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3 **TITLE:**

4 Paleolimnological fingerprinting of the impact of acid mine drainage after 50 years of chronic
5 pollution in a southern Finnish lake

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22 **Abstract:**

23 Acid mine drainage (AMD) is acknowledged to have long-lasting impacts on aquatic environments.
24 Hence, mines have also been detected to pose problems years after closure due to the leaching of
25 toxic drainage initiated by sulfide oxidation. To assess the effects of chronic but relatively low volume
26 acid mine drainage derived from the Haveri copper–gold mine operating between 1938 and 1960 on
27 a freshwater bay in southern Finland, we compared cladoceran assemblages from the pre-mining
28 period with contemporary populations using paleolimnological approaches and multiple sediment
29 cores. The cladoceran community of the pre-mining era differed significantly from the contemporary
30 community of the lake (ANOSIM $R = 0.91$; $p = 0.0001$), but closely resembled the contemporary

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COBE

fferences in species

32 compositions between pre-mining and contemporary samples are most likely caused by
33 eutrophication and not by the AMD impact. Because AMD at our study site is most intense during
34 the spring snowmelt period, cladocerans may avoid seasonal pollution peaks through winter
35 dormancy. Possible pollution peaks resulting from heavy rains during the summer may have negative
36 impacts on the cladoceran community, but such short-term impacts are probably rapidly counteracted
37 by immigration from cleaner areas of the lake.

38 **Keywords:**

39 Acidic mine drainage, impacts, cladocera, paleolimnology, chronic pollution, Finland

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43 **1 Introduction**

44 Industrial pollution is acknowledged as a major cause of surface water degradation. The mining
45 industry is one of the major polluters of freshwater ecosystems, and one source of this pollution is
46 abandoned mines. Leaching from sludge ponds and acidic drainage from tailings are known to inflict
47 serious damage on receiving ecosystems (Kelly 1988; Wolkersdorfer and Howell 2005). Acidic mine
48 drainage (AMD) is one of best-known environmental challenges in the mining industry. In short, the
49 acidic leachate is formed when sulfidic minerals are exposed to atmospheric oxygen and water. The
50 effluents are usually rich in iron (Fe) and sulfate (SO_4^{2-}), and sometimes also in toxic substances, such
51 as potentially toxic metals (e.g. Akcil and Koldas 2006).

52 In Viljakkala, SW Finland (Fig. 1), the AMD originating from the abandoned Haveri copper–gold
53 (Cu–Au) mine tailings dump drains into the bay head of Lake Kirkkojärvi. The effluent leaching from
54 the tailings into Lake Kirkkojärvi has a pH of 3.9 and extremely high metal concentrations
55 (Parviainen 2009). Kihlman and Kauppila (2010) investigated the impacts of Haveri mine pollution
56 on protist and diatom communities using continuous sedimentary records retrieved from the deep
57 area of Lake Kirkkojärvi. They concluded that metal pollution had impacted the lake biota during the
58 1960s and 1970s, whereas the most recent samples indicated eutrophication. Thus, the impact of
59 AMD in Lake Kirkkojärvi is currently relatively minor and is probably masked by eutrophication.
60 However, the bay area adjacent to the tailings may still suffer from chronic pollution, which remains
61 unnoticed when only the central basin is considered. Moreover, shallow littoral areas such as bay
62 environments, which are usually neglected in pollution studies, are well-known hotspots for lake
63 biodiversity and highly important for lake functioning (e.g. Hampton et al. 2011). For this reason,
64 shallow lakes and wetlands are also regarded as sites of special importance in European Commission
65 policies (e.g. Gattenlöhner et al. 2004). In fact, Lake Viljakkalanselkä, which is part of the same lake
66 complex as Lake Kirkkojärvi (Fig. 1), is rich in protected bird and amphibian species. For example,
67 the nearby Alholampi bay, located 1 km south of Lake Kirkkojärvi (Fig. 1), is part of the Natura 2000
68 network, hosting numerous protected bird, invertebrate, amphibian, and vertebrate species (Pitkänen
69 2007). Due to its proximity, the sheltered bays of Lake Kirkkojärvi could thus provide additional
70 habitats for the biota mentioned above.

