Daphniid zooplankton assemblage shifts in response to eutrophication and metal contamination during the Anthropocene

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1   **Title:** Daphniid zooplankton assemblage shifts in response to eutrophication and metal

2   contamination during the Anthropocene

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15   **Abstract:** Human activities during the Anthropocene result in habitat degradation that has been

16   associated with biodiversity loss and taxonomic homogenisation of ecological communities.

17   Here we estimated effects of eutrophication and heavy metal contamination, separately and in

18   combination, in explaining zooplankton species composition during the past 125-145 years using

19   analysis of daphniid diapausing egg banks from four lakes in the Northeastern USA. We then

20   examined how these community shifts influenced patterns of diversity and homogenisation.

21   Analysis of past lake production (via subfossil pigments) and metal contamination (via

22   sedimentary metals) demonstrated that eutrophication alone (19-39%) and in combination with

23   metal pollution (17-54%) explained 36-79% of historical variation in daphniid species relative

24   abundances in heavily fertilised lakes. In contrast, metal pollution alone explained the majority

25   (72%) of historical variation in daphniid assemblages at the oligotrophic site. Several species

26   colonisation events in eutrophying lakes resulted in increased species richness and gamma

27   diversity through time. At the same time, daphniid assemblages in three eutrophied lakes became

28   more similar to each other (homogenised) but this pattern was only seen when accounting for

29   species presence/absence. We did not observe consistent patterns of divergence between the

30   assemblages in the eutrophying lakes and the low-nutrient reference site. Given the pervasive

31   nature of fertilisation and metal pollution and the sensitivity of cladocerans to these factors, we

32   suggest that many inhabited lake districts may already exhibit similar patterns of daphniid

33   assemblage shifts.

34   ***Key words***: biotic homogenisation, Jaccard similarity, eutrophication, *Daphnia*, heavy metals,

35   palaeolimnology

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Introduction

We have entered the Anthropocene, an era during which human activities can profoundly

influence the Earth's geological, chemical, and biological processes at a global scale [1]. One

pervasive signature of land use change in this human dominated era is the increasing release and

accumulation of chemical pollution in the environment. At the same time, biodiversity loss has

been precipitous, and this has long been cited as a consequence of widespread anthropogenic

environmental changes, including chemical pollution [2,3]. However, it can be difficult to

causally link pollution with changes in species diversity. In addition, increasing biotic

homogenisation of communities in human impacted environments is an emerging pattern gaining

increasing recognition [4,5]. As developed landscapes begin to predominate on Earth, sensitive

endemic species are often lost while human-tolerant species spread, a pattern that can reduce

biotic diversity at regional scales. Such assemblage shifts have been observed in a number of

groups and habitat types around the world including plants [6], molluscs [7], fish [8], amphibians

and reptiles [9]. One hypothesis is that habitat degradation associated with agricultural and urban

activity homogenises the physical landscape and thus its constituent ecological communities

[10,11].

Human activities routinely change nutrient influx to lakes and can have transformative

impacts on aquatic ecosystems [12]. Elevated nutrient input directly increases phytoplankton

productivity and can favour blooms of toxic cyanobacteria [13], while increased phytoplankton

biomass indirectly alters aquatic ecosystems by creating deep-water anoxia which can cause

extensive fish kills [14] and phosphorus (P) release from sediments [12]. While eutrophication

may proceed steadily, the ecological response of higher trophic level taxa such as invertebrates

and fish can be complex, non-linear, or even threshold-like [15–17]. These long-term effects of

61   nutrient accumulation on lake communities have been well documented (e.g., [18–20]), but are

62   typically studied in isolation from other stressors such as heavy metals (but see [21]). Yet,

63   nutrients and other chemical contaminants of freshwaters occur together, thus it is important to

64   understand their joint impacts on ecological communities.

65   Industrial development has increased the release of heavy metals (e.g., mercury [Hg],

66   cadmium [Cd], lead [Pb], chromium [Cr], copper [Cu], zinc [Zn]) into the environment [22]

67   where they accumulate in lake basins, even in remote locations (e.g., [23–25]). Moreover, Cu has

68   been widely applied as an algaecide in eutrophic lakes [26]. While heavy metals occur naturally,

69   at elevated concentrations most heavy metals, even essential elements such as Zn [27], are toxic

70   to living organisms. Toxicological testing has generated a large body of information about short-

71   term effects of individual metals on organisms in controlled laboratory environments (e.g. US

72   EPA's ECOTOX database: https://cfpub.epa.gov/ecotox). Yet how effects of heavy metals play

73   out over multi-decadal time scales in field conditions remains uncertain and represents an

74   important research frontier for ecotoxicology [28].

75   Freshwater zooplankton are often sensitive to alterations of the chemical environment

76   and offer an excellent model system to study the unique and interactive effects of eutrophication

77   and metal pollution. In particular, *Daphnia* species are keystone herbivores in lake food webs

78   [29] that respond strongly to eutrophication because of changes in resource availability [30],

79   phytoplankton size structure and grazing sensitivity [15], and exposure to cyanobacterial toxins

80   [31], among other factors. Daphniids are also sensitive to heavy metal exposure, although

81   susceptibility to individual elements varies among species [32]. Despite these observations,

82   relatively little is known of how planktonic invertebrate community composition has varied with

83   anthropogenic forcing over a century of eutrophication and metal contamination in much of the

84   industrialised world (but see [21,33]).

