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# 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 ($\delta^{13}C$, $\delta^{15}N$). Variation in $\delta^{13}C$ 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. $\delta^{13}C$ 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 $\delta^{15}N$ 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 $\delta^{13}C$ 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

Benthic food webs have been underresearched compared to pelagic food webs, but they are of great importance for energy flux and organic-matter cycling in lakes (Vadeboncoeur et al. 2002). In clear-water oligotrophic lakes, primary production is usually dominated by benthic periphyton (Ask et al. 2009). Conversely, in eutrophic, turbid lakes, most of the primary production is by phytoplankton because the euphotic zone does not reach the bottom of the lake. Thus, light restricts periphyton production (Vadeboncoeur et al. 2003). Nevertheless, benthic secondary production often is extremely important in these lakes. Phytoplankton assemblages in eutrophic lakes frequently comprise poorly grazed colonial or toxic cyanobacteria (Scheffer et al. 1997). Instead of being consumed by zooplankton, this phytoplankton production is assimilated into the microbial loop by bacterioplankton (Agasild and Nõges 2005, Zingel et al. 2007) or provides food for benthic detritivores after it sediments to the bottom of the lake. In shallow lakes, the main benthic consumers are bacteria and macroinvertebrates like chironomid larvae and bivalve mollusks (Wetzel 2001). Thus, in shallow, well-mixed lakes, benthic food chains appear to play an important role in the cycling of organic matter (OM) produced by pelagic phytoplankton (Weidel et al. 2008).

Shallow, eutrophic Lake Võrtsjärv (Estonia) exemplifies the coupling between pelagic primary production and benthic secondary production. The Võrtsjärv benthic zone can be divided into 2 main areas: a sheltered, macrophyte-rich zone from the southern end of the lake (Väike-Emajõgi river mouth) to the broadening of the lake basin south of Tondisaar islet; and a much larger, mostly unvegetated area that occupies the rest of the lake (Feldmann and Mäemets 2004). In the main basin, wind-induced resuspension of sediments causes high turbidity in the water column (Secchi depth < 1 m) and restricts the euphotic zone to a depth of ≤2 m (Reinart 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 ($\delta^{13}C$ and $\delta^{15}N$;
77 Peterson and Fry 1987), are expected to reflect these patterns. In general, $\delta^{13}C$ 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, $\delta^{15}N$ 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) $\delta^{13}C$ 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 $4^{th}$-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

neutral values (SAS Institute 1991; Uryu et al. 2001). For a model with 3 categorical variables, when comparisons are made within 1 variable, the weights of the other 2 variables are neutralized. Categorical variables were time (month), space (site), and trophic level (functional feeding group). Tukey's Honestly Significant Difference (HSD) tests were done on the model outputs (i.e., adjusted values of $\delta^{13}C$ and $\delta^{15}N$) to test monthly, site, and functional group differences. All analyses were done with JMP (version 10; SAS Institute Inc., Cary, North Carolina).

## RESULTS

### $\delta^{13}C$ and $\delta^{15}N$ of invertebrate taxa and OM sources

Of the 200 invertebrate samples analyzed, 143 were collectors, 41 were filterers, and 16 were predators. The lipid-corrected $\delta^{13}C$ signatures of the samples varied from –57.7 ± 1.8‰ in unidentified *Chironomus* larvae (Diptera) to –30.2 ± 1‰ in *Hydroporus* (Coleoptera). The largest taxonomic group, *Chironomus plumosus* (Linnaeus), which accounted for nearly ½ of the analyzed samples, had a mean $\delta^{13}C$ signature of –37.1 ± 0.4‰ (Fig. 2). The 2$^{nd}$ largest taxon sample, *Unio tumidus* Philipsson, had a slightly higher signature (–35.6 ± 0.5‰). Thus, despite the apparent amplitude and taxonomic richness observed, most sample $\delta^{13}C$ values clustered within the range –36 to –38‰, indicating a low number of food sources or low variability of the food-source signatures. $\delta^{15}N$ signature ranged from 0.6‰ in unidentified *Chironomus* to 12.3‰ in Zygoptera (Odonata). Though this amplitude is less than that of $\delta^{13}C$, the main taxa exhibited greater differences in $\delta^{15}N$ than in $\delta^{13}C$. The $\delta^{15}N$ of the 2 most numerous taxa, *Chironomus plumosus* and *Unio tumidus*, were 7.4 ± 0.1‰ and 10.8 ± 0.3‰, respectively. Predatory and filterer taxa exhibited generally higher $\delta^{15}N$ 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). $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ ($p = 0.49$), and LSM
227 (adjusted) values were within the range of standard error (Fig. 4C, Table 4). LSM $\delta^{13}C$ 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 $\delta^{15}N$ ($p < 0.0001$) for. There
231 was a significant difference between collector and filterer LSM $\delta^{15}N$ values, but not between
232 predators and the 2 other feeding functional groups (Fig. 2). LSM $\delta^{15}N$ values were 7.11 ±
233 0.36‰ for collectors, 10.28 ± 0.49‰ for filterers and 8.81 ± 0.76‰ for predators. The average
234 $\delta^{15}N$ 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 $\delta^{13}C$
239 signatures of consumers within sampling sites. Samples collected from in-shore sites and rivers
240 generally had $\delta^{13}C$ 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 $\delta^{13}C$ signatures and taxonomic richness during years
243 when macrophyte cover was scarce and turbidity high (Boll et al. 2012). Lower consumer $\delta^{13}C$