71 Zooplankton (e.g. Jeppesen et al. 2011), and cladocerans (water fleas) in particular (Eggermont and
72 Martens 2011), are regarded as good indicators of environmental change. Over the past decades,
73 cladocerans have extensively been used in ecotoxicology (Sarma and Nandini 2006) and also in other
74 fields of environmental stress research (Suhett et al. 2015). Because cladocerans are sensitive to heavy
75 metal pollution (Brix et al. 2001; Von Der Ohe and Liess 2004), they can be used to track the
76 ecological impact of metal-contaminated mine drainage. However, two problems are usually
77 associated with research on cladoceran species assemblages in relation to decades of pollution. First,
78 long-lasting sampling programs, which could be used to assess the long-term effects of AMD, are
79 practically non-existent. Secondly, the cladoceran community fluctuates considerably during the year
80 (Whiteside 1974; George and Edwards 1974), which hinders both sampling and the interpretation of
81 the results. The paleolimnological approach, however, can overcome both of these problems, because
82 cladocerans leave well-preserved, identifiable sub-fossil remains in the lake sediment (Korhola and
83 Rautio 2001). These remains can then be used to construct both the past cladoceran communities and
84 past environmental conditions. Cladocerans have been used in paleolimnology since the 1950s (Frey
85 1986), and have successfully been applied in mining pollution research. For example, Bradbury and
86 Megard (1972) used cladoceran subfossils to assess the impact of 19th century iron mining in the
87 United States. Kerfoot et al. (1999) detected a pronounced decline in the flux of *Bosmina* remains
88 due to copper mine effluent. Doig et al. (2015) concluded that cladoceran abundance was severely
89 reduced due to metal-contaminated mine water, and Sienkiewicz and Gąsiorowski (2016) assessed
90 the impact of acidification and natural neutralization on cladoceran assemblages in a mining pit lake
91 located along the border between Poland and Germany.

92 The working hypothesis of the present study was that even though the strongest impacts of AMD are
93 no longer detectable in the deeper, central area of the Lake Kirkkojärvi, impacts can still be detected
94 in the proximal tailings area. To assess the contemporary effect of the chronic AMD, which has
95 already lasted over 50 years since the closure of the Haveri mine (Parviainen et al. 2012), we
96 compared the pre-mining and contemporary cladoceran communities in a small bay in the close
97 vicinity of the tailings dump (Fig. 1). In addition, this study aimed to verify the spatial boundaries of
98 the ecologically damaged area through multiple sampling. Our results provide new information on
99 the effects of AMD on lake biota at northern latitudes, where species assemblages and environmental
100 conditions undergo large seasonal changes. This information is relevant to stakeholders and decision
101 makers regarding protection planning, remediation actions, or water usage restrictions in cases where
102 littoral areas of lakes are under threat of pollution.

103 **2 Materials and Methods**

104 2.1 Characteristics and environmental history of Lake Kirkkojärvi

105 Lake Kirkkojärvi is located in SW Finland (61.7148 N 23.2677 E WGS84) at 83 m a.s.l. (Fig. 1).
106 Annual averages for air temperature and precipitation are 4–5 °C and 600–650 mm y⁻¹, respectively
107 (Finnish Meteorological Institute 2015), and the average winter ice-cover period in the region is
108 approximately five months from December to April (Korhonen 2005). The lake area is 0.72 km², the
109 maximum depth is about 8.5 meters, and the mean depth is 2.5 meters. Lake Kirkkojärvi is a sub-
110 basin of a larger lake, Lake Viljakkalanselkä (5 km²), which in turn is connected to Lake Kyrösjärvi
111 (92 km²) (Fig. 1). According to the national lake monitoring data, the nutrient concentrations were
112 higher in Lake Kirkkojärvi than in the neighboring Lake Viljakkalanselkä during the 1960s and
113 1970s. Furthermore, the water is substantially clearer in Lake Viljakkalanselkä compared to Lake
114 Kirkkojärvi. The water of Lake Kyrösjärvi is characterized by higher concentrations of humic
115 compounds. The town of Viljakkala has a population of approximately 2000 and the land cover in
116 the region is characterized by farmland and forests. According to historical maps, the cultivated area
117 increased in the Lake Kirkkojärvi catchment between 1848 and 1955, but has been constantly
118 declining since 1955, mainly as a result of the expansion of the town center. According to Vänni
119 (1928), the water level of Lake Kirkkojärvi was lowered by approximately 3 meters during multiple
120 occasions in the 19th century. This decreased the lake area and water volume, and probably resulted
121 in increased erosion and transport of mineral matter into the lake. Moreover, the lowering of the water
122 level terminated the water exchange between Lake Kirkkojärvi and the more humic Lake Kyrösjärvi
123 due to the drying of the connecting channel located between the former Inkula island and Viljakkala.
124 In addition, prior to the lowering of the water level, the northern connection to Lake Viljakkalanselkä
125 was deeper and wider (Fig. 1). These pronounced changes in the hydrology of Lake Kirkkojärvi
126 during the 19th century may have also affected the lake water characteristics due to changes in
127 morphometry, which regulates many important processes and variables in a lake system (Håkanson
128 2004). In addition, shallow lakes are generally more prone to changes than deep lakes because of their
129 lower heat (Shutner et al. 1983) and dilution (Janse et al. 2008) capacity. However, no data are
130 available regarding the water quality of Lake Kirkkojärvi during 19th century.