85   In this study, we use lake sediment archives to examine long-term (ca. 145 years)

86   ecological responses of daphniid zooplankton assemblages to nutrient fertilisation and heavy

87   metal contamination. Both stressors are common consequences of anthropogenic land use, are

88   known to affect zooplankton, and leave unique signatures in lake sediments, allowing their

89   effects on lake biota to be studied over long time spans [34,35]. We estimated the relative

90   influence of these pollutants on temporal patterns of daphniid species composition in these lakes

91   and determined whether the effects of these contaminants resulted in the loss of diversity or

92   assemblage shifts seen in other taxonomic groups in human dominated landscapes.

93

94   **Materials and methods**

95   *Lake selection***:** Four study lakes in Connecticut, USA, were chosen representing a wide range of

96   nutrient influx over the past eighty years [36,37] (electronic supplementary material (ESM) 1:

97   table S1). Based on historic total phosphorus (TP) records, Black Pond has remained

98   oligotrophic and serves as a low-nutrient reference site, whereas Alexander Lake has become

99   mesotrophic, in part due to P supply from sediments. Cedar Pond and Roseland Lake have both

100   become hypereutrophic and are listed as impaired for recreation (both lakes) and the support of

101   aquatic life (Cedar) by the United States Environmental Protection Agency. Typical of lakes in

102   the region (ESM1: table S2), all study lakes are relatively small and shallow, but vary in physical

103   and chemical characteristics as well as the degree of historical changes in land use within their

104   watersheds [38] (ESM1: text S1, table S1). We restricted our study to lakes without landlocked

5

105   alewives (*Alosa pseudoharengus*) to avoid confounding influences of change in trophic regimes

106   arising from management of zooplanktivorous fish stock [39,40] (ESM1: text S2).

107   *Sediment collection and dating:* Methods for sediment collection, sectioning, storage, and

108   establishment of geochronology are described in detail elsewhere [38]. Briefly, 12.5-cm diameter

109   sediment cores were collected from the deepest basin of each lake by SCUBA divers. Cores were

110   sectioned at 1.5-cm intervals and stored in the dark at 4°C for examination of daphniid

111   diapausing egg densities. Sediments for examination of photosynthetic pigments and nutrients

112   were frozen. Rogalski [38] estimated sediment ages for these study lakes based on changes in

113   ^{210}Pb radioisotope activity with depth (ESM2: figure S1).

114   *Heavy metal analysis*: Sediment concentrations of Cd, Cr, Cu, Pb, and Zn were measured

115   by inductively coupled plasma-mass spectrometry (Thermo Finnigan Element 2 high resolution

116   ICP-MS) following extraction by hot block acid digestion. Sediment Hg concentrations were

117   measured by atomic absorption spectrometry (Direct Mercury Analyzer DMA80). Additional

118   methodological details are published elsewhere [38].

119   *Nutrient and subfossil pigment analyses:* Total carbon and total nitrogen composition of

120   sediments were measured on whole freeze-dried sediments. Samples of 2-10 mg dry mass were

121   packed into tin capsules and introduced into a NC-2500 elemental analyser. Nitrogen and C

122   components of sediments were oxidised completely at 1000°C in a furnace to convert organic

123   constituents into simple nitrogen-based gases and CO_2. Elemental mass ratios were estimated

124   using combustion data. Sedimentary pigments were extracted, filtered, and dried under N_2 gas

125   following the standard procedures of Leavitt and Hodgson [35] (ESM2: text S3).

126   *Daphniid community structure***:** Historical changes in species composition were

127   quantified using sedimentary banks of diapausing eggs for taxa in the Daphniidae family [41], a

128   group of filter-feeding crustacean zooplankton that are keystone herbivores in lakes [29] and

129   preserve well in lake sediments [20,41]. Diapausing eggs (encased in ephippia) were isolated by

130   filtering whole sediments through 50-μm mesh and examining the retained filtrate under a

131   dissecting microscope at 10× power. All ephippia were examined with a compound microscope

132   at 100× and 400× power to compare gross morphology and finer details of specimens. In most

133   cases, ephippia were identified from sediment subsamples until at least 50 specimens (median

134   73) per sediment slice were enumerated, beginning with surface sediment and examining

135   alternate sediment sections over the past 125-145 years. For the earliest time period from Black

136   and Alexander lakes, only 42 and 46 ephippia were available for counting, respectively.

137   We identified ephippia to species by hatching animals from a subset of viable eggs

138   representing all morphological types found in the four lakes. Intact ephippia were incubated in

139   COMBO freshwater medium [42] using spring light and temperature conditions (14:10h light:

140   dark, 15°C). Hatchlings were cultured in COMBO medium and fed *Scenedesmus obliquus* until

141   they reached maturity and could be identified to species. We then matched daphniid species

142   identity to the six ephippial morphotypes based on characteristics of the ephippia from which the

143   hatchlings originated (ESM3: text S4). Historical species densities in subfossil daphniid

144   assemblages were based on combined counts of empty and intact ephippia present in the

145   sediments, unless ephippia were crushed or too decomposed to be identified (1.1% of counts).

