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

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## PROCEEDINGS B

### **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

36

37

## 38 **Introduction**

39 We have entered the Anthropocene, an era during which human activities can profoundly  
40 influence the Earth's geological, chemical, and biological processes at a global scale [1]. One  
41 pervasive signature of land use change in this human dominated era is the increasing release and  
42 accumulation of chemical pollution in the environment. At the same time, biodiversity loss has  
43 been precipitous, and this has long been cited as a consequence of widespread anthropogenic  
44 environmental changes, including chemical pollution [2,3]. However, it can be difficult to  
45 causally link pollution with changes in species diversity. In addition, increasing biotic  
46 homogenisation of communities in human impacted environments is an emerging pattern gaining  
47 increasing recognition [4,5]. As developed landscapes begin to predominate on Earth, sensitive  
48 endemic species are often lost while human-tolerant species spread, a pattern that can reduce  
49 biotic diversity at regional scales. Such assemblage shifts have been observed in a number of  
50 groups and habitat types around the world including plants [6], molluscs [7], fish [8], amphibians  
51 and reptiles [9]. One hypothesis is that habitat degradation associated with agricultural and urban  
52 activity homogenises the physical landscape and thus its constituent ecological communities  
53 [10,11].

54 Human activities routinely change nutrient influx to lakes and can have transformative  
55 impacts on aquatic ecosystems [12]. Elevated nutrient input directly increases phytoplankton  
56 productivity and can favour blooms of toxic cyanobacteria [13], while increased phytoplankton  
57 biomass indirectly alters aquatic ecosystems by creating deep-water anoxia which can cause  
58 extensive fish kills [14] and phosphorus (P) release from sediments [12]. While eutrophication  
59 may proceed steadily, the ecological response of higher trophic level taxa such as invertebrates  
60 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 algacide 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

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 <sup>210</sup>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<sub>2</sub>. Elemental mass ratios were estimated  
124 using combustion data. Sedimentary pigments were extracted, filtered, and dried under N<sub>2</sub> 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- $\mu$ m mesh and examining the retained filtrate under a  
131 dissecting microscope at 10 $\times$  power. All ephippia were examined with a compound microscope  
132 at 100 $\times$  and 400 $\times$  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 ( $\text{CuSO}_4$ ) 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 ehippia* 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 2b-d), while both species were absent from unproductive Black  
255 Pond (figure 1). *D. pulicaria* 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 3a).  
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 3b), 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 3a). The assemblages in Alexander Lake and Cedar Pond  
277 diverged from the assemblage in the low nutrient site, Black Pond (figure 3b), 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<sub>4</sub> algacide  
337 was applied to Cedar Pond during this period of *D. pulicaria*'s absence. Similarly, we  
338 hypothesize that application of CuSO<sub>4</sub> 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 3a). 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 3a). Daphniid assemblages in eutrophying Alexander  
376 Lake and Cedar Pond diverged from the assemblage in the unproductive reference site, Black  
377 Pond (figure 3b); however, daphniids in Roseland became more similar to those in Black Pond.  
378 Although speculative, we suggest the annual application of CuSO<sub>4</sub> 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  $\text{CuSO}_4$  algacide 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

411 **Data accessibility** Supporting data have been uploaded to Dryad (doi:10.5061/dryad.2vh5c) in  
412 keeping with the journal's policies.

413 **Competing interests** We have no competing interests.

414 **Author contributions** MAR and DKS contributed to the conception and design of this study.  
415 MAR and PRL contributed to data acquisition, and MAR, DKS, and PRL contributed data  
416 analysis and interpretation. MAR drafted the manuscript and all authors contributed to revisions.  
417 MAR, DKS, and PRL agreed on the final version to be published and are accountable for the  
418 accuracy and integrity of the work.

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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 ( $\alpha < 0.05$ ) 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

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.