131 The Haveri Cu–Au mine was established in 1938. Waste rock and tailings material, totaling 1 400 000
132 tons, were deposited onto the former lake bed and restricted by dam structure (Fig. 1). In the late
133 1950s, the dam system broke down and a direct waterway from the tailings to Lake Kivijärvi opened
134 up. At the same time, tailings were also dumped directly into the bay (Räisänen et al. 2015), which
135 resulted in a dramatic decrease in the water depth at the head of the bay. The tailings contain high

136 concentrations of potentially toxic metals. For example, the average of Cu concentration is currently
137 831 mg/kg (Parviainen 2009). In the 1960s, mining ceased and the Haveri mine was abandoned.
138 Because of the unsuccessful and insufficient rehabilitation measures, AMD started almost
139 immediately and, according to sedimentological studies, the most intense metal load into the lake
140 occurred during the 1960s and 1970s (Parviainen et al. 2012). In 2006, the pH of the water in the
141 tailings drainage ditch, which drains into the head of the bay, was 3.5, and high concentrations of
142 dissolved metals (e.g. Al 23 600 $\mu\text{g L}^{-1}$, Co 722 $\mu\text{g L}^{-1}$, Cu 1660 $\mu\text{g L}^{-1}$, Ni 708 $\mu\text{g L}^{-1}$, and Zn 763
143 $\mu\text{g L}^{-1}$) were still recorded (Parviainen 2009). However, the AMD was found to be relatively rapidly
144 diluted on entering Lake Kirkkojärvi. In 2006, the pollutant concentrations at the mouth of the bay
145 were already clearly lower than in the drainage ditch (Al 88.8 $\mu\text{g L}^{-1}$, Co 0.4 $\mu\text{g L}^{-1}$, Cu 8.2 $\mu\text{g L}^{-1}$, Ni
146 3.3 $\mu\text{g L}^{-1}$, and Zn 2.5 $\mu\text{g L}^{-1}$). However, the sedimentary concentrations of pollutants, such as Cu,
147 are still clearly above pre-mining levels (Parviainen et al. 2012), suggesting ongoing although clearly
148 reduced pollution.

149 2.2 Study site

150 The study site was a bay that lies in the western part of Lake Kirkkojärvi and is the closest water body
151 adjoining the tailings disposal site. The bay is approximately 300 m long and 130 m wide (Fig. 1).
152 The maximum depth in the middle part of the bay is 3 meters, whereas the western part of the bay
153 comprises very shallow wetland (<0.5 meters). The bay has a shallow sill (1 m) across the inlet, which
154 partly restricts the entrance of material from the main basin. Vegetation is most abundant in the
155 western section of the bay and mainly consists of *Equisetum fluviatile* (L.), whereas *Potamogeton*
156 *natans* (L.), *Sparganium emersum* (Rehmann), and *Nuphar lutea* (L.) are relatively sparse.