146   *Relative importance of eutrophication and metals*: We used multivariate statistical

147   analyses to quantify the relative importance of heavy metal contamination and eutrophication in

148   explaining variation in daphniid species composition through time. Owing to the large number of

149   variables describing changes in metal contamination and eutrophication, principle components

150   analysis (PCA) was conducted on data from each lake to reduce each type of pollution to a

151   univariate index (ESM4: table S3). We used PC1 axis loading scores of metals and of

152   eutrophication as explanatory variables in a redundancy analysis (RDA) predicting daphniid

153   zooplankton assemblage structure (Hellinger-transformed species abundances). In Cedar Pond,

154   temporal patterns of Cu contamination greatly differed from the other metals and were not

155   reflected in the metals PC1. In this case, Cu was excluded from the metals PCA and was

156   included as a separate predictor, in addition to the eutrophication and metals PC1 scores.

157   Following Borcard et al. [43] and using adjusted R^2, we used variance partitioning to determine

158   the amount of variance in community structure explained by metals, eutrophication, and a

159   combination of these stressors [44]. Monte Carlo permutation tests (10,000 permutations) were

160   used to test the significance of these associations. RDA and variance partitioning were conducted

161   separately for each lake using data from 6-9 time periods per lake.

162   *Taxonomic similarity analysis*: Studies of taxonomic homogenisation typically compare

163   community similarity between two time intervals to gauge whether the communities have

164   become more similar over time [45]. We used this approach to compare overall changes in

165   daphniid community composition among the four study lakes since ca. 1860, a period of

166   significant change in regional metal contamination and eutrophication [23,38,46]. We recognize

167   that estimates of historical change in the relative abundance of daphniids in an egg bank reflect

168   both variation in the past density of animals in the water column as well as their tendency to

169   produce ephippia through sexual reproduction, a factor which can vary through time and among

170   species [47]. We estimated overall changes in community similarity using the Jaccard similarity

171   index (*J*). This measure based on species presence and absence is commonly used in studies of

172   biotic homogenisation [48] and allowed us to characterise community changes related to the

173   colonisation or local extinction of species. Similarity values range from 0 to 1, with 1

174   representing complete overlap in species composition and 0 representing no shared species

175   between two sites. We tested whether daphniid communities became more taxonomically

176   homogeneous over time among pollution-impacted lakes by subtracting historic baseline *J* values

177   (ca. 125-145 years BP) from modern (surface sediment) values. We also assessed whether the

178   communities in the heavily eutrophied lakes diverged from to the reference community in Black

179   Pond over time.

180   Our data set also enabled a more detailed examination of temporal shifts in daphniid

181   composition in the four lakes since ca. 1860. We conducted PCA using Hellinger transformed

182   daphniid species densities in all four lakes. PCA biplots allowed us to visualize the timing and

183   nature of any patterns of convergence and divergence in community composition among the four

184   study lakes. In addition, we calculated the Euclidean distance between historic and modern

185   daphniid assemblages in each lake, using the first three PC axis scores (based on a scaling that

186   preserves Euclidean distances in multidimensional space). We subtracted modern from historic

187   PCA distances, such that a positive difference indicated an increase in similarity between

188   assemblages over time. We conducted PCA, RDA, and variance partitioning using the package

189   *vegan* [49] with the statistical software R version 3.0.2 [50].

190   *Species richness trends:* We made rarefied estimates of species richness at each time

191   period based on random subsamples of 42 ephippia (smallest sample size of any of the lakes/

192   time periods). This analysis was conducted using the rarefy function in the R package *vegan*.

193

194   **Results**

195   *Heavy metal contamination*: The timing and magnitude of metal contamination differed among

196   lakes and metals [38] (figure 1, ESM4: figure S2). Metal contamination typically increased

197   beginning in the early 1900s and either levelled off or continued to rise over time. The exception

198   is Cedar Pond, where metals peaked earlier and declined in recent decades. The largest changes

199   occurred in Cu, Cd, and Hg, metals known to be highly toxic to cladoceran zooplankton [51,52].

200   Concentrations of Cu increased rapidly between the 1940s-90s and remained elevated in

201   Roseland Lake owing to consistent annual application of hundreds of kg of copper sulphate

202   ($CuSO_4$) to manage nuisance phytoplankton blooms (personal communication, Putnam County,

203   CT Water Department). Based on changes in sediment Cu contamination, we believe this

204   practise also occurred in Cedar Pond but ceased after the 1980s, although documentation of

205   practises is unavailable. Cd and Hg contamination likely entered the lakes through regional

206   atmospheric deposition resulting from industrial activity, including fossil fuel burning [22,53].

207   Overall, sediment concentrations of copper in Roseland Lake (ca. 1980s-modern sediments),

208   copper in Cedar Pond (at its peak in ca. 1982) and lead in Cedar Pond (ca. 1916-1969) reached

209   levels above probable effect concentrations (i.e. harmful effects to aquatic life are considered

210   likely; ESM4: figure S2) [54].

