1   *Article title:* Taxonomic and functional differences between winter and summer crustacean

2   zooplankton communities in lakes across a trophic gradient

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Abstract

Despite increasing interest in winter limnology, few studies have examined under-ice
zooplankton communities and the factors shaping them in different types of temperate lakes. To
better understand drivers of zooplankton community structure in winter and summer, we
sampled thirteen lakes across a large trophic status gradient for crustacean zooplankton
abundance, taxonomic and functional community composition, and C/N stable isotopes. Average
winter zooplankton densities were one-third of summer densities across the study lakes.
Proportionally, cladocerans were more abundant in summer than winter, with the opposite
pattern for calanoids and cyclopoids. In green (eutrophic) lakes, zooplankton densities were
higher under the ice than in brown (dystrophic) and blue (oligotrophic) lakes, suggesting better
conditions for zooplankton in productive lakes during the winter. Overall, zooplankton
communities were more similar across lakes under the ice than during the open water season.
Feeding group classification showed a decrease in herbivore abundance and an increase in
predators from summer to winter. C/N stable isotope results suggested higher lipid content in
overwintering zooplankton and potentially increased reliance on the microbial loop by winter
zooplankton. Our results show substantial variation in the seasonality of zooplankton
communities in different lake types and identify some of the factors responsible for this
variation.

Introduction

Increasing air temperatures associated with climate change are leading to a decrease in ice-
cover extent and duration on many lakes (O'Reilly *et al.*, 2015; Sharma *et al.*, 2019). The
decreasing lake ice cover significantly affects lake heat budgets, mixing regimes, light
availability, and various chemical and biological processes, both during winter and in the
following open-water seasons (Salonen *et al.*, 2009; Ye *et al.*, 2019; Ozersky *et al.*, 2021).
Additionally, changes in the timing of lake freezing and thawing may impact the life cycle of
many aquatic species, including effects on timing of growth, reproduction and feeding behavior
(Winder and Schindler, 2004; Stine *et al.*, 2009).

Predicting how lake ecosystems will respond to changes in winter conditions is difficult
because, historically, the ice-covered period has received much less attention from ecologists
than the open water period (Hampton *et al.*, 2017). Until recently, it was often assumed that

55   winter is a time of dormancy and that winter processes have little effect on the open water period

56   (Sommer *et al.*, 1986). However, previous studies suggest that life under the ice is active and that

57   winter conditions can be important for regulating phytoplankton and zooplankton biomass and

58   species composition in subsequent seasons (Weyhenmeyer *et al.*, 1999; Adrian *et al.*, 2006;

59   Salonen *et al.*, 2009; Bertilsson *et al.*, 2013; Bruesewitz *et al.*, 2015; Hampton *et al.*, 2017;

60   Wollrab *et al.*, 2021). Given that more than half of the lakes around the globe are covered with

61   ice during the winter (Weyhenmeyer *et al.*, 2011) and the rapid shortening of the ice-cover

62   period (Magnuson *et al.*, 2000; Blank *et al.*, 2009; Benson *et al.*, 2012), a better understanding of

63   winter lake ecology is emerging as an important research priority.

64   Zooplankton are a key component in lake food webs and play an important role in cycling

65   of nutrients and organic matter via grazing on phytoplankton. They are also an important food

66   source for higher trophic levels. Open-water zooplankton abundance and community

67   composition fluctuates seasonally depending on physical factors (temperature), as well as

68   bottom-up (food availability) and top-down (predation) biological controls (Sommer *et al.*,

69   2012). Seasonal open-water studies have shown that zooplankton community succession is

70   influenced by lake trophic status and differs between eutrophic and oligotrophic lakes (Sommer

71   *et al.*, 1986; Vanni and Temte, 1990; Sommer *et al.*, 2012). However, relatively little is known

72   about winter zooplankton communities, how they interact with lower and higher trophic levels

73   and how lake trophic status affects winter zooplankton communities.

74   Lake trophic status may be broadly characterized using the lake color paradigm, which

75   classifies lakes by water color based on concentrations of colored dissolved organic matter

76   (CDOM) and total phosphorus (TP) into brown (dystrophic, high CDOM and low TP), green

77   (eutrophic, low CDOM and high TP), and blue (oligotrophic, low CDOM and low TP) lakes

78   (Williamson *et al.*, 1999; Webster *et al.*, 2008; Leech *et al.*, 2018). Bottom-up and top-down

79   interactions between zooplankton, their food sources, and visual predators vary along both

80   CDOM and primary productivity gradients (Vinyard and O'Brien, 1976; Benndorf *et al.*, 2002;

81   Wissel *et al.*, 2003; Yuan and Pollard, 2018). For example, Leech *et al.* (2018) showed that

82   zooplankton biomass and community structure differ significantly between blue, brown, green

83   and murky (high CDOM and TP) lakes with highest zooplankton abundance in green and murky

84   lakes. Overall, the lake color paradigm offers a more holistic means of categorizing the lake

85   environment for plankton communities and exploring zooplankton seasonal dynamic in diverse

86   lake types beyond the more traditional oligotrophic/eutrophic classification.

87   Here, we examined seasonal patterns of the abundance, community structure, and aspects

88   of feeding ecology of crustacean zooplankton in thirteen seasonally-frozen north temperate lakes

89   during summer and winter. Study lakes varied in trophic status and represented various color

90   categories, including three green lakes, six blue lakes, and four brown lakes. By comparing

91   winter and summer zooplankton communities in diverse lake types, we sought to better

92   understand plankton seasonal dynamics under different environmental conditions. Our main

93   questions were: 1) how do environmental factors that may affect zooplankton communities differ

94   within and between lakes in winter compared to summer; 2) how does zooplankton community

95   composition, abundance and trophic structure vary between winter and summer in different lake

96   types; and 3) to what extent do environmental factors in different lake types shape the

97   zooplankton community in summer and winter seasons?

98

99   **Methods**

100   ***Study sites***

101   Thirteen lakes (fourteen stations) in Minnesota and Wisconsin (Fig. 1, Table 1) were

102   sampled during the ice cover season in late winter and again during the summer stratified season.

103   According to Minnesota Department of Natural Resources data (MN DNR,

104   https://www.dnr.state.mn.us/lakefind/index.html), ice cover in the study region formed at the end

105   of November and lasted until the end of April/beginning of May during the study years (2015

106   and 2018). We sampled lakes during academic spring breaks in 2015 (ten locations between

107   March 16 and March 20) and 2018 (four locations between February 27 and March 1) when

108   more time was available for planning and conducting research and when lakes ice was safe to

109   operate on. Summer samples were collected in mid-summer, between July 8 and July 23 (ten

110   locations in 2015 and 4 locations in 2018) when all lakes were stratified. Lakes were chosen to

111   represent a range of physical, chemical and biological conditions in order to assess how winter

112   and summer conditions differ in diverse north temperate lakes. The two Lake Superior stations

113   included a moderate depth (47-m) site in the Apostle Islands region and a shallower (8-m)

114   location in Chequamegon Bay (Fig. 1). Lakes LaSalle and Portsmouth are meromictic lakes with

115   anoxic conditions below ca. 40 m depth. We categorized study sites according to the lake total

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116   phosphorus, dissolved organic carbon, and chlorophyll *a* concentrations into blue (oligotrophic),

117   green (eutrophic) and brown (dystrophic) lakes (Williamson *et al*., 1999; Webster *et al*., 2008;

118   Leech *et al*., 2018). Lakes in our study with integrated values of TP concentrations ≤ 1 μM L^{-1}

119   (except for Portsmouth Lake), DOC ≤10 mg L^{-1}, and chl *a* ≤5 μg L^{-1} were assigned to blue

120   (oligotrophic) lakes. Meromictic Portsmouth Lake had integrated TP concentration of 1.2 μM L^{-1}

121   due to elevated TP values in monimolimnion. Brown lakes had integrated values for TP >0.5 but

122   ≤1.3 μM L^{-1}, DOC >10 mg L^{-1}, and chl *a* ≤10 μg L^{-1}. Green lakes had TP concentrations ≥1.3

123   μM L^{-1}, DOC ≤20 mg L^{-1}, and chl *a* >10 μg L^{-1} (Table 1).