157 2.3 Sediment and water sampling

158 We used the top–bottom (after–before) approach, where the bottom sample is assumed to represent a
159 time period before a given point of interest, i.e. the time before the commencement of the mining
160 operations, whereas the top sample represents the contemporary situation. The use of two snapshots
161 enables the comparison of environmental conditions between two time periods over a large area
162 compared to the time-consuming sequential study of a whole core (e.g. Michelutti et al. 2001;
163 Weckström et al. 2003; Korosi et al. 2012). A total of 18 samples from 9 sites were retrieved from
164 the AMD-impacted bay with a Russian peat corer in May 2016. The core length ranged from 0.35 m
165 to 1.35 m. Each core was sectioned using a metal slate, and the samples were stored in plastic zip-
166 lock bags and kept at $\sim 4\text{ }^{\circ}\text{C}$ prior to analysis. The top sample (1 cm) represents the contemporary
167 period, whereas the bottom sample (1 cm) was retrieved from varying sediment depths (0.20–1.30

168 m). The bottom samples were assumed to represent the time before mining on the basis of the
169 sediment characteristics, such as pronounced layers of tailings material, and earlier studies (Kihlman
170 and Kauppila 2010). We did not use any conventional dating methods (e.g. radiometric methods) to
171 date the bottom samples, because the direct dumping of tailings material has probably resulted in
172 considerable resuspension of lake sediments. To determine whether the ecological effects of AMD
173 could be detected along a spatial gradient from the area near the tailings towards the mouth of the
174 bay, we organized the sampling to cover the whole bay, except for the shallow (<0.5 m) bay head,
175 where the ice cover reached the lake bottom. In addition, we collected two surface sediment samples
176 from presumably unpolluted reference sites to investigate the contemporary cladoceran communities
177 in the bay environments, which are currently under a different type of human impact. The Lake
178 Kirkkojärvi reference site (R1; Fig. 1) is probably affected by farming due to the high proportion of
179 fields in the surroundings of the sampling site. According to old maps, the nearshore area has been
180 under cultivation at least since 1800 AD. Aquatic vegetation is abundant and consists of the same
181 species as in the polluted bay. In contrast, the Lake Viljakkalanselkä reference site (R2; Fig. 1) is
182 characterized by high cliffs and a lack of farming and other intensive human activity in the nearby
183 land areas, and the sparse aquatic vegetation is restricted to a narrow zone near the shoreline.

184 To verify whether AMD is still leaching from the tailings area, we measured the water pH (Merck
185 pH-indicator paper) in the gullies flowing on top of tailings dump. In addition, we collected water
186 samples from the polluted bay (sites 1 and 9; Fig. 1) and from Lake Viljakkalanselkä (W; Fig. 1) to
187 assess the current concentrations of nutrients and selected metals (Al, Cu and Ni). These same
188 elements exhibited clearly elevated levels in samples retrieved from the ditches on the top of the
189 tailings in 2006–2007 (Parviainen 2009) (Fig. 1, Table 1). The water sample reference site W was
190 chosen to represent a similar environment in terms of water depth and catchment land cover as in the
191 main study bay. Water samples (0.5 L) were retrieved from the depth of 0.5 meters into polyethylene
192 bottles in July 2016 and stored in the dark at 4 °C prior to analysis. The water samples were analyzed
193 for total ionic concentrations in accordance with ISO 17294-2:2003 at the Metropolilab Helsinki
194 environmental laboratory, which is an accredited testing laboratory (FINAS T058).

195 2.4 Cladoceran analysis

196 Cladoceran analysis and species identification were conducted according to Korhola and Rautio
197 (2001) and Sarmaja-Korjonen and Szeroczyńska (2007). Briefly, sediment samples of approximately
198 1 cm³ were first mixed with 10% KOH and heated to ca. 90 °C on a hotplate for 45 minutes. The
199 samples were sieved through a 50-µm mesh using tap water and the residue was pipetted into test

200 tubes and centrifuged. Excess water was removed and permanent microscopic slides were prepared
201 with safranin-stained gelatin glycerol for microscopic analysis. Cladoceran body components were
202 identified and the number of individuals was based on the most numerous component. Here, a
203 minimum of 133 individuals were enumerated per sample.

204 2.5 Numerical analysis

205 To examine the differences between pre-mining and contemporary cladoceran assemblages, we used
206 analysis of similarity (ANOSIM), which is a non-parametric test for significant differences between
207 groups (Clarke 1993). Proportional cladoceran data were $\log(x+1)$ transformed prior to ANOSIM
208 calculations to stabilize the variance. In addition, we conducted the non-metric multidimensional
209 scaling (NMDS) procedure to examine the variation in our samples. The Bray-Curtis dissimilarity
210 index was used in the ANOSIM test and in NMDS. The latter is suitable for biological community
211 data containing zero values (Minchin 1987). We used Pearson's correlation method to assess the
212 relationship between cladoceran species and both distance from the tailings and water depth. The
213 Shannon H' diversity index was used to rate species diversity in samples. Numerical analysis was
214 conducted using PAST statistics software 3.1. (Hammer et al. 2001).