211   *Eutrophication*: The degree and timing of anthropogenic eutrophication also varied

212   among lakes and roughly followed trajectories expected from regional monitoring programs

213   (figure 1, table S1, ESM4: figure S3). Analysis of multiple pigment and nutrient biomarkers

214   suggests that eutrophication was minimal in Black Pond, moderate in Roseland Lake, and

215   substantial in Alexander Lake and Cedar Pond. Eutrophication proceeded steadily over the past

216   century in Cedar Pond and Roseland Lake and was more pronounced over the past 50 years in

217   Alexander Lake (figures 1, S3). In all cases, sediment C: N ratio declined to some extent during

218   the past 100 years, consistent with increased deposition of phytoplankton biomass [55]. Subfossil

219   pigment concentrations were relatively unchanged during the past 125-145 years in Black Pond

220   (23% increase in total pigments), increased moderately in Roseland Lake (172% increase), and

221   rose substantially in Alexander Lake and Cedar Pond (334% and 405%, respectively) (figure

222   S3). Increases in the pigment okenone, produced by obligately anaerobic photosynthetic purple

223   sulphur bacteria, indicate that anoxia extended into the photic zone with increasing regularity in

224   Cedar Pond (after 1900) and Alexander Lake (after 1950). The relative concentration of subfossil

225   pigments produced by cyanobacteria (myxoxanthophyll, canthaxanthin, echinenone) increased in

226   Cedar and secondarily Roseland, but remained unchanged in Alexander Lake and Black Pond

227   (ESM4: figure S3).

228   *Daphniid community composition*: Five *Daphnia* (*D. ambigua*, *D. catawba*, *D. mendotae*,

229   *D. parvula* and *D. pulicaria*) and one *Ceriodaphnia* species were identified in the four lakes.

230   *Ceriodaphnia* ephippia were rarely viable and were therefore identified to the genus level. *D.*

231   *mendotae* and *D. pulicaria* colonised Cedar Pond by the 1930s, while *D. mendotae* colonised

232   Roseland and Alexander Lakes by the 1970s and 1990s respectively (figure 1). *D. pulicaria* was

233   absent from the sediment record in Cedar Pond beginning in the 1960s and was seen again in the

234   sediments ca. 2004-2011. *D. pulicaria* was present in low abundances from the beginning of the

235   sediment record in Alexander Lake. Overall, large-bodied *D. pulicaria* and *D. mendotae* were

236   rare (1-5% relative abundance) for as long as 80 years before rapidly increasing in abundance

237   over the past 10-30 years. Aside from these colonisation and population expansion events,

238   daphniid assemblages remained remarkably consistent in species relative abundances,

239   particularly prior to 1950 (figure 1). Local extinction occurred in only one instance

240   (*Ceriodaphnia* in Roseland, which comprised <1% of the community in 1904). *Ceriodaphnia*

241   was generally uncommon when present and declined in abundance in all lakes.

242   *Influence of environmental change on community structure*: Variance partitioning with

243   redundancy analysis revealed that the abundance of daphniid species in nutrient-polluted lakes

244   was strongly associated with variation in nutrients, either alone or in concert with metal

245   pollution. Eutrophication alone explained 18.7-39.1% of historical community variation, while

246   the combined (statistically-inseparable) effects of eutrophication and metals explained an

247   additional 17.3-53.6% of the variation (table 1). In contrast, variation in the daphniid assemblage

248   in oligotrophic Black Pond was associated strongly with changes in metal contamination alone

249   (adj. R^2 = 72%). Copper was a significant predictor (p=0.047, F=2.92) of daphniid species

250   abundances in Cedar Pond in an RDA model that included eutrophication (PC1). Variation

251   partitioning showed that copper explained 14.8% (adj. R^2) of the variation in the daphniid

252   assemblage in Cedar that was not explained by eutrophication (table 1). RDA biplots indicate

253   that two species, *D. mendotae* and *D. pulicaria*, were associated with states of high

254   phytoplankton abundance (figure 2*b-d*), while both species were absent from unproductive Black

255   Pond (figure 1). *D. puliciaria* was also associated with low copper time periods in Cedar Pond

256   (figure 2c). *Ceriodaphnia* was characteristic of low metal and low nutrient regimes (figure 2).

257   *Community similarity among lakes*: The daphniid assemblages in strongly eutrophying

258   lakes (i.e., Alexander, Roseland, and Cedar) became more similar to one another over the past

259   125-145 years, as evidenced by a positive change in *J* over this time interval (figure 3*a*).

260   Community comparisons between the eutrophying lakes and the low-nutrient reference

261   community in Black Pond were less consistent. The daphniid assemblages in Cedar Pond and

262   Alexander Lake diverged from that in Black Pond (figure 3*b*), while the daphniid assemblage in

263   eutrophied Roseland Lake became more similar to that in Black Pond.