124   ***Sample collection***

125   Sampling was conducted from the surface of the ice in February and March and from a

126   small boat during the ice-free period in July. During both seasons, we collected water column

127   temperature, dissolved oxygen, pH, total dissolved solids, fluorescent dissolved organic matter,

128   and conductivity profiles (Shchapov *et al.,* 2021) using a YSI EXO2 multiparameter sonde (YSI

129   In., Yellow Spring, OH, USA), water samples from different depths for chemical analyses and

130   zooplankton samples for determination of community composition and stable isotopes

131   (δ^{13}C/δ^{15}N) analysis (SIA).

132   Several physical characteristics were measured during winter and summer periods. During

133   the ice cover period, we visually estimated the percentage of snow cover on the ice. Average

134   snow depth was determined from measurements of 5 locations within several meters from the

135   sampling site. The thickness and properties of ice (layering) were recorded as well. Light

136   attenuation through snow, ice and water was measured with either a LI-COR probe equipped

137   with a quantum LI-192 cosine sensor (LI-COR Biosciences., NE, Lincoln, USA) or a

138   submersible hyperspectral irradiance sensor (TriOS Ramses, Rastede, Germany). Light

139   attenuation of ice was measured by paired measurements in air and by submersing the light

140   sensor through the hole in the ice and placing it as close as possible to the underside of the ice. If

141   any snow was present, measurements were repeated after carefully removing approximately 1 m^2

142   of snow. Light attenuation in the water column was determined from measurements of light

143   levels at resolution of 0.5 m from water surface to depths of 5-10 m. The hole in the ice was

144   covered by an opaque black plastic sheet to avoid light contamination during winter water

145   column light attenuation measurements. We calculated the euphotic depth for each sampling

146   location and period while accounting for light attenuation (Table 2) by the water column as well

147   as by snow and ice cover.

148   Water samples were collected for chl *a*, total phosphorus (TP), dissolved organic carbon

149   (DOC), and seston $\delta^{13}C$/ $\delta^{15}N$ SIA with a 3.7 L Van Dorn water sampler at several discrete

150   depths in each lake (Table 1). At minimum, water was collected at lake surface (or immediately

151   under the ice in winter) and 0.5 m above lake bottom. Water was collected into 2 L acid-washed

152   bottles and stored in the dark until return to the lab for analyses.

153   Crustacean zooplankton samples were collected using zooplankton net tows (0.5 m mouth

154   diameter, 64 μm mesh size, 1.5 m length), from 1.5 m above the lake bottom to the surface to

155   determine the total abundance of zooplankton. We did not use flow meters on our nets, which

156   could result in underestimates of zooplankton abundance due to net clogging and reduced net

157   efficiency. Potential net clogging due to high biomass of phytoplankton can be a problem

158   especially in deeper humic and eutrophic lakes (Mack *et al*., 2012). However, we do not believe

159   net clogging to be a major issue in our study due to relatively shallow depths of productive and

160   humic lakes (Table 1), and the high ratio (>4:1) of the net surface area to the net mouth area

161   (Gannon, 1980). One sample from each sampling date was fixed with 90% ethyl alcohol upon

162   collection and then transferred to 70% ethyl alcohol for storage until taxonomic identification

163   and counting. A second zooplankton sample was cleaned from algae and debris and kept alive

164   for ~2 hours in filtered lake water following collection to allow gut clearance; these zooplankton

165   were then frozen for later determination of bulk C and N content and C/N stable isotope

166   composition.

167   ***Lab Analyses***

168   Dissolved Organic Carbon (DOC) samples were filtered through pre-combusted Whatman

169   GF/F filters into pre-combusted 40 ml amber glass vials. Concentrations of DOC were

170   determined using a Shimadzu TOC-V autoanalyzer (Shimadzu Co., Kyoto, Japan). TP was

171   determined using a potassium persulfate digestion method to convert phosphorus to

172   orthophosphate (Murphy and Riley, 1962; Wetzel and Likens, 1991). Samples were then

173   analyzed using a SEAL Analytical AQ400 autoanalyzer with US EPA119-A method for TP

174   (Murphy and Riley, 1962). Phytoplankton abundance was estimated as chl *a* concentration. Chl *a*

175   was filtered onto 0.2 μm nitrocellulose filters and extracted into 90% acetone solution

176   (Welschmeyer, 1994). After an 18-hour extraction period in the dark, extracts were analyzed

177   using a Turner Designs 10-AU fluorometer (Turner Design, Sunnyvale, CA) using an excitation

178   wavelength of 436 nm and emission of 680 nm.

179   Seston samples from different depths were filtered onto pre-combusted Whatman GF/F

180   filters and frozen at -20°C. Afterward, filters were dried overnight and rolled in tin capsules for

181   determination of bulk C and N content and C/N stable isotope composition. Bulk zooplankton

182   samples for C and N stable isotopes analysis were kept at -20°C. Samples were freeze-dried,

183   thoroughly homogenized and weighed into tin capsules. C/N SIA on seston and zooplankton

184   samples was performed using Elemental Analysis – Isotope Ratio Mass Spectrometry (EA-

185   IRMS) at the Large Lakes Observatory facilities. For both seston and bulk zooplankton SIA

186   analysis we used acetanilide, B-2153 soil, B-2153 soil, caffeine, and RM8548 standards and run

187   them repeatedly after every ten samples. The analytical errors calculated on seston replicates for

188   δ^{13}C and δ^{15}N were 0.36 ‰ and 0.53 ‰, respectively.

189   Preserved zooplankton samples were subsampled using a Stempel pipette and transferred

190   into a Bogorov chamber for counting. We counted and identified samples using an Olympus

191   SZH10 stereoscopic microscope (at 7x-70x magnification). Identification was based on the

192   zooplankton key from Balcer *et al*. (1984) and Haney *et al*. (2013). Subsamples were identified

193   and counted until at least 300 individuals were processed. We did not count or scan the whole

194   sample for rare species due to relatively high zooplankton abundance, especially in samples

195   collected during the summer season. We identified adult copepods and cladocerans to species

196   level. Copepodites were distinguished between calanoids and cyclopoids only. Cladocerans were

197   counted without age stage determinations. Nauplii were not separated by stage or taxonomic

198   groups (cyclopoid vs. calanoid) and are included only in the total abundance analysis. They were

199   excluded from community composition and feeding group analyses due to their high density and

200   inability to separate them into those groups. Additionally, we assigned adult species to three

201   feeding groups: herbivores, omnivores and predators based on information from Balcer *et al*.

202   (1984) and Haney *et al*. (2013). We calculated abundance as individuals per liter (Ind. L^{-1}).

203   Rotifers were present in our samples but were not counted or identified.

204   ***Data analysis***

205   We used the R statistical computing environment (version 3.6.2) for analyses of our data (R

206   Core Team 2017). All graphics were created using the *ggplot2* package (Wickham, 2009).

207   The downwelling attenuation coefficient of PAR (K_D) in water was calculated using the

208   Beer-Lambert Law. K_D of ice ($K_{D\ ICE}$) was calculated following Eq. 1, and where present the K_D

209   of snow ($K_{D\ SNOW}$) was calculated following Eq. 2 where Z_{ICE} and Z_{SNOW} are the depth of ice and

210   snow, respectively (m). To account for large seasonal differences in daily incident irradiance,

211   euphotic depths are not calculated as the depth of 1% surface light but rather the depth where

212   mean daily PAR is 1 μmol m^{-2} s^{-1} which corresponds to the approximate minimum light

213   requirement of phytoplankton (Silsbe *et al.*, 2016). Mean daily PAR in the summer and winter

214   (485 and 90 μmol m^{-2} s^{-1}) was determined from remotely-sensed PAR climatology (MODIS

215   Aqua) for our region (90 – 95°W, 40 – 45°N). Euphotic depths in the summer and winter are

216   calculated using Eq. 3 and 4 respectively.