values were argued to be caused by consistently higher $CO_2$ concentration in the water column (and thus, more $^{13}C$ discrimination in primary producers) during turbid-water years. However, in Võrtsjärv, POM probably is supplemented by another, more negative source, e.g., methane-oxidizing bacteria (MOB; <–60‰; Grey et al. 2004, Jones and Grey 2011, Yasuno et al. 2012). Some samples had even lower $\delta^{13}C$ signatures (<–40‰) that strongly suggested assimilation of C from MOB. $\delta^{15}N$ values of benthic consumers was relatively high, so uptake of terrestrially derived OM ($\delta^{13}C \approx -28$‰, $\delta^{15}N \approx 0$‰) is very unlikely (Peterson and Fry 1987, Cremona et al. 2009), as it is for submerged macrophytes.

Contrary to our 2nd hypothesis, no lake-wide gradient was observed in Võrtsjärv macroinvertebrate SI signatures. Only organisms collected at the southern sites L2 and L3 had significantly lower $\delta^{13}C$ signatures than organisms collected at other sites. The main cause of this difference lies in the greater abundance of strongly $^{13}C$-depleted Chironomidae (*C. plumosus* and unidentified *Chironomus*) relative to other taxa at these 2 sites. Whether on a south–north or offshore–onshore axis, the observed variations in $\delta^{13}C$ and $\delta^{15}N$ did not appear to be linked to any diet shift on a large scale, but rather seemed to correspond to some local site or community composition differences (greater abundance of Chironomidae). Therefore, and despite its large surface area, the benthic zone of Võrtsjärv is a very homogeneous ecosystem. Most of the benthic organisms evidently feed on a limited number of C sources, and the food web essentially comprises 2 trophic compartments, i.e., benthic collectors and filterers, and predators (Table S5). This monotonous foodweb structure, dominated by omnivorous chironomids and mussels, is typical of shallow turbid lakes with unvegetated bottoms (Hargeby et al. 1994) or of the "turbid state" experienced by clearer lakes (Boll et al. 2012). In the profundal (i.e., below compensation point) zone of lakes the low diversity of feeding modes among invertebrates corresponds to a

low diversity of available food sources (Jonasson 1972). However, contrary to what is observed in clearer lakes, the contribution of macrozooplankton (by the sinking of dead individuals or fecal pellets to the benthic zone) to the invertebrate diet is negligible in Võrtsjärv because of the low abundance of these organisms in the water column (Zingel et al. 2007). More generally, the importance of benthic consumers in shallow lakes is consistent with the results of previous studies in which an inverse relationship between lake mean depth and zoobenthos contribution to whole-lake secondary productivity was described (Jeppesen et al. 1997, Vadeboncoeur et al. 2002). Based on the depth–benthos relationship compiled by Vadeboncoeur et al. (2002; fig. 7 in their paper), Võrtsjärv, with a mean depth of only 2.8 m, should lie at one extreme end of the continuum with the contribution of zoobenthos to lake secondary production as high as 90%.