215 **3 Results**

216 3.1 Sediment properties

217 Sediment stratigraphy varied between sites. The upper 2- to 5-cm-thick layers of each core consisted
218 of brown organic sediment, while each core included a black section of varying thickness (5–50 cm)
219 below the organic surface layer. The black layer consisted of extremely fine mineral matter
220 corresponding to tailings material. In cores 3, 5, 6, 7, and 9 (Fig. 1), there was also a layer of watery
221 clay up to 30 cm thick, which transitioned again to a black layer and gradually to light brown sediment
222 (Fig. 2). In other cores without a distinct clay layer, the black layer gradually changed to brown
223 material. All bottom samples were taken from the brown layer below the transition zone, except for
224 sites 2 and 3, where the corer did not reach the brown sediment layer.

225 3.2 Water quality

226 Because the ditch leading from the tailings dump into the lake via the failed dam was dry, we could
227 not measure the dilution of AMD in the Kirkkojärvi bay during our sampling campaign. However,
228 the water on top of the tailings was clearly acidic with a pH of 3.5. Nutrient and metal concentrations
229 were slightly higher in the Kirkkojärvi bay compared to the Lake Viljakkalanselkä water quality

230 reference site W. The highest concentrations of nutrients and metals were detected in the mouth of
231 the polluted bay (Table 2). Our water sample results are highly similar to those of Parviainen (2009)
232 regarding metal concentrations in a lake water sample at the mouth of the bay during the dry season.

233 3.3 Cladoceran assemblages

234 Cladoceran subfossil remains were numerous and generally well preserved, except in bottom samples
235 from coring sites 2 and 3, which consisted of tailings material and did not contain any cladoceran
236 remains. However, because of the high similarity among the pre-mining samples, we decided not to
237 retrieve additional bottom samples from sites 2 and 3. The total number of individuals enumerated
238 was 1574 in all contemporary samples (9 samples) and 1279 in pre-mining samples (7 samples),
239 whereas the number of enumerated individuals at the reference sites was 133 for R1 (1 sample) and
240 166 for R2 (1 sample). Altogether, 38 taxa were recorded. The average number of taxa identified was
241 18 in the contemporary samples and 20 in the pre-mining samples. Planktonic species exhibited the
242 highest proportions in all samples. The overall proportion of planktonic taxa (as classified in Bjerring
243 et al. 2009) was lower in the top samples (71.1%, SD 8.3) compared to the bottom samples (76.7%,
244 SD 4.2). The species composition in the samples is presented in Table 3. The average Shannon
245 diversity index was 0.927 for the pre-mining period and 1.528 for the contemporary period. The
246 cladoceran community differed significantly between the pre-mining and contemporary samples
247 (ANOSIM $R = 0.9081$; $p = 0.0001$). However, as illustrated in NMDS plot, the cladoceran
248 composition was highly similar in the contemporary and pre-mining samples of the polluted bay (Fig.
249 3). Contemporary cladoceran communities in the non-agricultural reference site R2 closely resembled
250 the pre-mining samples of the polluted bay, whereas the cladoceran assemblage in the agriculturally
251 impacted reference site R1 was more similar to the contemporary communities in the polluted bay
252 (Fig. 3, Table 3). The most visible change in cladoceran species assemblages of pre-mining and
253 contemporary samples was the appearance or higher relative abundances of *Bosmina longirostris* and
254 *Alonella nana* in the contemporary samples, whereas in the pre-mining samples, these taxa were
255 practically absent. Moreover, *Eubosmina longispina*, *Rhynchotalona falcate*, and *Monospilus dispar*
256 had generally lower abundances in the contemporary samples (Table 3). In contrast to the pronounced
257 differences between pre- and post-mining cladoceran communities, differences between near-tailing
258 samples and the samples retrieved from the mouth of the bay were nearly non-existent (e.g. sample
259 numbers 2 and 9). This is clearly illustrated in the NMDS scatterplot, where the contemporary
260 samples are situated close to each other without any correlation with distance from the AMD source
261 (Fig. 3). In the species-level correlation test, only *A. nana* showed a significant correlation with

262 distance from the AMD (Pearson correlation -0.74; $p = 0.022$), as it was more abundant close to the
263 tailings regardless of the water depth.