217   (1) $K_{D\ ICE} = -\log\left(E_{Z\ ICE}/E_0\right) \cdot Z_{ICE}^{-1}$

218   (2) $K_{D\ SNOW} = \left[-\log(E_{Z\ ICE}/E_0) + K_{D\ ICE} \cdot Z_{ICE}\right] \cdot Z_{SNOW}^{-1}$

219   (3) $Summer\ Z_{EU} = -\log\left(1/485\right) \cdot K_D^{-1}$

220   (4) $Winter\ Z_{EU} = -\log\left(1/(90 \cdot exp^{-K_{D\ ICE} \cdot Z_{ICE} - K_{D\ SNOW} \cdot Z_{SNOW}})\right) \cdot K_D^{-1}$

221   We calculated depth-integrated values for selected limnological parameters (chl. *a*, DOC,

222   and TP) using trapezoidal integration (Eq. 5).

223   (5) DIV = $\sum$(c*i* + cb)/2 × (db - d*i*)/td,

224   where DIV is the depth-integrated value for a particular variable, c*i* is the sample

225   concentration value at depth *i*, cb the sample concentration value at depth below depth *i*, d*i* the

226   sample depth *i*, db the sample depth below depth *i*, and td the station total depth.

227   To assess differences between seasons for environmental parameters and zooplankton

228   community characteristics we used paired t-tests with adjusted p-values by using the Holm

229   multiple testing correction. Zooplankton and environmental data were log10 transformed in order

230   to meet the normality and equal variance assumptions for parametric t-tests. Pearson correlation

231   tests were used to investigate the relationship between environmental parameters during winter

232   and summer across all lakes. In order to assess the differences between lake types within or

233   between seasons, we used 90% confidence interval (CI) overlap of log10-transformed values as a

234   criterion of significant difference. We choose to use paired t-test and 90% CIs rather than

235   repeated measures ANOVAs due to small number of observations in lakes grouped by color, the

236 greater simplicity of the former approach and its relative conservativeness (Goldstein and Healy,

237 1995).

238   To visualize differences between winter and summer in terms of zooplankton abundance

239 and species composition, we used nonmetric multidimensional scaling (NMDS) from the *vegan*

240 package in R (Oksanen *et al.*, 2018). Community similarity was calculated using the Bray-Curtis

241 dissimilarity metric (*betadisper* function in vegan), generated from species abundance data.

242 Species abundance data was fourth-root transformed in order to reduce the influence of the most

243 abundant taxa (Clarke and Warwick, 2001). We also used permutational multivariate analysis of

244 variance (PERMANOVA; *adonis* function in vegan) to test for significant differences in

245 zooplankton community composition between winter and summer seasons. In order to identify

246 the species that contributed most to the dissimilarity between seasons, we used similarity

247 percentage analysis (SIMPER; *vegan* package; Oksanen *et al.*, 2018). We set a 40% similarity

248 threshold within groups. Additionally, we examined the relationship between zooplankton

249 species abundance and environmental variables (using *envfit* function in *vegan*) for each season

250 among different lake types.

251

252 **Results**

253 *Environmental variables*

254   Snow, ice, and light conditions varied between seasons across all lakes (Fig. 2a; Table 2).

255 Among sampled lakes, only four had snow on the ice surface during sampling in 2018, with

256 snow depth ranging between 20 and 36 cm. Lakes sampled in 2015 did not have snow

257 accumulation on ice surface due to lower snow amounts in the study region in 2015 compared to

258 2018 (MN DNR, https://www.dnr.state.mn.us/climate/snowmap/index.html). Ice thickness

259 varied between 47 and 67 cm across all study locations. Lake euphotic depths differences

260 between seasons were significant (p=0.0002), with higher values in summer (10.9±7.1 SD m)

261 than in winter (6.6±6 m). Blue lakes, on average, had the highest euphotic depth (16.7±5.2 m) in

262 summer and a relatively high value (11.3±4.5) in winter. Green lakes had low euphotic depths

263 during winter (3±1.8 m) with about 2 m change between seasons. Brown lake euphotic depths

264 were substantially lower in winter (0.8±1.1 m) compared to summer (5.1±3.2 m). Light

265 attenuation results (Table 2) among lakes showed that snow and ice absorb and reflect most of

266 the incoming light in winter. This is especially evident for lakes sampled during winter 2018,

267   when ice was covered with snow. We found that, across all lakes, water column k_d values were

268   significantly higher (df=13, p=0.03) in summer (1.1±1.6) than in winter (1±1.5).

269   Most of the water chemistry parameters were significantly different between winter and

270   summer (Fig. 2b-d). The exception were depth-integrated dissolved organic carbon (DOC)

271   values which were not significantly different between seasons (df=13, p=0.4) with winter values

272   of 16.3±23.4 mg L^{-1} and 12.3±9.3 mg L^{-1} in summer. However, high DOC brown lakes seemed

273   to have larger summer-winter variation in DOC concentrations compared to blue and green lakes

274   (Fig. 2b). The 90% confidence intervals for winter DOC values in brown lakes (10.5–93.3 mg L^{-}

275   ^{1}) showed significant difference to DOC values in blue lakes (2.3-5.5 mg L^{-1}), and no difference

276   with green lakes (5.6-17 mg L^{-1}). Summer DOC 90% CIs indicate similar results to winter (Table

277   S1). Total phosphorus (TP) concentrations were significantly higher (df=13, p=0.03) in summer

278   compared to winter with average values across all lakes of 0.8±0.7 μM L^{-1} in winter and 1.3±1.3

279   μM L^{-1} in summer (Fig. 2c). We found that TP 90% CIs were overlapping across all lake types in

280   winter, while in summer green lakes (1.2-6.9 μM L^{-1}) were slightly overlapping with brown

281   lakes (0.4-1.3 μM L^{-1}) but were significantly different (0.3-1 μM L^{-1}) from blue lakes. Depth-

282   integrated chl a concentrations were significantly lower in the winter compared to the summer

283   (df=13, p=0.03,) with average values of 1.4±1.4 and 9.1±16.4 μg L^{-1}, respectively (Fig. 2d).

284   According to 90% CIs, winter chl a values were only significantly different between green (1.6-

285   4.5 μg L^{-1}) and brown (0-1.6 μg L^{-1}) lake types, but not with blue (0.6-1.9 μg L^{-1}) lakes. In

286   summer, green lakes differed significantly (8.7-100 μg L^{-1}) from brown (0.8-7.1 μg L^{-1}) and blue

287   (0.9-2 μg L^{-1}) lake types. Overall, seasonal differences in chl a concentrations in green and

288   brown lakes were more pronounced than in blue lakes.

289   ***Zooplankton communities and abundance***

290   Total zooplankton and nauplii densities varied between seasons (Fig. 3). Zooplankton

291   density (adult and copepodites) (±SD) was significantly different (df=13, p<0.001) between

292   summer and winter with values of 14.1±14 and 4.6±4.9 Ind L^{-1}, respectively (Fig. 3a). Green

293   lakes on average had the highest total zooplankton density (9.8 Ind L^{-1}) compared to brown (5.3

294   Ind L^{-1}) and blue (1.9 Ind L^{-1}) lakes in winter. In summer zooplankton densities increased across

295   all lakes with the highest density again in green (34.6 Ind L^{-1}), then brown (13.2 Ind L^{-1}), and

296   blue (5.9 Ind L^{-1}) lakes. Winter 90% CIs for total zooplankton densities overlapped and were not

297 significantly different between all lake types (Table S1), while summer CIs showed that green

298 lakes differ significantly (13.5-76.2 Ind L^{-1}) from blue (2.5-8.2 Ind L^{-1}), but not brown (4-27.2

299 Ind L^{-1}) lakes. Nauplii density did not change significantly (df=12, p=0.06) between seasons,

300 with average values 14.3±28.7 Ind L^{-1} in winter and 12.8±11.2 Ind L^{-1} in summer (Fig. 3c). Blue

301 lakes had lower average nauplii densities in winter (2.1±1.9 Ind L^{-1}) than summer (8.4±6.6 Ind L^{-}

302 ^{1}), while green (41.3±56.2 Ind L^{-1}) and brown (12±13.2 Ind L^{-1}) lakes on average had higher

303 nauplii densities under the ice compared to the open-water period (27±15.2 and 9.9±6.8 Ind L^{-1},

304 respectively). For both seasons, we found that nauplii 90% CIs overlapped for all lake types and

305 were not significantly different (Table S1).

