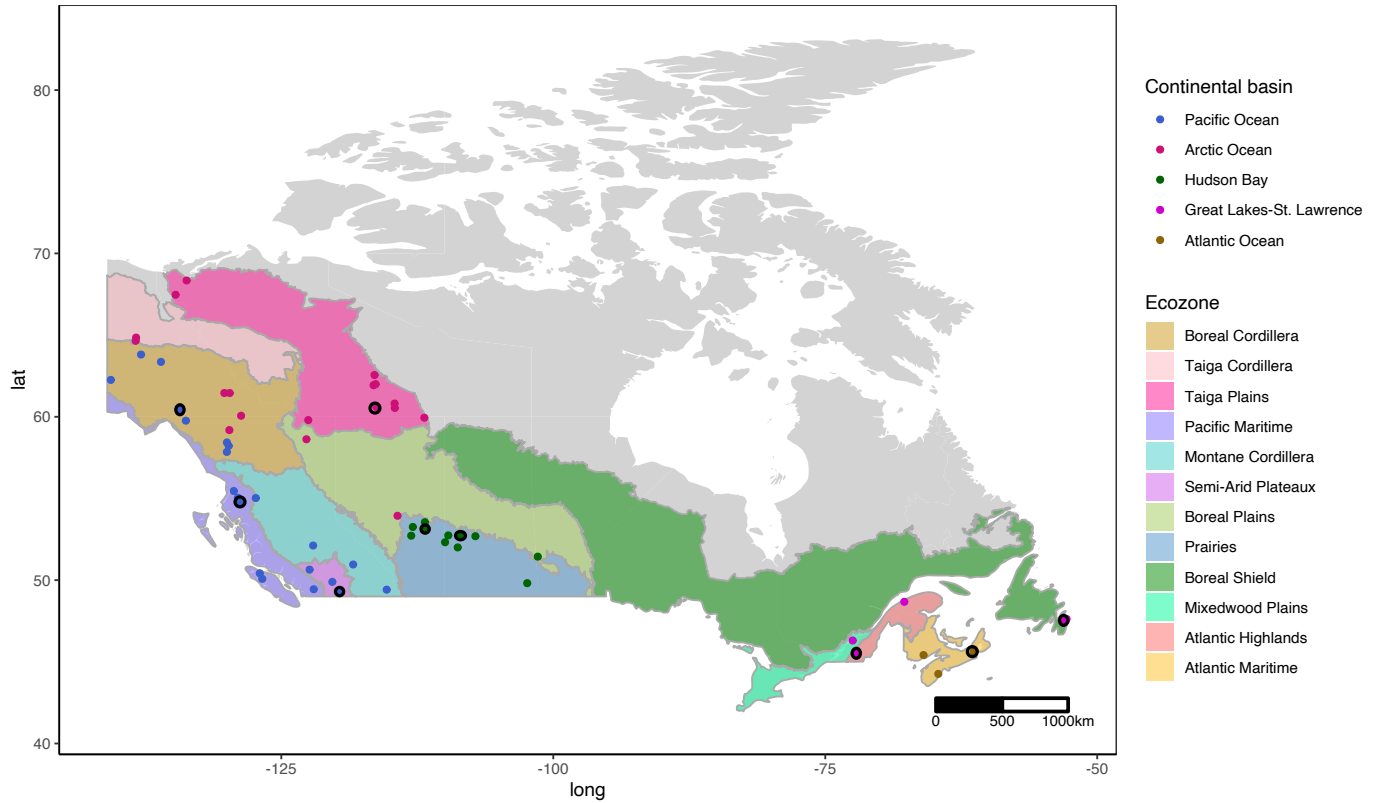
Figure 6. Map of Canadian lakes with significant LCBD values (n=62) for crustacean zooplankton. Sites are contoured by ecozone and colored by continental basin. After correcting for multiple comparisons, no sites remained significant at $p<0.05$; although the 9 sites circled in black were borderline significant ($p=0.06$).