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4   **Benthic foodweb structure in a large shallow lake studied by stable isotope analysis**

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Abstract: The benthic foodweb structure of Lake Võrtsjärv, a large (270 km^2), shallow, and turbid Estonian lake, was evaluated based on C and N stable-isotope signatures (δ^{13}C, δ^{15}N). Variation in δ^{13}C between sampling sites was not related to site proximity to the littoral zone or the more vegetated southern part of the lake, but rather appeared to be influenced by in-situ site peculiarities. δ^{13}C was stable temporally and between functional feeding groups, a result implying that the whole benthic food web of the lake relies largely on the same C source admixture, essentially particulate organic matter (POM). Thus, the foodweb composition of Lake Võrtsjärv is remarkably homogeneous given the lake's large surface area. Apparent trophic-level δ^{15}N fractionation between total collectors and total predators (mean 1.7‰) was lower than the value of 3.4‰ generally adopted in foodweb studies, but the higher value was valid for specific prey–predator links. The low δ^{13}C signature of some chironomid samples indicated probable assimilation of methane-oxidizing bacteria (MOB) by these sediment-dwelling invertebrates. However, the lack of similar ^{13}C depletion in benthic filterers (mussels) indicated that the MOB layer is essentially confined to the sediments and does not reach the water column, which probably constrains transfer of methane-derived C through the food web to fish in this lake. Our study demonstrates that the benthic food web of shallow turbid lakes like Võrtsjärv is simplified and is mostly sustained by phytoplanktonic C sources.

Key words: carbon and nitrogen stable isotopes, benthic food webs, Lake Võrtsjärv, macroinvertebrates, carbon source, methane-oxidizing bacteria, trophic levels

37   Benthic food webs have been underresearched compared to pelagic food webs, but they

38   are of great importance for energy flux and organic-matter cycling in lakes (Vadeboncoeur et al.

39   2002). In clear-water oligotrophic lakes, primary production is usually dominated by benthic

40   periphyton (Ask et al. 2009). Conversely, in eutrophic, turbid lakes, most of the primary

41   production is by phytoplankton because the euphotic zone does not reach the bottom of the lake.

42   Thus, light restricts periphyton production (Vadeboncoeur et al. 2003). Nevertheless, benthic

43   secondary production often is extremely important in these lakes. Phytoplankton assemblages in

44   eutrophic lakes frequently comprise poorly grazed colonial or toxic cyanobacteria (Scheffer et al.

45   1997). Instead of being consumed by zooplankton, this phytoplankton production is assimilated

46   into the microbial loop by bacterioplankton (Agasild and Nõges 2005, Zingel et al. 2007) or

47   provides food for benthic detritivores after it sediments to the bottom of the lake. In shallow

48   lakes, the main benthic consumers are bacteria and macroinvertebrates like chironomid larvae

49   and bivalve mollusks (Wetzel 2001). Thus, in shallow, well-mixed lakes, benthic food chains

50   appear to play an important role in the cycling of organic matter (OM) produced by pelagic

51   phytoplankton (Weidel et al. 2008).

52   Shallow, eutrophic Lake Võrtsjärv (Estonia) exemplifies the coupling between pelagic

53   primary production and benthic secondary production. The Võrtsjärv benthic zone can be

54   divided into 2 main areas: a sheltered, macrophyte-rich zone from the southern end of the lake

55   (Väike-Emajõgi river mouth) to the broadening of the lake basin south of Tondisaar islet; and a

56   much larger, mostly unvegetated area that occupies the rest of the lake (Feldmann and Mäemets

57   2004). In the main basin, wind-induced resuspension of sediments causes high turbidity in the

58   water column (Secchi depth < 1 m) and restricts the euphotic zone to a depth of ≤2 m (Reinart

59   and Herlevi 1999). Low irradiance in the water column selects for shade-tolerant phytoplankton

60   species, and benthic primary production is severely light limited (Nõges et al. 2004b). In

61   Võrtsjärv, most of the primary producer biomass is constituted by planktonic cyanobacteria

62   (Nõges and Nõges 2012). Diatoms are present, but they are mostly planktonic (Heinsalu et al.

63   2008), so over most of the lake bottom, epipelic and epipsammic primary production is assumed

64   to be very low. Thus, benthic food webs in Võrtsjärv are probably heterotrophic, sustained by C

65   from the microbial loop, sedimenting phytoplankton, and particulate organic matter (POM)

66   originating from the littoral and inflow rivers (Nõges et al. 2004a). A study of Võrtsjärv fish

67   communities revealed that, in 2009–2011, benthivorous fish composed ~50% of the species and

68   70% of the fish total biomass (Järvalt et al. 2011). These findings demonstrate the importance of

69   the benthic food web in transferring OM from phytoplankton to fish in this lake. However,

70   pelagic and littoral components of Võrtsjärv have received extensive attention, but detailed

71   knowledge of the benthic foodweb structure is lacking (Kangur et al. 2004).