306   The absolute and relative abundances of main zooplankton groups changed between

307 seasons and among sampled lakes (Fig. 4). The density of calanoid copepods was not

308 significantly different (df=11, p=0.7) between winter and summer across lakes, averaging

309 1.6±2.7 and 1.8±2.5 Ind L^{-1}, respectively (Fig. 4a). However, the relative abundance of calanoids

310 varied greatly between seasons with relatively more calanoids under the ice (32.6±24.2 %) than

311 in summer (19.7±24.5 %) (Fig. 4d). Cyclopoid average density was not significantly different

312 (df=13, p=0.08) between summer and winter with higher values in summer (3.4±5.1 Ind L^{-1})

313 than in winter (1.4±1.7 Ind L^{-1}) (Fig. 4b). Cyclopoids were relatively more abundant in winter

314 (52.6±24.6 %) compared to summer (32.1±24.3 %) (Fig. 4e). Cladoceran average density was

315 significantly different (df=10, p=0.006) between seasons with higher values in summer (5.2±8.5

316 Ind L^{-1}) compared to winter (0.8±1.4 Ind L^{-1}) (Fig. 4c). The relative abundance of cladocerans

317 also was higher in summer (48.2±32.2 %) than in winter (14.8±13.1 %) (Fig. 4f). Among all

318 lakes, green lakes had the highest relative abundance of cladocerans in winter (20.7±4.8 %). In

319 the summer, the highest cladoceran percentage was in brown lakes (52.8±12.3 %). Our 90% CI

320 analysis for main zooplankton groups densities showed that only cyclopoids in blue lakes during

321 winter were significantly different from brown lakes (Table S1).

322   Across all lakes, we identified 21 distinct species in summer compared to 14 species in

323 winter. Our data showed that the average number of species per lake in winter (4.4±1.7 spp.) was

324 significantly (df=13, p=0.01) lower than in summer (6.6±2.6 spp.) (Fig. 3d). We used NMDS

325 ordination to visualize differences in zooplankton communities in winter and summer across

326 study lakes (Fig. 5). Multivariate homogeneity of group dispersions analysis showed greater

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327   dispersion around the median for summer zooplankton communities (0.43) than for those in

328   winter (0.35) suggesting that zooplankton communities are more similar among lakes in winter

329   than in summer. Ordination of the Bray-Curtis dissimilarity matrix of all zooplankton species

330   revealed that winter and summer communities differed significantly in their composition

331   (permANOVA p=0.0096). Differences between green, brown, and blue lakes were significant as

332   well (permANOVA p<0.001). SIMPER analysis showed that densities of *Daphnia longiremis*

333   (11.4 %), *Bosmina longirostris* (9.8 %), *Leptodiaptomus sicilis* (9.5 %), *Diacyclops thomasi*

334   (8.1%) and *Skistodiaptomus oregonensis* (8.2 %) contributed the most to dissimilarity between

335   winter and summer seasons, together accounting for ~47% of the community difference between

336   seasons. Altogether, on average, these species constituted more than 90% of the total

337   zooplankton abundance across all lakes in winter, while in summer, they contribute about 68% of

338   total abundance. Chl *a*, TP and TN were the strongest environmental covariates with multivariate

339   dissimilarity trends for zooplankton communities in summer and winter (Fig. 5). In summer (Fig

340   5c), chl *a* (R^2=0.49, p=0.0157) concentration and TP (R^2=0.44, p=0.0073) increased in the

341   direction of green lakes. Both vectors also pointed away from *Holopedium gibberum*, *B.*

342   *longirostris* and *Limnocalanus macrurus* and towards *Skistodiaptomus pygmaeus*,

343   *Onychodiaptomus birgei*, *Onychodiaptomus sanguineus*, *Leptodiaptomus siciloides*, *S.*

344   *oregonesis*, *Daphnia pulicaria*, *Daphnia parvula*, *Daphnia retrocurva*, and *Mesocyclops edax*. In

345   winter (Fig. 5b), the community dissimilarities no longer trended with chl *a* or TP but instead

346   gained correlation with TN (R^2=0.58, p=0.0059). The TN vector did not trend with a specific

347   lake type. It appeared to point away from *B. longirostris* and *L. sicilis* and towards *Alona*, *S.*

348   *pygmaeus*, *Acanthocyclops vernalis*, *S. oregonensis*, *Tropocyclops prasinus mexicanus* and *D.*

349   *longiremis*.

350   The absolute densities and relative abundances of different zooplankton feeding groups

351   varied in lakes of different types between winter and summer (Fig. 6). The average density of

352   herbivores was significantly higher in summer than in winter with values in summer equal

353   6.91±8.9 and in winter 2.1±3.6 Ind L^{-1} (Fig. 6a). The relative abundance of herbivores was also

354   higher in summer (67.3±24 %) than in winter (47±24.5 %). Interestingly, in some lakes (Parkers,

355   LaSalle, Side, and Portsmouth) the relative abundance of herbivores was lower in summer

356   compared to winter (Fig. 6b). The average density of predatory zooplankton across all lakes was

357   not significantly different between winter and summer with values 1.21±1.5 and 3.06±5.2 Ind L^{-}

358 [1], respectively (Fig. 6d). However, the relative abundance of predators across all lakes was

359 higher in winter (49.1±22.1 %) compared to summer (29±25.5 %) (Fig. 6e).

360   Seston and zooplankton C and N stable isotope values showed divergent interseasonal

361 patterns (Fig. 7). Depth-integrated seston $\delta^{13}C$ was not significantly different between seasons

362 with average values (±SD) for summer equal -30.3±1.8 ‰ and winter -31.7±2.7 ‰ (Fig. 7a). On

363 the other hand, zooplankton $\delta^{13}C$ values were significantly more depleted during the winter (-

364 36.1±4.4 ‰) than in summer (-31.3±4.6 ‰) (Fig. 7d). Seston $\delta^{15}N$ signatures were significantly

365 more enriched in winter (4.7±4.7 ‰) compared to summer (1.3±2.6 ‰) (Fig. 7b). Average

366 zooplankton $\delta^{15}N$ values followed a similar pattern to seston and also were significantly more

367 enriched in winter (8.8±3.6 ‰) compared to summer (5.3±4.1 ‰) (Fig. 7e). Seston carbon to

368 nitrogen (C/N) ratio did not differ significantly between winter (8.8±2.6) and summer (8.2±2.8),

369 while zooplankton C/N ratio were significantly higher in winter (5.8±1.1) than in summer

370 (3.8±1.4) (Fig. 7c and 7f).

371

372 **Discussion**

373   Our objectives were to compare winter and summer zooplankton communities across

374 diverse north temperate lakes. We found that, across all study lakes, winter zooplankton

375 abundance was approximately one third of the open water period densities (Fig. 3). However,

376 zooplankton were still active under the ice, and high nauplii densities indicate potential

377 reproduction by copepods. We also observed interesting patterns of seasonal change in

378 zooplankton community structure. Zooplankton communities were more similar across our study

379 lakes during winter than in summer (Fig. 5). In addition, we found that calanoid and cyclopoid

380 copepods dominated zooplankton numbers under the ice while cladocerans were dominant in the

381 summer (Fig. 4). Feeding group classification and stable isotope analysis suggested that

382 herbivory was a more important energy pathway in the summer, whereas predation may be more

383 important under the ice. It is important to emphasize that all our study lakes were sampled only

384 once in winter and once in summer. Thus, our results represent a snapshot of late winter and

385 mid-summer conditions for the study lakes and do not consider successional patterns, which may

386 lead to significant intraseasonal changes in zooplankton communities (e.g., Sommer *et al.* 2012),

387 but are poorly understood for the under-ice period. Although limited to one sampling date in

388 each season, our study offers new information about zooplankton abundance, community

389 composition, and trophic structure under the ice across diverse northern lakes, a topic rarely

390 represented in the limnological literature.