264

265 **4 Discussion**

266 The origin of the clay section in samples 3, 5, 6, 7, and 9 (Fig. 1) is unknown, but may be related to
267 the disposal of tailings and consequent disturbance of the original bottom material or to the
268 construction of the dam structures. The sediments underlying the tailings are comprised of silt and
269 clay (Parviainen 2009), which may have been mobilized during the construction work. Flood events
270 (Thorndycraft et al. 1998) and other catchment-related disturbances (Dearing 1991) are also known
271 to induce pronounced resuspension and sedimentation processes resulting in distinct zones in the
272 sediment record. The relatively thin layer of natural lake sediment above the tailings material
273 compared to the deep section of the lake (Kihlman and Kauppila 2010) indicates different
274 sedimentation dynamics in the shallow bay (e.g. Boggs 2006). Acidic mine water is clearly still being
275 produced on top of the tailings dump, as predicted by Parviainen (2009). However, at least during the
276 dry season, relatively little AMD leaks into Lake Kirkkojärvi. Nevertheless, even in the absence of
277 direct AMD inflow, metal concentrations are higher in the polluted bay than in Lake Viljakkalanselkä
278 (W). This is probably caused either by continuous dam leaching or other sources, such as sedimentary
279 release or runoff from the catchment area. The slightly lower metal concentrations at sampling site 1
280 are probably connected to the dense beds of *E. fluviatile*, which is known to have an extremely high
281 potential for metal accumulation (Bateman 1999). This sampling site is also more sheltered compared
282 to site 9, for example, which in turn is more exposed to wave-induced resuspension of sediment due
283 to the larger effective fetch (see e.g. Dearing 1997). The difference in cladoceran species numbers
284 between the impacted bay and the reference sites can most probably be explained by the higher
285 number of counted individuals in the impacted bay. Because of this, rare species were probably
286 missed at the reference sites. In all samples, the cladoceran community was largely composed of
287 species that are common in North European lakes (e.g. Bjerring et al. 2009).

288 4.1 Comparison of pre-mining and contemporary communities

289 It is not clear whether our bottom samples were deposited prior to the period of repeated water level
290 manipulations in the latter half of the 19th century, but because the sedimentation rate in Lake
291 Kirkkojärvi is approximately 0.5 cm y^{-1} (Kihlman and Kauppila 2010), and because black tailings
292 material was probably deposited very rapidly, the depths of the bottom samples (5 to 15 cm below

293 the tailings material) are probably not enough for them to originate from the first half of the 19th
294 century. The water depth at the study site was certainly affected by the dumping of tailings material
295 in the 1950s, which resulted in bottom elevation, particularly at the head of the bay. This may explain
296 the higher proportion of planktonic species in pre-mining samples, as the planktonic/littoral ratio is
297 generally considered a good indicator of water level fluctuations (e.g. Korhola et al. 2005; Nevalainen
298 et al. 2011). At the species level, the change in the planktonic/littoral ratio resulted from an increased
299 abundance of *Alonella nana*, which is regarded as a macrophyte-associated species (Bjerring et al.
300 2009; Adamczuk 2014). This is consistent with the high abundance of aquatic vegetation at the head
301 of the bay.