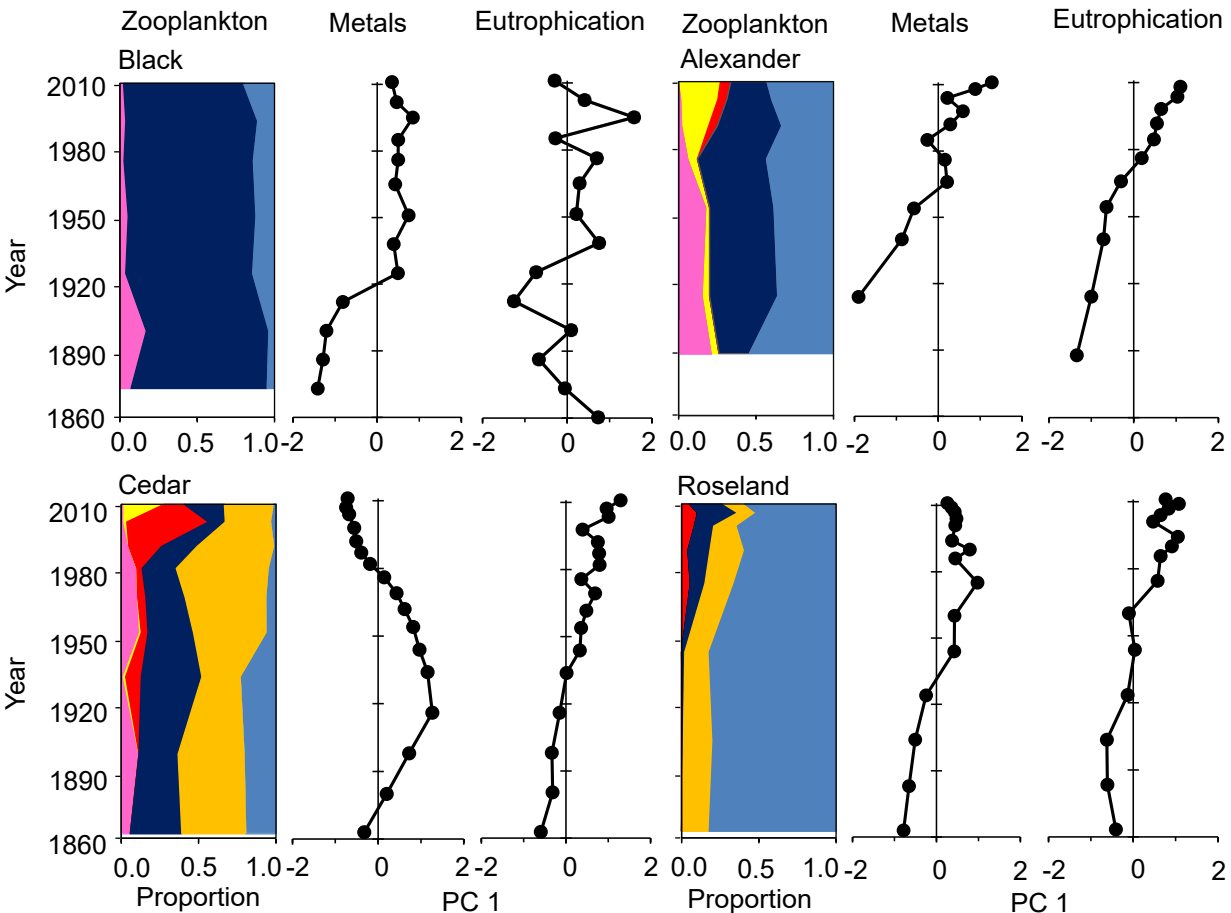
Lake	Index	Adj R <sup>2</sup>	F	P value
	Nutrients	0.000	1.00	0.416
Black	Nutrients + Metals	-0.185 <sup>a</sup>	--	--
	Metals	0.716	8.64	0.039
Alexander	Nutrients	0.254	5.10	0.064
	Nutrients + Metals	0.536	--	--
	Metals	-0.038	0.38	0.844
Cedar	Nutrients	0.346	4.636	0.017
	Nutrients + Metals	0.135	--	--
	Metals	-0.069	0.280	0.855
	Nutrients + Copper	-0.160	--	--
Cedar	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 <sup>a</sup>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



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*Ceriodaphnia*
 *D. ambigua*
 *D. catawba*
 *D. mendotae*
 *D. parvula*
 *D. pulicaria*