264   PCA showed that over the past century the assemblages in Alexander Lake, Cedar Pond,

265   and Roseland Lake followed similar trajectories along PC axis 3 (figure 4, ESM4: table S4), a

266   pattern which reflects the colonisation and increase of *D. pulicaria* and *D. mendotae* in these

267   eutrophying lakes through time. The assemblage in the low nutrient site Black Pond did not

268   change along the PC3 axis, as neither of these species were ever present in the sediment record.

269   At the same time, the assemblages in Alexander Lake, Cedar Pond and Black Pond shifted

270   towards the left along PC axis 1, indicating an increase in *D. catawba* and a decrease in

271   *Ceriodaphnia* and *D. ambigua*. The assemblage in Roseland shifted in the opposite direction

272   along the PC1 axis. Additional information on daphniid assemblage shifts along PC axes 1-2 and

273   2-3 are provided in figure S4 (ESM4).

274   Euclidean distances calculated using the first three PC axis scores showed that overall the

275   Alexander and Roseland daphniid assemblages became more similar while diverging from the

276   assemblage in Cedar Pond (Figure 3*a*). The assemblages in Alexander Lake and Cedar Pond

277   diverged from the assemblage in the low nutrient site, Black Pond (figure 3*b*), while the

278   assemblage in eutrophying Roseland Lake became more similar to that in Black Pond.

279   *Species richness*: The low-nutrient reference lake Black Pond maintained three daphniid

280   species throughout the ca. 150-year record. Species richness increased in the three eutrophying

281   lakes, with two species gained in Roseland and Cedar and one gained in Alexander over the

282   historic record. Similar patterns were recorded using rarefied species richness estimates (ESM4:

283   figure S5).

284   **Discussion**

285   Land use change resulting in habitat loss and modification is often associated with biodiversity

286   loss and homogenisation of the world's biota [2,4]. Here we demonstrate that nutrient and heavy

287   metal pollution, two widespread forms of chemical pollution associated with anthropogenic

288   activities, explained key changes in the composition of daphniid zooplankton assemblages over

289   the past ~140 years. However, contrary to expectations, these responses resulted in an overall

290   increase in species richness in eutrophying lakes owing to colonisation by large-bodied daphniid

291   taxa. While we observed some evidence of homogenisation of daphniid assemblages in three

292   eutrophying lakes, this pattern depended on the similarity measure used, and patterns of

293   divergence from the low-nutrient reference site were inconsistent. Overall, eutrophication was

294   the dominant force explaining historical changes in community structure in fertilised lakes (table

295   1, figure 2 *b-d*). Contamination by metals alone explained subtle shifts in species relative

296   abundances in the oligotrophic reference lake. While caution is warranted when extrapolating

297   based on patterns observed in four study lakes, the pervasive nature of lake eutrophication

298   [12,46], and metal contamination [22], combined with the sensitivity of cladocera to both factors

299   [15,32], suggest that daphniid zooplankton assemblages in other lake regions with ubiquitous

300   agricultural or urban development may have followed similar trajectories [12].

301   Although daphniid species composition and relative abundances responded to both

302   eutrophication and metal contamination in study lakes, effects of nutrients were paramount in the

303   nutrient-rich sites, both uniquely and in combination with metal contamination (table 1). We

304   observed colonisation by *D. mendotae* and *D. pulicaria* in eutrophying lakes and shifts in relative

305   abundance of other taxa (figures 1, 2 and 4), but no extirpation of native species. Elevated

306   phytoplankton abundance due to eutrophication is expected to favour large-bodied *Daphnia* such

307   as *D. pulicaria* and *D. mendotae* [30], consistent with their absence from the oligotrophic

308   reference lake and low densities prior to eutrophication. This species sorting from the regional

309   species pool fostered a gain of taxonomic diversity, both in terms of species richness per site

310   (alpha) and the total number of species found across sites (gamma). Further, our estimates of

311   changes in historical diversity may represent a minimum value, as we did not correct for

312   increased sediment accumulation rates and dilution of subfossils which can occur in both modern

313   sediments and with eutrophication [56].

314   Temporal patterns of *D. mendotae* and *D. pulicaria* colonisation and population

315   fluctuations appeared to exhibit a threshold response to fertilisation. Although colonisation of

316   these species occurred early in the sedimentary record of eutrophying lakes, substantial increases

317   in relative abundances of these taxa were restricted to the past 10-30 years, after eutrophication

318   had progressed substantially (figure 1). Large-bodied *D. mendotae* and *D. pulicaria* may require

319   high algal abundance to achieve stable dominance [57,58]. Interestingly, colonisation and

320   establishment of *D. galeata*, a species closely related to *D. mendotae*, also appears to require a

321   eutrophication threshold in European lakes [33].

322   Spatial dispersal of *Daphnia* resting eggs undoubtedly enabled the colonisation of new

323   species in the eutrophying lakes; however, it seems unlikely that an increase in dispersal alone

324   would explain the recent rapid increase in *D. mendotae* and *D. pulicaria*. Owing to their large

325   body size, increased dispersal opportunities might make colonisation by these species more

326   likely, but other smaller taxa in our assemblages should disperse even more frequently. In a

327   study of colonisation patterns of Midwestern USA reservoirs, *D. pulicaria* and other large-

328   bodied cladocerans were also slower to colonise [59]. Allen *et al*. attributed this pattern to

329   reduced dispersal ability in larger-bodied cladocerans [59]; however, it is also likely that the

330   increasing eutrophic status of these reservoirs favoured establishment of these large species.