302 In northern Europe, *E. longispina* is more common in acidic environments than *B. longirostris*
303 (Uimonen-Simola and Tolonen 1987; Bērziņš and Bertilsson 1990; Nilssen and Sandoy 1990), for
304 which reason the higher relative abundance of *B. longirostris* in the contemporary samples of the
305 studied bay may suggest that the effect of mine effluent is not so significant at our study site.
306 Moreover, European populations of *B. longirostris* are regarded as intolerant of copper pollution
307 (Koivisto et al. 1992; Bossuyt and Janssen 2005) and should not be able to thrive if Cu pollution is
308 still high enough to cause biological damage. The literature considering the acidity preferences of
309 littoral species is more limited, but some general classification proposals exist. According to Krause-
310 Dellin and Steinberg (1986), *R. falcata* is an acidophilic species, whereas *M. dispar* is classified as
311 alkaliphilous. *M. dispar* was present in lower proportions in our contemporary samples, but the
312 similar change within *R. falcata* undermines any generalizations regarding the impact of AMD and
313 littoral taxa. *Chydorus sphaericus*, which was more abundant in the contemporary samples, is known
314 to tolerate acidic mine pollution relatively well (Belyaeva and Deneke, 2007; Sienkiewicz and
315 Gąsiorowski, 2016). However, as the abundance of *C. sphaericus* was also high at the reference site
316 R1, its recent success in Lake Kirkkojärvi probably results from factors other than mine pollution.
317 An interesting species in this regard is also *A. nana*, which was more frequent in contemporary
318 samples and had the highest contemporary abundance at the head of the bay, where the AMD impact
319 is strongest. Many cladoceran species are known to adapt to copper pollution (Bossuyt and Janssen
320 2005; Agra et al. 2011), but no information regarding the tolerance or adaptation of *A. nana* to AMD
321 or metals is currently available. However, *A. nana* was also common at reference site R1, and the
322 high relative abundance near the bay head may be due to other factors than its pollution tolerance,
323 such as the high amount of aquatic vegetation in the shallow head of the bay.

324 The observed low ecological impact of mine pollution may be connected to the seasonality of both
325 AMD and cladocerans. The highest influx of AMD into the investigated part of the bay occurs during
326 the spring snowmelt, when most of the cladoceran species are still absent (e.g. Koksvik 1995).
327 Cladoceran numbers are usually very low during winter, as communities emerge later in spring from
328 dormant eggs (ephippia). This coincidence may explain the lack of a pollution effect on cladocerans
329 at our study site. Potentially high mortality among overwintering individuals is later compensated by
330 resting egg hatchlings. In addition, rainy seasons may also affect the AMD input (Parviainen 2009),
331 which probably results in marked fluctuation in AMD-associated water parameters. The role of
332 dilution with almost neutral water is crucial to the attenuation of AMD toxicity (Filipek et al. 1987;
333 Yu and Heo 2001; Navarro Torres et al. 2011). This is mainly because pH is one of the most important
334 regulators of the bioavailability of metals in AMD (Chapman et al. 1983; John and Leventhal 1995),
335 and the damaging effect of acidity itself also decreases with increasing neutrality. Moreover,
336 cladocerans are capable of escaping from polluted environments (Lopes et al. 2014), and even if the
337 summer community of cladocerans is greatly altered by AMD during occasional heavy rains, the
338 effect may not be visible in our samples if the bay is rapidly recolonized by cladocerans from less-
339 impacted areas of the lake. Generally, cladocerans are passively transported by wind-induced water
340 movements, but are also known to exhibit horizontal migration (e.g. Burks et al. 2002) and are rapid
341 colonizers of newly formed habitats, even when a water connection does not exist (e.g. Louette and
342 De Meester 2005). In addition, based on the almost neutral pH and low Cu concentrations in water
343 samples 1 and 9, the submerged tailings do not affect the water quality. This is also suggested by the
344 presence of *B. longirostris*, which is a Cu- and pH-sensitive species. In addition, the black color of
345 the tailings suggests that no notable alteration or metal release has taken place, probably due to the
346 anoxic conditions (e.g. Salomons 1995).

347 Because the observed changes in alkaliphilous and acidophilous cladoceran species are not in close
348 agreement with what would be expected if AMD had a strong effect on the community, there must
349 be other explanations for the detected community change. Kihlman and Kauppila (2010) noted clear
350 changes in diatom and lacustrine protists communities in sedimentary samples retrieved from the
351 deepest part of Lake Kirkkojärvi covering the last ca. 70 years. Ecological shifts were seen to coincide
352 with sedimentary metal peaks of the 1960s and 1970s, and were interpreted to indicate the most
353 intense AMD release from the tailings. However, diatom and lacustrine protist communities have not
354 returned to their pre-mining composition, but instead to a community structure that indicates nutrient
355 enrichment rather than pollution (Kihlman and Kauppila 2010).