72   Benthic primary production is very low in Võrtsjärv because the compensation depth is

73   well above the bottom of the lake, so benthic invertebrate consumers are mostly sustained by a

74   limited number of sources: POM derived from sedimented phytoplankton and detrital material

75   originating from macrophyte decay (Nõges et al. 2004a). Thus, markers of consumer food source

76   and trophic level, like C and N stable isotopes (SI) signatures, respectively (δ^{13}C and δ^{15}N;

77   Peterson and Fry 1987), are expected to reflect these patterns. In general, δ^{13}C varies little with

78   trophic level and constitutes a good tracer for organic-matter source (Peterson and Fry 1987,

79   Cremona et al. 2009). On the other hand, δ^{15}N increases 1 to 4‰ between food source and

80   consumer (Post 2002, Cremona et al. 2010) and is a good indicator of the trophic level of an

81   organism. Therefore, we used C and N SI analysis to assess the structure of Lake Võrtsjärv

82   benthic food webs. Our working hypotheses were that: 1) δ^{13}C signatures of benthic consumer

83   taxa at the same site would show little variation because of their similar dependence on

84   homogeneous C sources; and 2) $\delta^{13}C$ and $\delta^{15}N$ signatures would show a shift between sites along

85   a south–north, on-shore/off-shore axis as the contribution of sedimented phytoplankton to the

86   total OM increases relative to the littoral or riverine contributions.

87

88   **METHODS**

89   **Study site**

90   Lake Võrtsjärv (surface area at mean water level ca 270 km^2) is a eutrophic, polymictic

91   lake in southern Estonia. The lake is shallow (mean depth = 2.8 m) and, because of poor outflow

92   conditions and unregulated flow, experiences important water-level fluctuations (Nõges et al.

93   2003) that affect its surface area and volume (Järvet 2004). The lake has well buffered water (pH

94   = 8.1) with medium hardness (335 µS/cm; Tuvikene et al. 2004). Two 4th-order rivers (Õhne and

95   Väike Emajõgi) are the largest inflows to the lake. The pH in their lower reaches (8.0 and 8.1,

96   respectively) is similar to that in the lake (Järvekülg 2001). Sapropel (lake mud) forms about ⅔

97   of the upper part of the bottom sediments, dominating particularly in the southern part of the lake

98   (Raukas 2004). The distribution of aquatic vegetation is uneven. The southern tip of the lake is

99   heavily vegetated, but vegetation becomes gradually sparser as the lake widens and is more wind

100   exposed. The northern part of the lake is essentially vegetation free, except along the shores,

101   because of unfavorable conditions (Fig. 1). Tondisaar islet can be regarded as a boundary point,

102   south of which conditions are better for macrophyte colonization (Feldmann and Mäemets 2004).

103

104   **Sample collection**

105   Foodweb component samples were collected during the ice-free periods of 2008–2010.

106   Eight lake (L1–8) and 3 inflow river (S1–3) sites (Table S1) were selected for invertebrate

107   sampling (Estonian State Environmental Monitoring 2011). The lake sites stretched along a

108   south–north gradient from the southernmost part of the lake to north of Tondisaar islet (Fig. 1).

109   The river sites comprised downstream reaches of the 2 main inflow rivers Väike Emajõgi and

110   Õhne. The main substratum in the rivers and in the lake is fine sand and silt. Sites S1–3 and L1–

111   5 were covered with macrophytes, with the 2 dominant species being emergent *Phragmites*

112   *australis* (Cav.) Trin. ex Steud. and submerged *Myriophyllum spicatum* L. 1753. Sites L6 and 7

113   did not have noticeable macrophyte presence, whereas site L8 had very low macrophyte density.

114   Flow at the river sites was very low or absent (0–0.5 m/s; Estonian Institute of Hydrology and

115   Meteorology). Invertebrate samples were collected with a 0.5-mm-mesh hand net with different

116   handle lengths (2–6 m) and by Borutski and Zabolotski grabs. In the laboratory, taxa were

117   identified to the lowest practical taxonomical unit, which was often genus for Chironomidae

118   (*Chironomus* in offshore areas, *Stictochironomus* in nearshore areas). The following taxonomic

119   keys were used for identifying invertebrates: Killeen et al. (2004) for mussels, Timm (2009) for

120   annelids, and Nilsson (1996, 1997) for insects. Individual taxa were isolated with steel forceps

121   under a microscope. Chironomid larvae and unionid mussels formed most of the material.