331   In eutrophying Cedar Pond, elevated Cu concentrations were associated with the

332   disappearance of *D. pulicaria* for several decades; this species reappeared and flourished when

333 Cu levels approached baseline levels (figures 1, 2c). As densities of *D. pulicaria* were near the

334 detection limit throughout much of the record in Cedar, it is possible that this species was not

335 absent but rather extremely rare in the 1960s-90s. The rapid rise in Cu in Cedar in the 1950s-

336 1980s, particularly separately from other metals, is consistent with the idea that $CuSO_4$ algaecide

337 was applied to Cedar Pond during this period of *D. pulicaria*'s absence. Similarly, we

338 hypothesize that application of $CuSO_4$ may have prevented *D. pulicaria* from establishing and

339 flourishing in eutrophying Roseland Lake.

340   Interestingly, with the exception of copper in Cedar Pond, pollution with metals alone

341 had no measurable unique effect on daphniids in fertilised lakes, even though the magnitude of

342 change in metal influx was apparently similar to or greater than that of nutrients associated with

343 eutrophication, and several metals reached potentially-toxic levels (ESM4: figure S2). Effects of

344 metal contamination alone resulted in subtle shifts in community structure only in oligotrophic

345 Black Pond. Overall*, Ceriodaphnia* was strongly negatively associated with metal increases

346 (figure 2) and has declined in all lakes where present (figure 1). This is consistent with

347 laboratory findings that *Ceriodaphnia* usually exhibits high sensitivity to chemicals during *in*

348 *vivo* toxicity trials [32]. One possible explanation for the relative lack of importance of metals is

349 that rapid evolutionary responses of the *Daphnia* assemblages have obscured ecological impacts

350 of metals. However, an empirical study of *Daphnia* populations in these lakes actually found

351 evidence of maladaptation to metal contamination [60].

352   Despite the paramount effect of nutrients, variance partitioning analysis suggested that

353 daphniid community composition was sensitive to the combined effects of fertilisation and metal

354 pollution (table 1). This could occur because synchronous changes in metal contamination and

355 eutrophication make it impossible to statistically tease apart their relative influences. However, it

356   is also possible that metals and eutrophication have a synergistic effect on daphniid species

357   patterns. Such a synergy could arise because eutrophication favours lower oxygen content in

358   deep water, thereby elevating metal release from sediments [61]. Consistent with this

359   interpretation, we note that the carotenoid okenone from obligately-anaerobic purple sulphur

360   bacteria was more common in both Cedar Pond and Alexander Lake after several decades of

361   fertilisation, indicating repeated periods of anoxia in these lakes. In addition, fertilisation was

362   accompanied by other changes in water chemistry that may have influenced metal toxicity [46].

363   For example, laboratory analyses show that daphniid metal sensitivity increases when

364   accompanied by low concentrations of calcium [62], an element which has declined in Alexander

365   Lake, Roseland Lake and Black Pond after ca. 1970 [46]. Although further research is needed to

366   differentiate among these mechanisms, together our findings suggest that complex, hierarchical

367   interactions between eutrophication and metal pollution may control daphniid community

368   composition.

369   Colonisation by *D. pulicaria* and *D. mendotae* led to an increase in Jaccard similarity in

370   the three eutrophying lakes; however, this consistent homogenisation pattern was not observed in

371   the multivariate ordination of the daphniid assemblages (figure 3*a*). Shifts in daphniid

372   composition in the eutrophying lakes followed similar trajectories along PC axis 3 (figure 4).

373   However, the Euclidian distance, which incorporates variation along the first three PC axes

374   (figures 4 and S4), showed that eutrophying Alexander and Roseland became more similar,

375   while both diverged from Cedar (figure 3*a*). Daphniid assemblages in eutrophying Alexander

376   Lake and Cedar Pond diverged from the assemblage in the unproductive reference site, Black

377   Pond (figure 3*b*); however, daphniids in Roseland became more similar to those in Black Pond.

378   Although speculative, we suggest the annual application of $CuSO_4$ to control primary

379   productivity in Roseland caused the daphniid assemblage to resemble that of the low-nutrient

380   lake, an undesirable effect as large-bodied *Daphnia* species are effective biological controls of

381   phytoplankton production [63].

382   This study focused on daphniid zooplankton because they are keystone organisms in

383   freshwater food webs [29], are well preserved in sediments [41], can be identified to species by

384   hatching and culturing diapausing embryos, and are known to be sensitive to both nutrient and

385   metal pollution [15,30–32]. However, many other zooplankton species inhabit these lakes, and it

386   remains unknown how they may have responded to eutrophication and metal contamination.