The mean $\delta^{15}N$ enrichment observed in Võrtsjärv between the collector and predator trophic levels (1.7‰) was lower than the typical trophic fractionation value of ~3.4‰ that has been reported by many previous investigators (e.g., Minagawa and Wada 1984, Vander Zanden et al. 1999, Post 2002). Several factors could contribute to this discrepancy. First, an apparent trophic enrichment <3.4‰ could indicate consumer omnivory, i.e., feeding at >1 trophic level (Zah et al. 2001). Aquatic invertebrates are often opportunistic in their feeding behavior, and taxa like chironomids that have primitive chewing mouth parts can graze on particles and cells of a wide size range (Tall et al. 2006). Low trophic enrichment can also be caused by invertebrate-specific assimilation rates that differ from those of vertebrates (Webb et al. 1998, McCutchan et al. 2003). The overall fractionation value we obtained is very close to values of 1.8‰ reported by Anderson and Cabana (2005) and 1.6‰ by Cremona et al. (2010) for Québec benthic invertebrates. However, our collector isotope values were mainly for Chironomidae, and 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 $\delta^{15}N$ value of
295 7.4‰ for *C. plumosus* and a 3.4‰ trophic enrichment above this value would give a $\delta^{15}N$ value
296 of 10.8‰, which is actually slightly lower than most of the predator $\delta^{15}N$ values we obtained.
297 Furthermore, the average $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{13}C$ signature of benthic consumers was more negative than the POM, sediment, and

macrophyte signatures despite a slight positive $\delta^{13}C$ fractionation (+0.4‰) from source to consumer (Post 2002). This lower benthic consumer signature can be accounted for only by the assimilation of a more negative OM source not included in our sampling and analysis of putative food sources. Some samples, mostly chironomids, exhibited an even lower $\delta^{13}C$ signature, in some cases approaching –60‰. This depleted $\delta^{13}C$ signature of chironomids relative to other taxa is most plausibly accounted for by a contribution to their diet of MOB found in sediments (Grey et al. 2004, Jones and Grey 2011). For example, *Chironomus plumosus* is a taxon characteristic of eutrophic and dystrophic lakes that experience $O_2$ depletion in the sediments (Jones and Grey 2011). These hypoxic conditions are likely to favor the activity of MOB and their subsequent assimilation by bottom-feeding invertebrates. The $\delta^{13}C$ values obtained for *C. plumosus* in Võrtsjärv are not nearly as low as those reported from some other lakes by Jones and Grey (2011), but nevertheless, they suggest some contribution of methane-derived C to the chironomid biomass in this lake. Some other bottom/sediment feeding taxa collected in Võrtsjärv, like the Oligochaeta, also exhibited a strongly depleted $\delta^{13}C$ signature (see Hershey et al. 2006 for similar findings in Oligochaeta). On the other hand *Stictochironomus* sp., which inhabits the vegetated littoral zone, exhibited a heavier C signature than *C. plumosus*, indicating a stronger reliance on POM and terrestrial detritus in its diet.

In some lakes, methane-derived C can be transferred through the food web to make a substantial contribution to fish biomass (Ravinet et al. 2010, Sanseverino et al. 2012). However, our findings suggest only limited potential transfer of methane–derived C to other parts of the food webs of Võrtsjärv. The lake is polymictic and its water column is well oxygenated throughout the year, so the MOB layer is certainly constrained in the deeper subsurface sediments (see fig. 4 by Jones and Grey 2011). This situation also means that zooplankton are

unlikely to graze MOB and transfer methane-derived C to the upper parts of the food webs. Hence, mobile top consumers in the lake like benthivorous fishes that rely heavily on chironomid larvae are likely to contain some methane-derived C in their biomass, but only to a small extent, and this situation is likely to be most evident for fish from the southern basin of the lake where the chironomids with particularly low $\delta^{13}C$ values were collected (Agasild et al., in press). Our results suggest that MOB should be taken into account as potential energy mobilizers in addition to phytoplankton and detritus in foodweb studies in Lake Võrtsjärv, but their role in C transfer to top consumers is probably a small one.

In summary, our results support previous research that benthic foodweb structure of turbid shallow lakes is remarkably homogenous and dominated by a few abundant taxa, mainly chironomid larvae and bivalve mollusks, with somewhat different feeding strategies but sustained by few OM sources. Võrstjärv benthic foodweb structure is similar to that of other productive turbid lakes of the hemiboreal region, i.e., lakes that are generally more light- than nutrient-limited, but we suggest that it stands at the very end of the benthic/pelagic continuum regarding the importance of benthos in secondary production. A turbid state, whether temporary or permanent, induces strong coupling between primary and secondary production in the pelagic and benthic zones, with primary production essentially carried out by phytoplankton and secondary production processed by benthic consumers. Three major OM sources (macrophytes, terrestrial detritus and benthic algae) that are used by consumers of nonturbid, not light-limited lakes are excluded in the simplified food webs of these lakes.