122   Sediment samples were collected only at L1 and L6 with a Willner sediment corer. In the

123   laboratory the 25-cm-long sediment core was sectioned into 2-cm (top layer), 3-cm, and three 5-

124   cm-thick slices, which were freeze-dried for the later analyses. POM from rivers and Võrtsjärv

125   was sampled once a month by sieving a depth-integrated water sample through a 100-µm net and

126   then collecting material onto precombusted (550°C, 3 h) GF/F filters. The 2 dominant

127   macrophytes (*P. australis* and *M. spicatum*) were collected manually 3 times during the

128   vegetated period (May, August, and October) in 2008 from L1.

129

130 **SI analyses**

131   Because of the high inorganic C content in Võrtsjärv lake water, $\delta^{13}C$ and $\delta^{15}N$ values of

132 sediment, *M. spicatum* (not *P. australis* because this species has aerial leaves), and POM filters

133 were measured in separate analyses. These samples were all acidified prior to the $\delta^{13}C$ analyses.

134 The freeze-dried sediment was treated with 12% HCl to remove inorganic C. Approximately 3

135 mg of dried, acidified (only for $\delta^{13}C$), and pure material was used for $\delta^{15}N$ and $\delta^{13}C$ analyses.

136 For sediments, average stable N and C signatures of the sliced 25-cm sediment core were used.

137 For macrophytes, the whole aboveground plant (leaves and stems) was used for SI analyses.

138 POM filters and macrophytes were maintained for 24 h in HCl vapors and then dried to constant

139 mass at 60°C. The collected material was dried, pulverized, and stored as a ground powder.

140   An average of 10 individuals from each invertebrate sample was used for SI analyses.

141 The shells of snails and mussels do not reflect the SI signature of their diets (McConnaughey et

142 al. 1997), so they were removed manually with forceps and only the soft tissue was used. Prior to

143 analysis, invertebrate samples were dried to constant mass at 60°C. Failure to allow organisms

144 clear their gut content could bias SI analyses done on whole individuals (Junger and Planas

145 1994), but the need to clear gut content has been questioned (Kaehler and Pakhomov 2001,

146 Jardine et al. 2005, Cremona et al. 2009). Therefore, we tested the extent to which gut clearance

147 would influence SI signatures of Lake Võrtsjärv Chironomidae because they made up the

148 overwhelming majority of the macroinvertebrate samples. Three groups of animals were kept

149 alive for 12 h before drying to permit clearance of their gut contents. The SI values for these

150 groups were compared with those of control groups dried immediately without gut clearance.

151 Comparison made with a nonparametric Mann–Whitney *U* test revealed no significant difference

152   between the treated and untreated groups ($p > 0.05$, $n = 10$–24; Table S2). Therefore, subsequent

153   samples were processed without gut clearance.

154   All SI analyses were done at the University of Jyväskylä (Finland) using a Flash EA 1112

155   elemental analyzer connected to a DELTAplus Advantage IRMS (Thermo Finnigan, Bremen,

156   Germany). SI ratios are expressed as δ values ($\delta^{13}C$, $\delta^{15}N$) in ‰ referenced to the international

157   standards for C (PeeDee Belemnite) and N (atmospheric N_2). Internal laboratory standards were

158   homogenized potato leaves for POM, macrophytes, and sediment and white muscle tissue of pike

159   for invertebrates. Lipids have more negative $\delta^{13}C$ values relative to other major biochemical

160   compounds, so the lipid-corrected value for C was used for statistical analyses (Logan et al.

161   2008). Both corrected and uncorrected data are presented in Table S3.

162

163   **Data treatment**

164   Benthic taxa were classified into 3 functional feeding groups (FFGs) according to their

165   feeding mode (Kangur et al. 2004): collectors, filterers and predators. The collector group

166   comprises organisms feeding on bottom detritus like POM and settled phytoplankton. Filterers

167   are taxa that obtain their food from the water column. In this study, they were particle feeders

168   like mussels. Predators feed on other live benthic invertebrates. Samples were not separated

169   according to year because most (75%) of our samples were collected in 2010 and because the

170   year effect on isotopic signature is weak (Cremona et al. 2010). Several taxa were not found

171   either during all time periods or at all the sites, so a general linear model (GLM) with factorial

172   test effect was used for data analysis. Adjusted values (i.e., Least Square Means, LSM) of $\delta^{13}C$

173   and $\delta^{15}N$ were used as the response variable. LSMs are predicted values from the model across

174   the level of categorical effects where the other model variables are controlled by setting them to

175 neutral values (SAS Institute 1991; Uryu et al. 2001). For a model with 3 categorical variables,

176 when comparisons are made within 1 variable, the weights of the other 2 variables are

177 neutralized. Categorical variables were time (month), space (site), and trophic level (functional