391 ***Large seasonal variations in environmental conditions***

392   Our results show large winter to summer changes in environmental parameters relevant to

393 zooplankton, as well as differences in how winter-summer conditions change in lakes of different

394 trophic status (Fig. 2). In all our study lakes, euphotic depth was lower in winter than in summer,

395 mainly due to presence of ice and snow cover (Fig. 2a). However, water column light attenuation

396 coefficients show that the water columns of study lakes were clearer in winter than summer

397 (Table 2). Ice characteristics and the presence of snow can have large impacts on light conditions

398 in the water column beneath. For example, snow-covered (<10 cm) ice with impurities and gas

399 bubbles can reduce PAR to less than 10% of surface values, and snow deeper than 13.5 cm has

400 been found to reduce light below levels required for phytoplankton growth (Bolsenga and

401 Vanderploeg 1992; Leppäranta, 2014; Pernica *et al.*, 2017). In the four study lakes that had

402 appreciable snow cover (range 20-36 cm), ice and snow cover substantially reduced light

403 penetration (to 2.4-3% of surface irradiance immediately under the ice). The total amount of

404 solar radiation reaching Earth's surface at the latitude of our study is 2-fold higher in July than in

405 March, further impacting the light environment in lakes during winter. Thus, lower light

406 conditions in winter can reduce phytoplankton biomass, contributing to the lower zooplankton

407 abundances and changes in trophic structure that we observed in winter (see next sections).

408 However, in the absence of deep snow, light levels can still be relatively high during winter,

409 supporting winter-period primary production. According to Gosselin *et al.* (1985), sea algae

410 showed photosynthetic activity at light level of 7.6 μmol m^{-2} s^{-1} and algae biomass increased at

411 light intensity of >20 μmol m^{-2} s^{-1}. In our study, 11 out of 14 sampling locations had winter light

412 intensities equal to or above those thresholds.

413   Along with light, phosphorus availability is important for ecosystem productivity as this

414 element can be a limiting factor for phytoplankton and, consequently, zooplankton production

415 (Hanson and Peters 1984; Schindler *et al.*, 2016). Overall, depth-integrated TP values were

416 higher in summer than during the ice-covered period across most study lakes (Fig. 2c), likely due

417 to lower concentrations of particulate P (in phytoplankton and suspended detritus). Lakes that

418 had higher TP concentrations in summer (green lakes) also had higher TP concentrations in

419   winter, which might be due to high concentartions of inorganic P, indicating their greater

420   potential to support winter phytoplankton production if light is not limiting.

421   Chl *a* concentrations (Fig. 2d), used as a proxy for phytoplankton biomass, were almost

422   universally lower in winter compared to summer (with exception of large, shallow, oligo-

423   mesotrophic Lake Mille Lacs). The lower values of chl *a* during the ice-cover period are likely

424   due to reduced light conditions, low temperatures, and potentially high grazing pressure by

425   zooplankton and low rates of water column mixing, which reduce nutrient replenishment to the

426   upper, illuminated water layer of ice covered lakes (Bengtsson, 2011; Bertilsson *et al.*, 2013).

427   While chl *a* concentrations were lower in winter (1.4±1.4 µg L^{-1}) than summer (9.1±16.4 µg L^{-1})

428   in all but one lake, green lakes retained relatively higher chl *a* concentrations in winter (2.7±0.7

429   µg L^{-1}) compared to other lake types. The observed, moderately high chl *a* values in productive

430   lakes during the ice-covered period suggest that nutrient limitation of phytoplankton may still be

431   important during winter, despite reduced light availability. Overall, these results show that more

432   productive lakes in summer also have higher phytoplankton biomass in winter. Hampton *et al.*

433   (2017) found a similar pattern, with a strong correlation between winter and summer chl *a*

434   concentrations across 101 lakes. Interestingly, in addition to having higher winter chl *a*

435   concentrations, green lakes also showed the greatest cumulative average change in chl *a* between

436   winter and summer (92.1% change), followed by brown lakes (90.1% change). In contrast,

437   relatively little change in chl *a* concentrations occurred between seasons in blue lakes (0.3%

438   change). The relative stability of phytoplankton abundance, measured here as chl *a*

439   concentrations, in blue lakes may potentially be explained by persistent year-round nutrient

440   limitation, or higher top-down pressure from wintertime zooplankton grazing.

441   Although pelagic primary consumers mainly depend on phytoplankton production (Hessen

442   *et al.*, 1989; Bern, 1994; Brett *et al.*, 2009), zooplankton can also utilize other energy sources

443   such as terrestrial DOC (Carpenter *et al.*, 2005; Kankaala *et al.*, 2010). Allochthonous C usually

444   is a poor C source for zooplankton due to low nutrition content in organic matter of terrestrial

445   origin (Brett *et al.*, 2009; Galloway *et al.*, 2014). However, poor-quality C can be upgraded by

446   bacterial action and transferred to higher trophic levels through the microbial loop (Bec *et al.*,

447   2003; Martin-Creuzburg *et al.*, 2005; Säwström *et al.*, 2009). The concentrations of DOC in our

448   study were higher in brown lakes during winter (42.1±32.8 mg L^{-1}) compared to summer

449   (21.8±11.8 mg L^{-1}), while in green and blue lakes, DOC was slightly higher in summer than

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450   under the ice (Fig. 2b). Seasonal changes in DOC concentrations can be explained by the lake's

451   size and watershed features, climate conditions, loading rates of allochthonous material along

452   with photodegradation losses (Wetzel, 2001; Pace and Cole, 2002; Vachon *et al.*, 2016; Hampton

453   *et al.*, 2017). The higher concentration of DOC in brown lakes during winter may be due to the

454   combined effects of extensive terrestrial input from the surrounding watershed prior to the ice-

455   cover period (Wetzel, 2001; Raymond and Saiers 2010) and low light conditions under the ice

456   resulting in reduced photodegradation and assimilation of DOC (Farjalla *et al.*, 2009; Vachon *et*

457   *al.*, 2016). The high DOC in brown lakes, where winter phytoplankton concentrations are

458   especially low, may play an important role for overwintering zooplankton through its

459   assimilation and trophic upgrading via the microbial loop (Rautio *et al.*, 2011).

460   ***Seasonal changes in zooplankton abundance and community composition***

461   Total adult and copepodite densities (hereafter referred to as total zooplankton) across all

462   lakes were lower during the ice-cover period than during open water and constituted 32% of

463   summer zooplankton numbers. Other studies also report reduction in zooplankton numbers

464   during the ice-covered period. For instance, Hampton *et. al.*, (2017) showed that, across 36 lakes,

465   winter zooplankton abundance was roughly 25% of summer values and other studies have also

466   demonstrated between 1.2 and 38-fold (9.4-fold on average) decrease in zooplankton densities

467   (Jensen, 2019; Kalinowska *et al.*, 2019; Kalinowska and Karpowicz 2020). In our study, green

468   lakes had the highest average zooplankton density in both seasons, followed by brown, and then

469   blue lakes (Fig. 3a and 3b). As with phytoplankton, the most significant change in average

470   zooplankton density between winter and summer was in green lakes (71.8% reduction), then

471   followed by blue (67.1%) and brown (60.2%) lakes. While winter and summer changes are

472   similar across all lake types, it appears that green (eutrophic) lakes can provide better conditions

473   for zooplankton under the ice. The presence of abundant *Daphnia* spp. (many of which had full

474   guts and eggs in brood pouches) and high number of nauplii in green lakes during winter

475   provides additional support for this. Cladocerans like *Daphnia* spp. typically undergo diapause

476   and are rare in lakes during the ice-covered period because of low temperatures and low

477   phytoplankton biomass. However, in systems with enough food under the ice, *Daphnia* can still

478   be active and reproduce (Tsugeki *et al.*, 2009; Hamrová *et al.*, 2011; Mariash *et al.*, 2017).

479   Nauplii abundance, which is indicative of reproduction by copepods, showed equal or even

480   higher densities under the ice than in summer for some lakes (Fig. 3c). We found the highest

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481   nauplii counts in one green (105 Ind L^{-1}) and brown (25.6 Ind L^{-1}) lake during the winter, while

482   in summer, maximum nauplii densities reached 39 and 20.2 Ind L^{-1} in green and brown lakes,

483   respectively. Thus, our results show that zooplankton remain active under the ice and can even

484   maintain high reproductive activity in some lakes. Alternatively, as seen in previous studies for

485   some cyclopoid copepods (Elgmork, 2006; Wærvågen and Nilssen 2010), high nauplii

486   abundance under the ice might be partly explained by the presence of overwintering nauplii

487   cohorts.