387   Similarly, it is difficult to extrapolate from four lakes, including a single reference lake, to a

388   broader regional trend. However, we note that surveys of regional lakes have demonstrated a

389   decline in transparency and an increase in nutrient concentration since 1930 [46]. Furthermore, a

390   2011 survey of 14 Connecticut lakes shows that *D. mendotae* and *D. pulicaria*, are usually absent

391   from oligotrophic lakes in this lake district (ESM5: text S5, table S5, figure S6). Thus, while we

392   have limited knowledge of historic regional daphniid compositional changes, the frequent

393   occurrence of these taxa associated with elevated nutrients, combined with the fact that many

394   lakes have eutrophied, support the idea that the shifts in daphniid composition observed in our

395   study lakes could be more widespread.

396   Chemical alteration of the environment by addition of nutrients and metals played a

397   significant role in explaining the patterns of daphniid zooplankton assemblage shifts documented

398   here. Eutrophication of lakes is ubiquitous in inhabited regions [12], while contamination with

399   metals affects lakes in both industrial landscapes [22] and more remote sites [25]. In addition,

400   application of $CuSO_4$ algaecide is a widespread management practice that still occurs in many

401   lakes in the US today, despite its high toxicity to aquatic life and limited effectiveness in

402 regulating symptoms of eutrophication [64]. In regions of the world where significant land

403 conversion and/or industrial activity began relatively early (e.g. Europe, Asia), anthropogenic

404 eutrophication and metal contamination have been impacting lake ecosystems for centuries (e.g.,

405 [65–67]). Given these observations, modest extrapolation of our findings suggests that

406 eutrophication and metal contamination may have driven long-term shifts in daphniid

407 zooplankton assemblages in inhabited catchments around the world. Whether such community

408 responses have occurred and how they might affect the functioning of *Daphnia* populations in

409 these lakes are questions that deserve further attention.

410

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606

607 **Figure legends:**

608 **Figure 1.** Changes in daphniid taxa relative abundances, heavy metal contamination, and

609 eutrophication in the four study lakes over the past 125 years+. Relative abundances of

610 daphniid taxa over time in the four lakes are based on analysis of diapausing egg banks.

611 Metals and eutrophication data represent the first PC scores for analyses conducted for each lake

612 ESM4: table S3 and figures S1-S2 provide additional details and plots of raw data.

613

614 **Figure 2**. RDA triplots showing significant (α<0.05) relationships between eutrophication

615 (E) or metal (M) PC1 scores, copper (Cu), in the case of Cedar Pond, and Hellinger

616 transformed daphniid species densities. High values for M and E indicate higher concentrations

617 of metals and greater eutrophication respectively. Since only one explanatory variable was

618 significant in the RDAs of Black, Alexander, and Roseland, it is only possible to display one

619 RDA axis for these plots. The X axes (RDA 1) show the relationship between eutrophication or

620 metals and daphniid composition, while the Y axes (PC 1) show unconstrained (residual)

621 variation. Species scores are represented by abbreviated species names: *Ceriodaphnia* = CER, *D.*

622 *ambigua* = AMB, *D. catawba* = CAT, *D. mendotae* = MEN, *D. parvula* = PAR, *D. pulicaria* =

623 PUL. Years indicate daphniid composition (site scores) at each time period.

624

625 **Figure 3**. Changes in similarity between modern and historic daphniid assemblages in the

626 study lakes. Community similarity measured with PCA is based on 3D Euclidean distances

627 using PC1-PC3 axis scores. For both Jaccard similarity (*J*) and PCA distance, increasing values

628 indicate greater similarity in modern sediments. Decreasing (negative) distances indicate

629 divergence over time. Panel *a* compares assemblages in eutrophying lakes; panel *b* compares

28

630   eutrophying lakes with the low-nutrient reference site, Black Pond. Lake abbreviations: B:

631   Black, A: Alexander, C: Cedar, R: Roseland. Modern J and PCA distances compare assemblages

632   in surface sediment (ca. 2011). Historic J and PCA distances compare assemblages ca. 145-115

633   years before present, matching sediment ages in each lake pair as closely as possible. Years used

634   in historic comparisons include: B-C 1873-1863, R-B 1863-1873, A-B 1888-1899, A-C 1888-

635   1898, A-R 1888-1863, R-C 1863-1863.

636

637   **Figure 4.** PCA biplot showing temporal shifts in daphniid assemblage composition in each

638   lake. The PCA is based on Hellinger transformed species densities estimated from sediment

639   diapausing egg banks. PC1 and PC3 are plotted to show as much variation in daphniid

640   community structure as possible, while ensuring that all six taxa were important in

641   explaining at least one axis of the plot. Biplots of PC axes 1-2 and 2-3 are provided in

642   ESM4 figure S4. ESM4 table S4 provides additional PCA results. Species scores are

643   represented by abbreviated species names: *Ceriodaphnia* = CER, *D. ambigua* = AMB, *D.*

644   *catawba* = CAT, *D. mendotae* = MEN, *D. parvula* = PAR, *D. pulicaria* = PUL. Site scores are

645   labelled with the approximate age of the sediment for that time period. Lines are drawn to show

646   the temporal changes in daphniid composition within each lake. Alexander=blue, Cedar=gold,