178 feeding group). Tukey's Honestly Significant Difference (HSD) tests were done on the model

179 outputs (i.e., adjusted values of δ^{13}C and δ^{15}N) to test monthly, site, and functional group

180 differences. All analyses were done with JMP (version 10; SAS Institute Inc., Cary, North

181 Carolina).

182

RESULTS

183

δ^{13}C and δ^{15}N of invertebrate taxa and OM sources

184

185 Of the 200 invertebrate samples analyzed, 143 were collectors, 41 were filterers, and 16

186 were predators. The lipid-corrected δ^{13}C signatures of the samples varied from -57.7 ± 1.8‰ in

187 unidentified *Chironomus* larvae (Diptera) to -30.2 ± 1‰ in *Hydroporus* (Coleoptera). The

188 largest taxonomic group, *Chironomus plumosus* (Linnaeus), which accounted for nearly ½ of the

189 analyzed samples, had a mean δ^{13}C signature of -37.1 ± 0.4‰ (Fig. 2). The 2nd largest taxon

190 sample, *Unio tumidus* Philipsson, had a slightly higher signature (-35.6 ± 0.5‰). Thus, despite

191 the apparent amplitude and taxonomic richness observed, most sample δ^{13}C values clustered

192 within the range -36 to -38‰, indicating a low number of food sources or low variability of the

193 food-source signatures. δ^{15}N signature ranged from 0.6‰ in unidentified *Chironomus* to 12.3‰

194 in Zygoptera (Odonata). Though this amplitude is less than that of δ^{13}C, the main taxa exhibited

195 greater differences in δ^{15}N than in δ^{13}C. The δ^{15}N of the 2 most numerous taxa, *Chironomus*

196 *plumosus* and *Unio tumidus*, were 7.4 ± 0.1‰ and 10.8 ± 0.3‰, respectively. Predatory and

197 filterer taxa exhibited generally higher δ^{15}N signatures than collectors.

198   C signatures of sediments were –30‰ for the southernmost site (L1) with no difference

199   between depth of sampling (Table S4) and –33‰ in the northernmost site (L6). Sediment

200   samples from L1 exhibited a slight depletion (~1.15‰) of the heavier isotope for $\delta^{15}N$ between

201   the surface and 25 cm. River and lake POM SI signatures were nearly identical, which is

202   indicative of similar environmental conditions between phytoplankton from tributaries and

203   Võrtsjärv (Fig. 2). According to the signatures of foodweb components, the main food sources

204   for invertebrate consumers appeared to be POM, a little sediment OM, and another, much more

205   ^{13}C-depleted source that was not sampled. Macrophyte SI values indicated that vascular plants do

206   not contribute substantially to the invertebrate dietary OM.

207

208   **Influence of categorical variables on SI signatures**

209   The GLM explained 60% and 69% of $\delta^{13}C$ and $\delta^{15}N$ variation, respectively (Table 1). For

210   both $\delta^{13}C$ and $\delta^{15}N$, sampling site appeared as the most important variable, followed by month

211   and FFG. No significant differences were observed between the center of the lake and the south

212   shore or between on-shore and off-shore areas (Fig. 3A–D). Significant differences in isotopic

213   signatures were observed among invertebrate samples from a few different sites (Fig. 4A, Table

214   2). These differences can be explained by the more negative $\delta^{13}C$ signature of *C. plumosus* and

215   unidentified *Chironomus* at these sites. Thus, the spatial variations in $\delta^{13}C$ and $\delta^{15}N$ in Võrtsjärv

216   appeared to correspond to local in-site peculiarities. River site (S1–3) isotopic signatures did not

217   differ from those of most lake sites (L1, 4, 5, 7, 8). $\delta^{13}C$ signature tended to decrease from the

218   eastern shore (L8) to the center (L6), but the differences were not statistically significant. For

219   example, benthos collected at sites L2 and L3 had a significantly lower $\delta^{13}C$ signature than

220   benthos collected from other sites, but samples collected from the most vegetated sites (L1–5)

221   did not differ significantly from samples collected at less-vegetated sites (L6–8). δ^{13}C signature

222   varied little between months. September was the only month that differed from the others (Fig.

223   4B, Table 3). Thus, spatial homogeneity of δ^{13}C paralleled temporal stability of the same

224   signature, a result implying reliance on the same C source by benthic consumers during most of

225   the growing season.

226   Output values from the GLM revealed that FFG did not affect δ^{13}C ($p = 0.49$), and LSM

227   (adjusted) values were within the range of standard error (Fig. 4C, Table 4). LSM δ^{13}C values

228   were -36.5 ± 0.9‰ for collectors, -37.2 ± 1.26‰ for filterers, and -34.8 ± 1.95‰ for predators.