488   Zooplankton species composition was different between winter and summer, with higher

489   species diversity during the summer. Across all lakes, we found 21 species during the open water

490   season compared to 14 species under the ice. We found 9 zooplankton species that appeared only

491   during the summertime and were absent or below the detection limit under the ice. Among those

492   species, a few had a relatively high abundance in some lakes during summer and accounted for

493   12% of multivariate dissimilarity between two seasons (*Diaphanosoma birgei*, *Mesocyclops*

494   *edax*, and *Holopedium gibberum*). When we compared winter-summer diversity for different

495   lake types, we found that blue lakes showed less change between winter (8 species) and summer

496   (10 species) compared to green (9 vs. 18 spp.) and brown (10 vs. 14 spp.) lakes.

497   Our results showed that zooplankton communities were more similar across lakes during

498   winter than during the open water period (Fig. 5). Butts and Carrick (2017) similarly reported

499   greater similarity among phytoplankton communities during the winter in a study of seven

500   diverse lakes in Michigan. Their explanation was that the harsh winter conditions (low light and

501   temperature, stable water column) provided a selective advantage to specific groups of

502   phytoplankton (e.g., mixotrophic flagellates) regardless of lake trophic status. Likewise, we

503   believe that the same increased similarity of environmental conditions during winter is

504   responsible for the greater zooplankton community similarity across lakes and lake types in

505   winter compared to summer. Additional studies of lake communities in winter are needed to

506   determine whether our findings and those of Butts and Carrick (2017) are generally true about

507   the way pelagic communities reorganize across seasons.

508   Along with changes in species composition, we also observed changes in coarse-level

509   taxonomic and feeding group composition across seasons and lake types (Fig. 4, Fig. 6).

510   Calanoid and cyclopoid copepods dominated during the ice-cover period across all lakes, while

511   the relative and absolute abundances of cladocerans were higher in summer across all lakes.

512   These results agree with other studies, which also show the dominance of copepods during the

513   winter ice cover (Rautio *et al.*, 2000, Rautio *et al.*, 2011; Hampton *et al.*, 2017, Perga *et al.*,

514   2021). That is likely due to the ability of copepods to accumulate fat reserves before winter and

515   use them throughout the ice-covered period (Mariash, 2012; Grosbois *et al.*, 2017). Cladocerans,

516   such as *Daphnia*, have lower ability to accumulate storage lipids (Smyntek *et al.*, 2008) and have

517   growth and reproduction optima at temperatures above 15°C (Lynch and Ennis, 1983; Gliwicz *et*

518   *al.*, 2001), which helps explain their lower densities under the ice. Nevertheless, our results

519   showed that cladocerans (mainly *Daphnia* spp.) were still present under the ice in many of our

520   study lakes, constituting on average 21% of total zooplankton abundance.

521   It is important to mention that zooplankton densities and species composition among

522   different lake types also can be affected by fish predation. It has been shown that planktivorous

523   fish can reduce the number of large-size zooplankton in eutrophic and hypertrophic lakes (Auer

524   *et al.* 2004), while in brown lakes, crustacean zooplankton densities are less affected by fish

525   (Wissel and Boeing, 2003). Although most studies of lacustrine zooplankton-fish interactions

526   were conducted in summer, several from fall, winter, and spring showed high predation pressure

527   on zooplankton by fish in different lake types (Vanni *et al.*, 1990; Jeppesen *et al.*, 1997, Jeppesen

528   *et al.*, 2004, Hansson *et al.*, 2007). Therefore, fish can have a strong top-down effect on

529   zooplankton and must be incorporated in future seasonal studies to better understand the

530   complete picture of lake trophic interactions.

531   ***Seasonal changes in zooplankton trophic structure***

532   Among feeding groups, during the ice-covered period, we observed high relative

533   abundance of predators (52% of total abundance) (Fig. 6). In contrast, during the open water

534   season, all lakes on average were dominated by herbivorous zooplankton (67.5%) with predators

535   (28.5%) following next. The elevated relative abundance of predators among all lake types in our

536   study suggests that predatory zooplankton may be better adapted to survive periods of low

537   phytoplankton biomass under the ice. These changes in densities of different feeding groups are

538   likely related to food availability, specifically higher phytoplankton availability in the summer

539   and, possibly, greater importance of the microbial loop in winter which may support predatory

540   species during that time. Zooplankton classified as predators are often omnivorous and this

541   flexibility in diet might also help explain their dominance during winter. For example, some

542   cyclopoid copepod species can alter their feeding behavior from herbivory to carnivory in

543 environments with low primary production, and also change their feeding strategies at different

544 life stages (Lampert, 1978; Santer, 1993).

545   To further investigate seasonal changes of zooplankton trophic dynamics, we used C and N

546 stable isotope analysis of seston and bulk zooplankton (Fig. 7). Stable isotopes of C are often

547 used to identify the source of organic matter in seston and zooplankton (France, 1995). Across

548 all sampled lakes, we found that seston C stable isotope values ranged in summer between -27.4

549 and -33.9 ‰, while in winter they ranged from -28.7 to -40.5 ‰. Among sampled lakes,

550 meromictic Lake LaSalle had the most depleted $\delta^{13}C$ depth-integrated value -40.5 ‰ in winter.

551 This outlier value might be explained by the effect of anoxic conditions below the 40 meters

552 depth and the development of methanogenic bacteria, which usually have a depleted $\delta^{13}C$

553 signature (Deines et $al.$, 2009). We found small seasonal variation in seston $\delta^{13}C$ among blue and

554 brown lakes. This may be explained by low phytoplankton production in blue lakes and high

555 concentartions of allochthonous organic matter in brown lakes despite the season (Yoshii et $al.$,

556 1999; Grey et $al.$, 2000; Gu and Shell, 1999; Gu et $al.$, 2011). In green lakes, the difference

557 between winter and summer was slightly higher (1.7 ‰), but still low. The slightly enriched

558 values of seston $\delta^{13}C$ in green lakes during summer (-29.5±1.7 ‰) might suggest higher

559 phytoplankton production rates during the summer compared to winter (McCusker et $al.$, 1999).

560   Stable isotopes of N can provide information about the trophic position of consumers or their

561 reliance on tissue catabolism during starvation (Vander Zanden and Rasmussen 2001). In our

562 study, we observed higher values of seston $\delta^{15}N$ in winter than in summer for all lakes. Notably,

563 this was evident in brown lakes where winter $\delta^{15}N$ ranged between 6.8-9.3 ‰, while in summer

564 $\delta^{15}N$ values were much lower and ranged between -0.6 and 3.3 ‰. High $\delta^{15}N$ values of seston

565 might suggest low efficiency of N recycling under the ice in low productivity brown lakes

566 (Karlsson et $al.$, 2004). Alternatively, high abundance of heterotrophic protists and low

567 phytoplankton biomass in winter seston samples of brown lakes may be a source of elevated

568 $\delta^{15}N$ values since heterotrophic protists rely more on the microbial loop and have a

569 correspondingly elevated trophic position (Tranvik, 1992; Karlsson and Säwström, 2009).

570 Similarly, zooplankton $\delta^{15}N$ values were also higher in winter compared to summer across all

571 lakes. This enrichment may indicate zooplankton starvation (and hence tissue catabolism, which

572 is reflected in enriched $\delta^{15}N$) or higher prevalence of carnivory during the ice-covered period

573   (Adams and Sterner, 2000). The seasonal enrichment of zooplankton δ^{15}N ($\pm$SD) was especially

574   large in low productivity blue (5$\pm$3.8 ‰ change) and brown (4$\pm$4.5 ‰ change) lakes. This

575   observation is in accordance with our results on feeding groups, where the percentage abundance

576   of predators is highest in blue and brown lakes during the wintertime (Fig. 6). The N stable

577   isotope results suggest the possible importance of the terrestrial organic matter, which may be

578   trophically upgraded through the additional trophic step of the bacteria-heterotrophic protist link

579   and potentially subsidize zooplankton communities under the low food conditions in winter.