356 In the absence of convincing evidence regarding the impact of AMD on cladoceran populations, there
357 are relatively strong indications that the community change in the contemporary lake sediments has
358 resulted from eutrophication. Many studies have suggested *C. sphaericus* and *B. longirostris* to be
359 indicators of eutrophication (e.g. Whiteside 1970; Korhola 1990; Korponai et al. 2011; Nevalainen
360 and Luoto 2013). In particular, the replacement of *Eubosmina* by *B. longirostris* is considered as a
361 typical result of eutrophication (Goulden 1964; Crisman and Whitehead 1978). The higher abundance
362 of *B. longirostris* is most probably related to food availability, as the food concentration has been
363 noted to correlate positively with most reproductive parameters of *B. longirostris* (Urabe 1991;
364 Mason and Abdul-Hussein 1991). In addition, *B. longirostris* is known to have the capacity to
365 withstand the toxins of blue-green algal, which often thrive in eutrophicated waters, better than many
366 other cladoceran species (Fulton 1988). The small-bodied *Bosmina* species have low food reserves
367 and rapidly die due to starvation (Goulden and Hornig 1980). Therefore, the food supply must be
368 permanently adequate to sustain the increasing population and allow it to dominate the cladoceran
369 community. Moreover, *R. falcata* was classified as an oligotrophic/acidophilic species by Bjerring et
370 al. (2009), and its disappearance during the last few decades from the bay of Lake Kirkkojärvi may
371 indicate eutrophication. In addition, the higher Shannon diversity in the contemporary samples may
372 indicate eutrophication, as demonstrated by Korponai et al. (2011). The fact that the contemporary
373 cladoceran community at the reference site located in the less eutrophic Lake Viljakkalanselkä (R2)
374 was found to resemble that of the pre-mining era in Kirkkojärvi bay also suggests possible
375 eutrophication of Lake Kirkkojärvi bay. Furthermore, the diatom-based total phosphorus and pH
376 models constructed by Kihlman and Kauppila (2010) indicate eutrophication instead of acidification.
377 The modeled phosphorus levels have increased from approximately 15 $\mu\text{g L}^{-1}$ to 21 $\mu\text{g L}^{-1}$ during the
378 past decades, whereas pH has modestly increased from 6.6 to 6.8. As noted by Kihlman and Kauppila
379 (2010), the eutrophication has been gradual and the community change has been directional. It is thus
380 likely that the increased nutrient concentrations derived from agricultural land use in the catchment
381 have been shaping the cladoceran communities of the lake more in the recent past than the AMD from
382 the closed mine. Lake Kirkkojärvi is thus an example of a lake where human impacts, such as
383 eutrophication, have turned a lake pollution case into a multiple-stressor problem (e.g. Ormerod et al.
384 2010).

385 **5 Conclusions**

386 Although harmful mine drainage water globally affects aquatic ecosystems, most lakes are also
387 subjected local low-intensity disturbances such as anthropogenic eutrophication. Multiple-stressor

388 scenarios are of particular importance in shallow lakes and nearshore areas. These are the most
389 vulnerable environments due to the low water volume, but are ecologically highly important for lake
390 functioning. In Lake Kirkkojärvi, the cladoceran community of the pre-mining era differed
391 significantly from the contemporary community of the lake, and closely resembled the contemporary
392 community of a nearby non-polluted reference site. The relatively weak, yet chronic, disturbance
393 signal due to AMD is most likely overridden by eutrophication. The cladocerans avoid the spring
394 peak period of AMD pollution through dormancy and are more strongly affected by water
395 characteristics in the summer. Moreover, the relatively thin layer of lake sediment above the tailings
396 sediment sequence is adequate to protect the cladoceran community from the harmful effects of toxic
397 substances. This is highly important in terms of remediation planning. The results of this study
398 emphasize the importance of local conditions and species life strategies and highlight the difficulty
399 in making any generalizations regarding pollution impacts, especially if they are assessed using lake
400 biota as environmental indicators.

401 **Compliance with Ethical Standards**

402

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404

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407

408 **Conflict of interest**

409

410 The authors declare that they have no conflicts of interest.

411

412 **Ethical approval**

413 This article does not contain any studies with human participants or animals performed by any of
414 the authors.

415 **References**

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605 **Figure captions**

606 **Fig. 1** Location of the study site and the sampling sites

607 **Fig. 2** Simplified sediment stratigraphy, sampling horizons, and thickness variation of different
608 zones

609 **Fig. 3** NMDS bi-plot for pre-mining samples (filled circles) and contemporary samples (open circles).
610 Stars represent reference samples R1 (agriculturally impacted) and R2 (non-agriculturally impacted).
611 Stress = 0.075.