229   This result implies that the Võrtsjärv benthic food web is sustained primarily by 1 C source or by

230   a fixed proportion of sources. On the other hand, FFG did affect δ^{15}N ($p < 0.0001$) for. There

231   was a significant difference between collector and filterer LSM δ^{15}N values, but not between

232   predators and the 2 other feeding functional groups (Fig. 2). LSM δ^{15}N values were $7.11 \pm$

233   0.36‰ for collectors, 10.28 ± 0.49‰ for filterers and 8.81 ± 0.76‰ for predators. The average

234   δ^{15}N enrichment between the collector group and the next trophic level represented by the

235   invertebrate predator group was therefore 1.7‰.

236

237   **DISCUSSION**

238   Our 1st hypothesis was supported because no difference was detected among δ^{13}C

239   signatures of consumers within sampling sites. Samples collected from in-shore sites and rivers

240   generally had δ^{13}C values between -30‰ and -40‰, which would correspond to a primary

241   uptake of POM (~-32‰). In a long-term study of a shallow Danish lake, benthic

242   macroinvertebrates had relatively lower δ^{13}C signatures and taxonomic richness during years

243   when macrophyte cover was scarce and turbidity high (Boll et al. 2012). Lower consumer δ^{13}C

244   values were argued to be caused by consistently higher CO_2 concentration in the water column

245   (and thus, more ^{13}C discrimination in primary producers) during turbid-water years. However, in

246   Võrtsjärv, POM probably is supplemented by another, more negative source, e.g., methane-

247   oxidizing bacteria (MOB; <–60‰; Grey et al. 2004, Jones and Grey 2011, Yasuno et al. 2012).

248   Some samples had even lower $\delta^{13}C$ signatures (<–40‰) that strongly suggested assimilation of

249   C from MOB. $\delta^{15}N$ values of benthic consumers was relatively high, so uptake of terrestrially

250   derived OM ($\delta^{13}C \approx$ –28‰, $\delta^{15}N \approx$ 0‰) is very unlikely (Peterson and Fry 1987, Cremona et al.

251   2009), as it is for submerged macrophytes.

252   Contrary to our 2nd hypothesis, no lake-wide gradient was observed in Võrtsjärv

253   macroinvertebrate SI signatures. Only organisms collected at the southern sites L2 and L3 had

254   significantly lower $\delta^{13}C$ signatures than organisms collected at other sites. The main cause of this

255   difference lies in the greater abundance of strongly ^{13}C-depleted Chironomidae (*C. plumosus* and

256   unidentified *Chironomus*) relative to other taxa at these 2 sites. Whether on a south–north or

257   offshore–onshore axis, the observed variations in $\delta^{13}C$ and $\delta^{15}N$ did not appear to be linked to

258   any diet shift on a large scale, but rather seemed to correspond to some local site or community

259   composition differences (greater abundance of Chironomidae). Therefore, and despite its large

260   surface area, the benthic zone of Võrtsjärv is a very homogeneous ecosystem. Most of the

261   benthic organisms evidently feed on a limited number of C sources, and the food web essentially

262   comprises 2 trophic compartments, i.e., benthic collectors and filterers, and predators (Table S5).

263   This monotonous foodweb structure, dominated by omnivorous chironomids and mussels, is

264   typical of shallow turbid lakes with unvegetated bottoms (Hargeby et al. 1994) or of the "turbid

265   state" experienced by clearer lakes (Boll et al. 2012). In the profundal (i.e., below compensation

266   point) zone of lakes the low diversity of feeding modes among invertebrates corresponds to a

267   low diversity of available food sources (Jonasson 1972). However, contrary to what is observed

268   in clearer lakes, the contribution of macrozooplankton (by the sinking of dead individuals or

269   fecal pellets to the benthic zone) to the invertebrate diet is negligible in Võrtsjärv because of the

270   low abundance of these organisms in the water column (Zingel et al. 2007). More generally, the

271   importance of benthic consumers in shallow lakes is consistent with the results of previous

272   studies in which an inverse relationship between lake mean depth and zoobenthos contribution to

273   whole-lake secondary productivity was described (Jeppesen et al. 1997, Vadeboncoeur et al.

274   2002). Based on the depth–benthos relationship compiled by Vadeboncoeur et al. (2002; fig. 7 in

275   their paper), Võrtsjärv, with a mean depth of only 2.8 m, should lie at one extreme end of the

276   continuum with the contribution of zoobenthos to lake secondary production as high as 90%.