647   Roseland=red, Black=black.
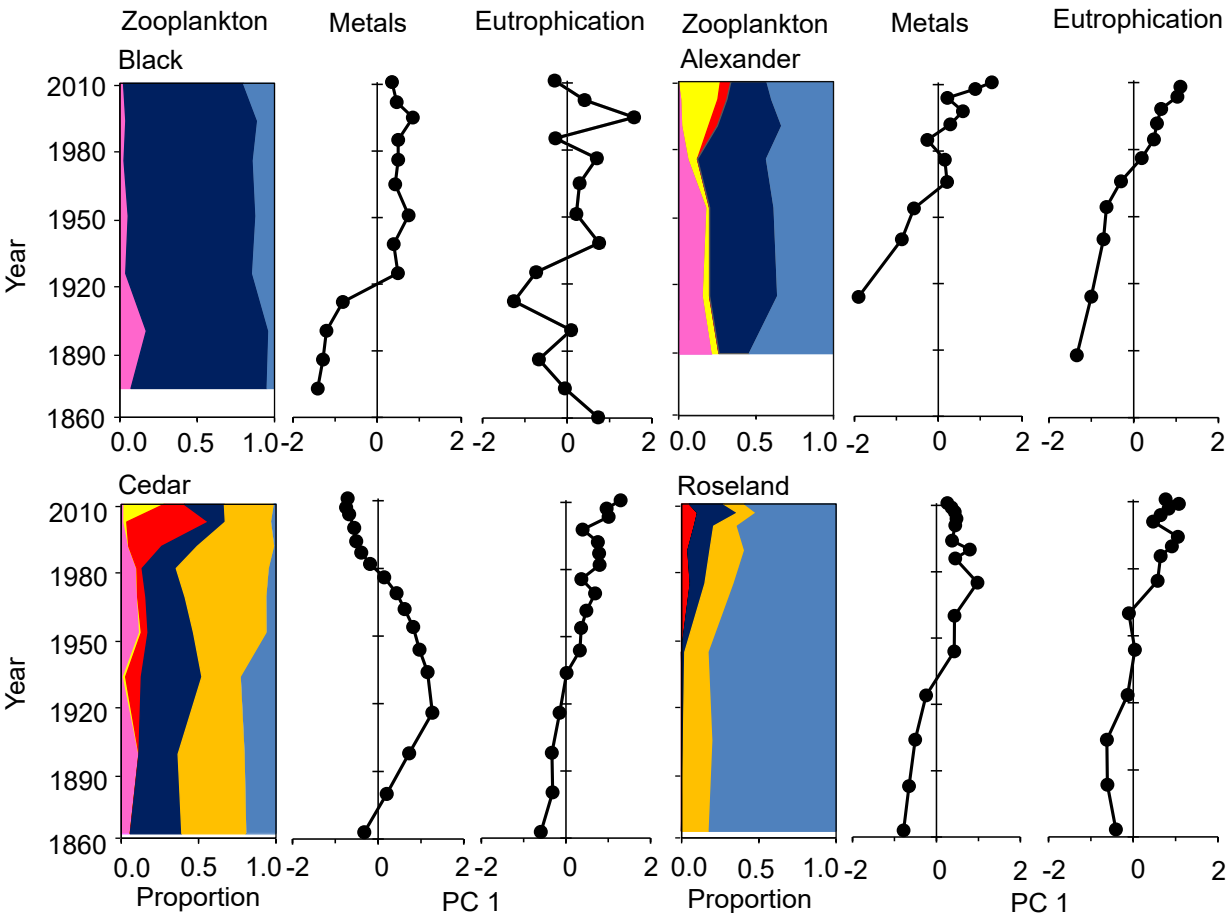
648

649

650 **Tables:**

651 **Table 1**. Variance partitioning of RDA-models relating daphniid assemblage structure

652 (Hellinger transformed species abundances at various time periods) with metal

653 contamination (PC1) and eutrophication (PC1) (electronic supplementary material, table S2).

654 Separate analyses were conducted for each lake. Amount of variation explained solely by

655 eutrophication and metals as well as their overlap is displayed. Overlap between the effects of

656 nutrients and metals cannot be statistically distinguished, as this variation could be explained by

657 two independent mechanisms running in parallel.

Lake	Index	Adj R^2	F	P value
	Nutrients	0.000	1.00	0.416
Black	Nutrients + Metals	-0.185[a]	--	--
	Metals	0.716	8.64	0.039
	Nutrients	0.254	5.10	0.064
Alexander	Nutrients + Metals	0.536	--	--
	Metals	-0.038	0.38	0.844
	Nutrients	0.346	4.636	0.017
	Nutrients + Metals	0.135	--	--
	Metals	-0.069	0.280	0.855
Cedar	Nutrients + Copper	-0.160	--	--
	Metals + Copper	-0.010	--	--
	Nutrients + Metals + Copper	0.039	--	--
	Copper	0.148	2.556	0.062
Roseland	Nutrients	0.391	10.54	0.011

Nutrients + Metals	0.380	--	--
Metals	-0.180	0.570	0.561

658   [a] Note that non-significant terms can have a negative adjusted R^2, which can affect the adjusted

659   R^2 of the significant terms.

660