580   Additional research is needed to better understand pelagic trophic dynamics during the ice cover

581   season and the role of winter-active zooplankton in coupling the classic and microbial food

582   webs.

583

584   Conclusion

585   Ongoing climate change is reducing the duration of the ice-cover period while prolonging the

586   open-water season on north temperate lakes (Sharma *et al.*, 2020). This may impact lake food

587   webs and energy flow among trophic levels (Wollrab *et al.*, 2021). Zooplankton are a crucial

588   component in the energy transfer from bacteria and phytoplankton to fishes in lakes. Therefore, it

589   is important to understand how seasonal changes alter zooplankton communities in lakes of

590   different trophic status, how zooplankton will be affected by the shortening of ice cover, and

591   how these changes will modify energy transfer from the base of the food web to long-lived

592   consumers. Our study represents only one sampling date in winter and summer across diverse

593   lakes and potentially might demonstrate different seasonal successional patterns among

594   zooplankton communities. Nonetheless, this study provides new insights into seasonal

595   zooplankton dynamics across lakes of different trophic states. We showed that zooplankton

596   communities in all lakes are still active under the ice despite low phytoplankton biomass. We

597   found that zooplankton community composition changed dramatically but predictably between

598   seasons. Finally, feeding group and stable isotope analyses suggest changes in the food sources

599   that support zooplankton during the winter. Nonetheless, many questions about winter in lakes

600   remain. Year-round limnological studies that examine community and trophic dynamics across

601   the entire food web (from bacteria to fish) are needed to improve prediction of what the future

602   holds for our changing lakes.

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614   **Data Archiving**

615   Original data for this study are available online via the Data Repository for University of

616   Minnesota (DRUM; Shchapov *et al.,* 2021). They include CTD profiles, water chemistry

617   parameters and zooplankton abundance and community composition data.

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Table and Figure legends

Table 1- Study lake characteristics, including color classification, size, depth, sampling depths, sampling dates, TP, DOC, and chl *a* depth-integrated summer concentrations.

Table 2 – Light attenuation coefficients for snow/ice and water column in winter and only for water column in summer along with snow and ice conditions for all lakes in wintertime. Nd – no data.

Fig 1 - Map of study locations. Lakes of different water color are indicated by different symbols.

Figure 2 - Depth-integrated water chemistry variables (b, c, d) and euphotic depth (a) in summer and winter for all lakes. Boxplots represent data recorded across all lakes in winter and summer; winter and summer values for individual lakes are connected by black lines. Scatterplots show the paired winter and summer lake data for each variable; p- and rho-values are from Pearson correlation tests. Each lake is represented by different shape and color; blue circles indicate blue lakes, brown triangles indicate brown lakes, green squares show green lakes. Scales for chemistry variables are logarithmic.

Figure 3 – Zooplankton abundance based on adult and copepodite counts (a and b) and only on nauplii numbers (c). Paired t-test results with adjusted p-values shown for abundance data. Scatterplot show the paired winter and summer zooplankton data for total zooplankton count; correlation coefficients (rho) and p-values are from Spearman rank correlation test. Each lake is represented by different shape and color; round blue points indicate blue lakes, brown triangles indicate brown lakes, and green squares show green lakes. Scales for density are logarithmic.

Figure 4 - Zooplankton taxonomic groups densities (a-c) and percentage abundance (d-f) based on the number of adult individuals between summer and winter seasons across all sampled lakes. Paired t-test results with adjusted p-values shown for abundance data. Each lake is represented

931   by different shape and color; round blue points indicate blue lakes, brown triangles indicate

932   brown lakes, and green squares show green lakes. Scales for density are logarithmic.

933

934   Figure 5 – NMDS of the Bray-Curtis dissimilarity plot for zooplankton species across all lakes in

935   winter (polygon filled with blue color) and summer (polygon filled with red color) (plate a); only

936   in winter (plate b), and only in summer (plate c). Blue-edged polygons represent blue lakes

937   (oligotrophic), brown-edged polygons represent brown lakes (high DOC), and green-edged

938   polygons show green lakes (eutrophic). Vectors on plates b and c represent correlations ($p<0.05$)

939   between environmental variables and the distribution of zooplankton species.

940

941   Figure 6 – Boxplots represent zooplankton herbivore and predator feeding groups densities and

942   percentage abundance based on the number of adult individuals in summer and winter across all

943   lakes; paired t-test results with adjusted p-values showed for abundance data. Scatterplots show

944   the paired winter and summer zooplankton data for herbivore and predator feeding groups;

945   correlation coefficients (rho) and p-values are from Spearman rank correlation test. Each lake is

946   represented by different shape and color; round blue points indicate blue lakes, brown triangles

947   indicate brown lakes, and green squares show green lakes. Scales for density are logarithmic.

948

949   Figure 7 - Depth-integrated SIA data for seston (top panel – a, b, and c) and bulk zooplankton

950   (bottom panel – d, e, and f) in the winter and summer across all lakes. Each lake is represented

951   by different shape and color; round blue points indicate blue lakes, brown triangles indicate

952   brown lakes, and green squares show green lakes. Results of paired t-test with adjusted p-values

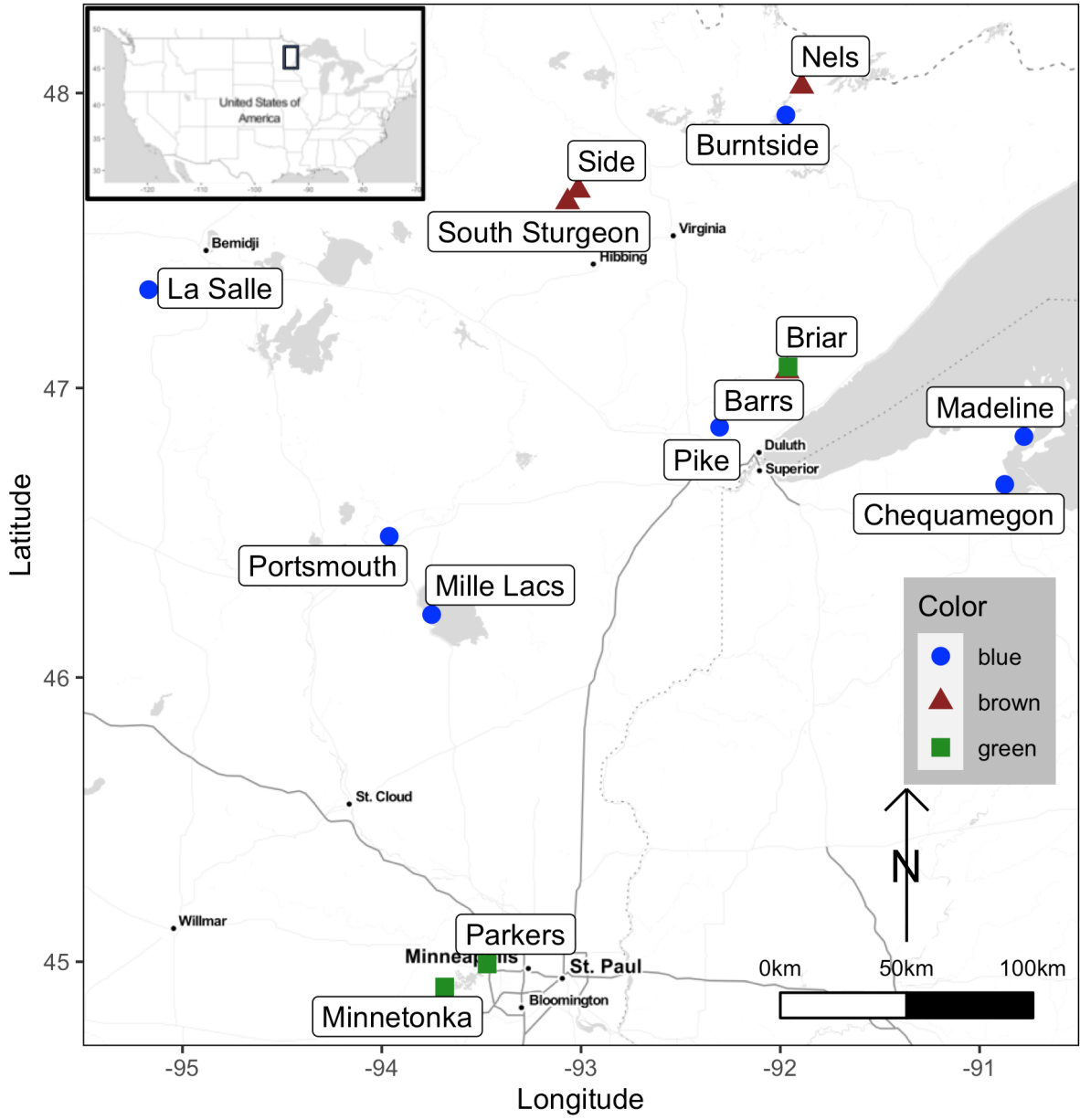953   showed on the top of each boxplot.