277   The mean $\delta^{15}N$ enrichment observed in Võrtsjärv between the collector and predator

278   trophic levels (1.7‰) was lower than the typical trophic fractionation value of ~3.4‰ that has

279   been reported by many previous investigators (e.g., Minagawa and Wada 1984, Vander Zanden

280   et al. 1999, Post 2002). Several factors could contribute to this discrepancy. First, an apparent

281   trophic enrichment <3.4‰ could indicate consumer omnivory, i.e., feeding at >1 trophic level

282   (Zah et al. 2001). Aquatic invertebrates are often opportunistic in their feeding behavior, and

283   taxa like chironomids that have primitive chewing mouth parts can graze on particles and cells of

284   a wide size range (Tall et al. 2006). Low trophic enrichment can also be caused by invertebrate-

285   specific assimilation rates that differ from those of vertebrates (Webb et al. 1998, McCutchan et

286   al. 2003). The overall fractionation value we obtained is very close to values of 1.8‰ reported

287   by Anderson and Cabana (2005) and 1.6‰ by Cremona et al. (2010) for Québec benthic

288   invertebrates. However, our collector isotope values were mainly for Chironomidae, and

289   especially *Chironomus plumosus* because these make up most of the benthic biomass in

290   Võrtsjärv. Kangur et al. (2004) estimated that Chironomidae represent 84% of bottom animal

291   abundance in the lake with *C. plumosus* alone constituting 73%. The invertebrate predators we

292   analyzed are not capable of consuming the bivalve mollusks that constitute the only other

293   significant component of the benthic biomass (Kangur et al. 2004), so chironomid larvae are

294   likely to be the principle prey for the invertebrate predators. We obtained a mean δ^{15}N value of

295   7.4‰ for *C. plumosus* and a 3.4‰ trophic enrichment above this value would give a δ^{15}N value

296   of 10.8‰, which is actually slightly lower than most of the predator δ^{15}N values we obtained.

297   Furthermore, the average δ^{15}N of *C. plumosus* is exactly 3.22‰ enriched relative to the upper

298   layer of sediments that constitute its natural food input. Thus, N trophic fractionation among

299   benthic invertebrates in Võrtsjärv is consistent with widely adopted values when considered in

300   the context of probable prey–predator interactions rather than as community-wide averages.

301   Among the 3 FFGs categorized in our study, the filterers exhibited the highest adjusted

302   δ^{15}N signature. In Lake Võrtsjärv, the filterer FFG essentially comprises freshwater mussels, and

303   some physiological and ecological characteristics specific to these taxa might explain their high

304   δ^{15}N signatures. Mussels are larger bodied and longer lived organisms than chironomids. They

305   have a very efficient rate of retention for particles >5 μm, which is the size of the large

306   flagellates and ciliates (Jørgensen et al. 1984, Thorp and Casper 2002) that constitute a high

307   proportion of the zooplankton biomass in Võrtsjärv (Zingel and Nõges 2010). The δ^{15}N signature

308   of some Võrtsjärv zooplankton is ~8.5‰ (H. Agasild, Estonian University of Life Sciences,

309   unpublished data), ~1.5 to 3.0‰ lower than that of the mussels and at the same level as that of

310   the chironomids, which suggests that mussels in Võrtsjärv might feed extensively on the

311   abundant protozoan zooplankton.

312   The δ^{13}C signature of benthic consumers was more negative than the POM, sediment, and

313   macrophyte signatures despite a slight positive δ^{13}C fractionation (+0.4‰) from source to

314   consumer (Post 2002). This lower benthic consumer signature can be accounted for only by the

315   assimilation of a more negative OM source not included in our sampling and analysis of putative

316   food sources. Some samples, mostly chironomids, exhibited an even lower δ^{13}C signature, in

317   some cases approaching –60‰. This depleted δ^{13}C signature of chironomids relative to other

318   taxa is most plausibly accounted for by a contribution to their diet of MOB found in sediments

319   (Grey et al. 2004, Jones and Grey 2011). For example, *Chironomus plumosus* is a taxon

320   characteristic of eutrophic and dystrophic lakes that experience O_2 depletion in the sediments

321   (Jones and Grey 2011). These hypoxic conditions are likely to favor the activity of MOB and

322   their subsequent assimilation by bottom-feeding invertebrates. The δ^{13}C values obtained for *C.*

323   *plumosus* in Võrtsjärv are not nearly as low as those reported from some other lakes by Jones

324   and Grey (2011), but nevertheless, they suggest some contribution of methane-derived C to the

325   chironomid biomass in this lake. Some other bottom/sediment feeding taxa collected in

326   Võrtsjärv, like the Oligochaeta, also exhibited a strongly depleted δ^{13}C signature (see Hershey et

327   al. 2006 for similar findings in Oligochaeta). On the other hand *Stictochironomus* sp., which

328   inhabits the vegetated littoral zone, exhibited a heavier C signature than *C. plumosus*, indicating

329   a stronger reliance on POM and terrestrial detritus in its diet.

330   In some lakes, methane-derived C can be transferred through the food web to make a

331   substantial contribution to fish biomass (Ravinet et al. 2010, Sanseverino et al. 2012). However,

332   our findings suggest only limited potential transfer of methane–derived C to other parts of the

333   food webs of Võrtsjärv. The lake is polymictic and its water column is well oxygenated

334   throughout the year, so the MOB layer is certainly constrained in the deeper subsurface

335   sediments (see fig. 4 by Jones and Grey 2011). This situation also means that zooplankton are

336   unlikely to graze MOB and transfer methane-derived C to the upper parts of the food webs.

337   Hence, mobile top consumers in the lake like benthivorous fishes that rely heavily on chironomid

338   larvae are likely to contain some methane-derived C in their biomass, but only to a small extent,

339   and this situation is likely to be most evident for fish from the southern basin of the lake where

340   the chironomids with particularly low δ^{13}C values were collected (Agasild et al., in press). Our

341   results suggest that MOB should be taken into account as potential energy mobilizers in addition

342   to phytoplankton and detritus in foodweb studies in Lake Võrtsjärv, but their role in C transfer to

343   top consumers is probably a small one.

344   In summary, our results support previous research that benthic foodweb structure of

345   turbid shallow lakes is remarkably homogenous and dominated by a few abundant taxa, mainly

346   chironomid larvae and bivalve mollusks, with somewhat different feeding strategies but

347   sustained by few OM sources. Võrstjärv benthic foodweb structure is similar to that of other

348   productive turbid lakes of the hemiboreal region, i.e., lakes that are generally more light- than

349   nutrient-limited, but we suggest that it stands at the very end of the benthic/pelagic continuum

350   regarding the importance of benthos in secondary production. A turbid state, whether temporary

351   or permanent, induces strong coupling between primary and secondary production in the pelagic

352   and benthic zones, with primary production essentially carried out by phytoplankton and

353   secondary production processed by benthic consumers. Three major OM sources (macrophytes,

354   terrestrial detritus and benthic algae) that are used by consumers of nonturbid, not light-limited

355   lakes are excluded in the simplified food webs of these lakes.

356

357 **ACKNOWLEDGEMENTS**

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522

523 **FIGURE CAPTIONS**

524 Fig. 1. Location of sampling sites on the main river inflows (S1−3, left) and in Võrtsjärv (L1-L8,

525   right). Dashed line represents lower limit of macrophyte distribution.

526 Fig. 2. Mean (±1 SE) C and N stable isotope signature biplot of foodweb components sampled in

527   Võrtsjärv.

528 Fig. 3. Adjusted values of sampling site mean $\delta^{13}C$ (A, B) and $\delta^{15}N$ (C, D) of benthic

529   macroinvertebrates calculated by the test-effect model plotted against distance from the

530   southern end of the lake (A, C) and distance from nearest shore (B, D). Only data from

531   the in-lake sites (L1−8) are shown in the plots.

532 Fig. 4. Mean (±1 SE) Least Square Means (LSM) adjusted values of isotopic signatures of

533   macroinvertebrates among sampling sites (see Fig. 1 for locations) (A), months (B),

534   functional feeding groups (C). The adjusted values were calculated by the test effect

535   model.

536

537 Table 1. Analysis of variance for the test effect model of $\delta^{13}C$ and $\delta^{15}N$ of benthic

538 macroinvertebrates with the variables site (L1–8), month (June, July, August, September,

539 October, November), and functional feeding group (FFG; collector, filterer, predator). For $\delta^{13}C$,

540 the analysis was done on lipid-corrected values. * indicates significant effect.

Model and variables	Sum of squares	df	F ratio	p	r^2
$\delta^{13}C$					0.60
Site	4605	10	21.39	<0.0001*	
Month	1720	5	15.98	<0.0001*	
FGG	22	2	0.51	0.6	
$\delta^{15}N$					0.69
Site	534	10	16.28	<0.0001*	
Month	155	5	9.46	<0.0001*	
FFG	117	2	17.85	<0.0001*	

541

542

543   Table 2. Results of Tukey's Honestly Significant Difference test ($\alpha = 0.05$) on the Least Square

544   Means (LSM) of C and N isotopic signatures for site categorical variables. Sites with the same

545   letter are not significantly different.

Site	$\delta^{13}C$	$\delta^{15}N$
L1	A	A
L2	CD	CD
L3	D	D
L4	A	ABCD
L5	A	ABC
L6	BCD	BCD
L7	A	AB
L8	A	A
S1	ABCD	ABCD
S2	AB	ABCD
S3	ABC	ABCD

546

547   Table 3. Results of Tukey's Honestly Significant Difference test ($\alpha = 0.05$) on the Least Square

548   Means (LSM) of C and N isotopic signatures for month categorical variable. Months with the

549   same letter are not significantly different.