954

Lake Color	Lake, State	Lake Size, km^2	Site depth, m	Water sampling depths, m	Summer sampling date	Winter sampling date	$\int TP$, µM L^{-1}	$\int DOC$, mg L^{-1}	$\int$ Chl a, µg L^{-1}
Blue	Burntside, MN	28.9	26	0, 5, 14, 25	13-Jul-15	19-Mar-15	0.4	7.3	1.6
Blue	La Salle, MN	0.9	60.5	0, 7, 20, 35, 57	14-Jul-15	20-Mar-15	0.9†	6	2.3
Blue	Mille Lacs, MN	536.1	8.5	0, 7	10-Jul-15	18-Mar-15	0.7	7.5	3.3
Blue	Pike, MN	2.0	13	0, 6, 12	8-Jul-15	16-Mar-15	0.7	10.5	2.5
Blue	Portsmouth, MN	0.5	93	0, 15, 25, 35, 80	10-Jul-15	18-Mar-15	1.8†	5.5	0.7
Blue	Superior (Chequamegon Bay), WI	82102.6	8	0, 7.5	9-Jul-15	17-Mar-15	0.2	6	0.7
Blue	Superior (Madeline Ice Road), WI	82102.6	47	0, 15, 30, 45	9-Jul-15	17-Mar-15	0.3	1.7	1.2
Brown	Barrs, MN	0.52	6	0, 2, 3.75, 5	12-Jul-15	16-Mar-15	0.5	22	6.9
Brown	Nels, MN	0.7	8	0, 7	13-Jul-15	19-Mar-15	0.5	14.5	1.9
Brown	Side	1.5	10	0, 2, 4, 8	23-Jul-18	2-Mar-18	1.2	12.8	6.8
Brown	South Sturgeon	0.8	10	0, 2, 4, 8	23-Jul-18	2-Mar-18	1	38.5	0.8
Green	Briar, MN	0.3	5.5	0, 4.5	12-Jul-15	16-Mar-15	2.6	13	13.8
Green	Minnetonka	2.3	10	0, 2, 4, 8	16-Jul-18	27-Feb-18	5.1	19.2	58.1
Green	Parkers	0.4	10	0, 2, 4, 8	16-Jul-18	27-Feb-18	1.9	9.25	31.8

956 † Lakes LaSalle and Portsmouth are deep, meromictic lakes with high TP concentrations in the monimolimnion. The summer depth-integrated TP
957 concentrations in the epilimnion are 0.5 µM L^{-1} (LaSalle) and 0.6 µM L^{-1} (Portsmouth).

958

Lake Color	Lake, State	k_d snow	k_d ice	k_d water in winter	k_d water in summer	Snow depth, m	Ice thickness, m
Blue	Burntside, MN	Nd	2.7	0.4	0.5	0	0.6
Blue	LaSalle, MN	Nd	2.3	0.5	0.5	0	0.58
Blue	Mille Lacs, MN	Nd	2.6	0.3	0.6	0	0.65
Blue	Pike, MN	Nd	1.7	0.4	0.5	0	0.67
Blue	Portsmouth, MN	Nd	2.5	0.2	0.3	0	0.53
Blue	Superior (Chequamegon Bay), WI	Nd	3.4	0.2	0.3	0	0.52
Blue	Superior (Madeline Ice Road), WI	Nd	2.2	0.2	0.3	0	0.5
Brown	Barrs, MN	Nd	1.2	1.6	1.1	0	0.6
Brown	Nels, MN	Nd	5.8	1.2	1.2	0	0.58
Brown	Side, MN	20.2	2.5	0.8	0.7	0.36	0.62
Brown	South Sturgeon, MN	37.7	6.5	6	6.4	0.32	0.47
Green	Briar, MN	Nd	3.9	0.5	1	0	0.5
Green	Minnetonka, MN	7.6	0.9	0.8	1.4	0.2	0.68
Green	Parkers, MN	12.2	1.8	0.7	1.2	0.21	0.62

959

34

1

Fig.1

a) Euphotic depth (m)

winter   summer
T-test, p.adj=0.0002
df=13, t=-6.3

Euphotic depth (m)
p<0.0001, rho=0.86

Winter
Summer

b) DOC (mg L^{-1})
T-test, p.adj=0.39
df=13, t=-0.88

winter   summer

DOC (mg L^{-1})
p<0.00001, rho=0.92

Winter
Summer

c) TP (µM L^{-1})
T-test, p.adj=0.03
df=13, t=-3.1

winter   summer

TP (µM L^{-1})
p=0.0005, rho=0.82

Winter
Summer

d) Chl *a* (µg L^{-1})
T-test, p.adj=0.03
df=13, t=-3.2

winter   summer

Chl *a* (µg L^{-1})
p=0.14, rho=0.42

Winter
1:1
Summer

3

4

Fig.2

a) Adult and Copepodite Total Densities, (Ind. L^{-1})

T-test, p.adj<0.001

df=13, t=-4.6

winter   summer

b) Adult and Copepodite Total Densities, (Ind. L^{-1})

p<0.009, rho=0.7

Winter

Summer

c) Nauplii Density (Ind. L^{-1})

T-test, p.adj=0.06

df=12, t=2.1

winter   summer

d) Species richness

T-test, p.adj=0.01

df=13, t=-3.2

winter   summer

5

6   Fig.3

7

8   Fig. 4

a) Stress: 0.15

season
■ summer
▲ winter

colour
● blue
● brown
● green

b) Stress: 0.09

S.pallidius
L.siciloides

B.longirostris

D.g.mendotae

L.minutus

D.thomasi

T.p.mexicanus

L.sicilis

S.oregonesis

D.longiremis

A.vernalis

L.macrurus

TN, R² = 0.59, p = 0.0078

S.pygmaeus
Alona

season
■ winter

colour
● blue
● brown
● green

c) Stress: 0.13

C.sphaericus

H.gibberum

B.longirostris

D.g.mendotae

T.p.mexicanus

D.birgei

C.flavicans

L.minutus

L.macrurus

M.edax

D.thomasi

A.vernalis

D.retrocurva

L.sicilis

TP, R² = 0.54, p = 0.0085

Chl.a, R² = 0.49, p = 0.0166

L.siciloides   *D.parvula*

D.pulicaria

S.oregonesis

O.sanguineus

O.birgei   *S.pygmaeus*

season
■ summer

colour
● blue
● brown
● green

9

10

Fig. 5

a) Herbivore (Ind. L^{-1})
T-test, p.adj=0.003
df=13, t=4

b) Herbivore (%)

c) Herbivore (Ind. L^{-1})
p=0.4, rho=0.26

d) Predator (Ind. L^{-1})
T-test, p.adj=0.2
df=13, t=-1.4

e) Predator (%)

f) Predator (Ind. L^{-1})
p=0.11, rho=0.45

11

12

Fig. 6

a) Seston δ¹³C — T-test, p.adj=0.06, df=13, t=-2.08
b) Seston δ¹⁵N — T-test, p.adj=0.007, df=13, t=3.22
c) Seston C/N — T-test, p.adj=0.92, df=13, t=0.11
d) Zooplankton δ¹³C — T-test, p.adj=0.0002, df=13, t=-5.41
e) Zooplankton δ¹⁵N — T-test, p.adj=0.0001, df=13, t=4.17
f) Zooplankton C/N — T-test, p.adj=0.0007, df=13, t=5.48

13

14

Fig. 7