Month	$\delta^{13}C$	$\delta^{15}N$
June	A	AB
July	A	ABC
August	A	ABC
September	B	A
October	A	C
November	A	BC

550

551

552   Table 4. Results of Tukey Honestly Significant Difference test ($\alpha = 0.05$) on the Least Square

553   Means (LSM) of C and N isotopic signatures for functional feeding groups (FFGs). FFGs with

554   the same letter are not significantly different.

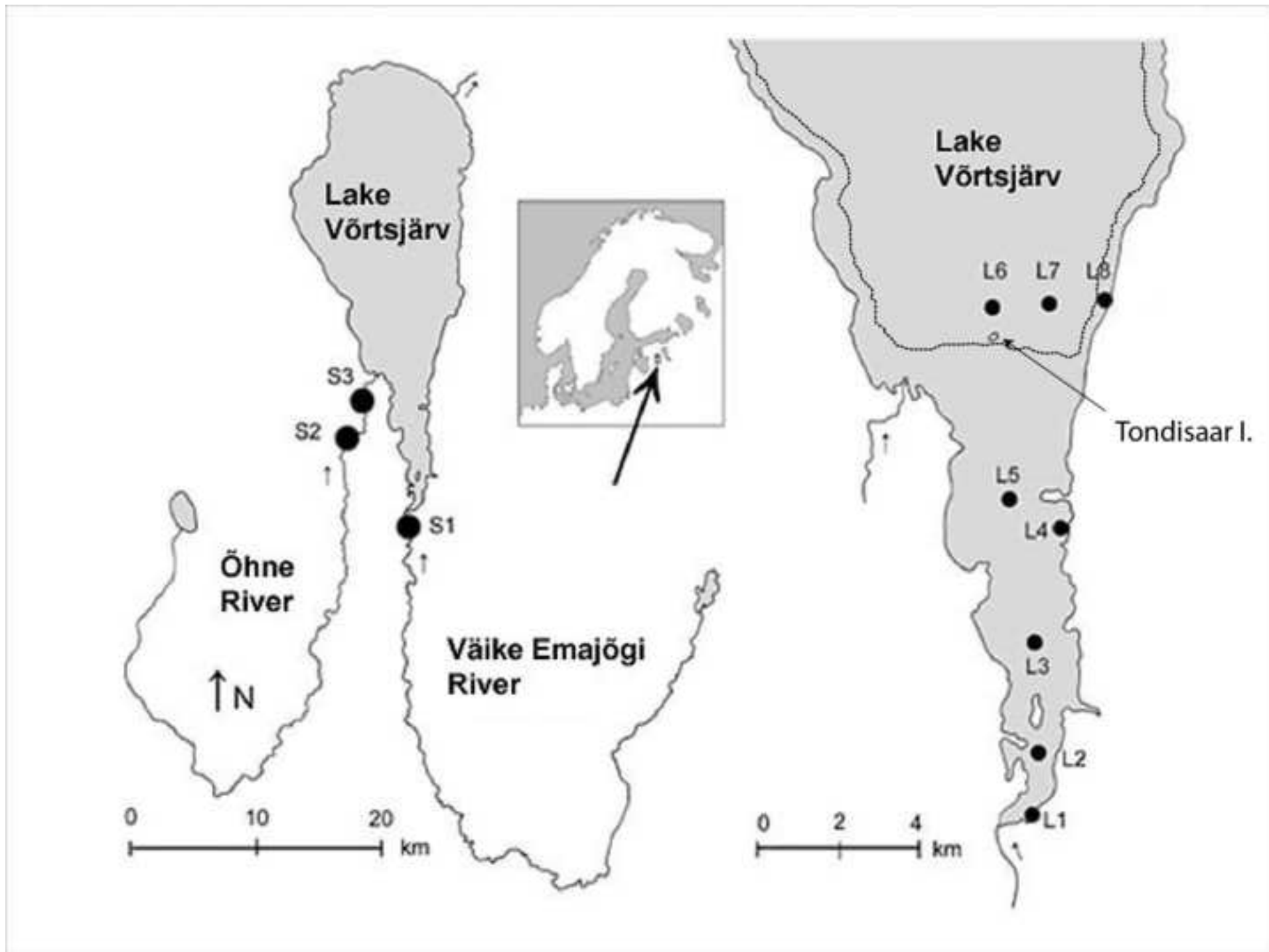| Functional group	$\delta^{13}C$	$\delta^{15}N$
Collector	A	B
Filterer	A	A
Predator	A	AB

555

Figure 1

Figure 2

Figure 3

Figure 4