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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

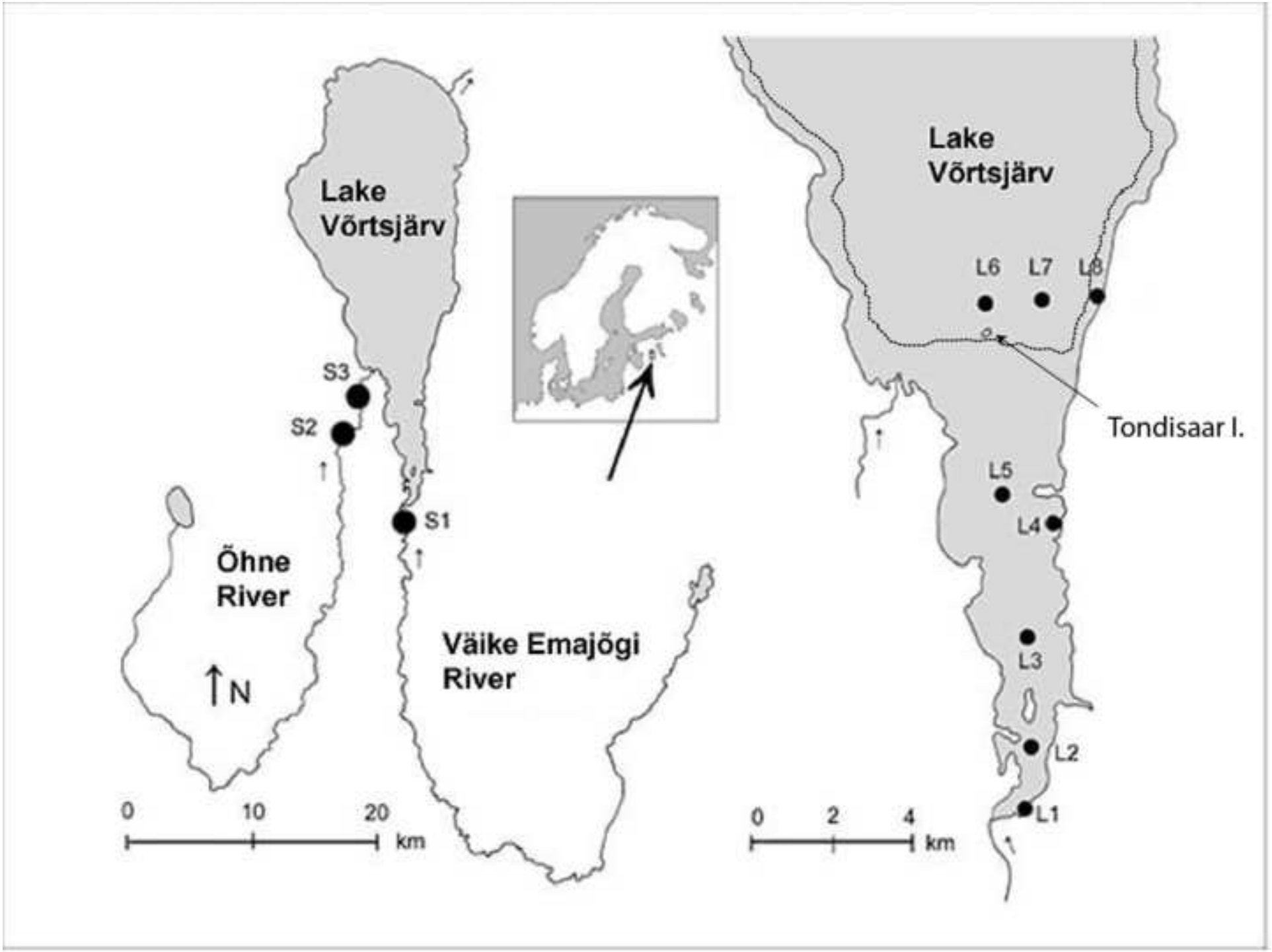

Lake
Võrtsjärv
S3
S2
S1
Õhne
River
Väike Emajõgi
River
N
0
10
20
km
Lake
Võrtsjärv
L6
L7
L8
Tondisaar I.
L5
L4
L3
L2
L1
0
2
4
km

Figure 2

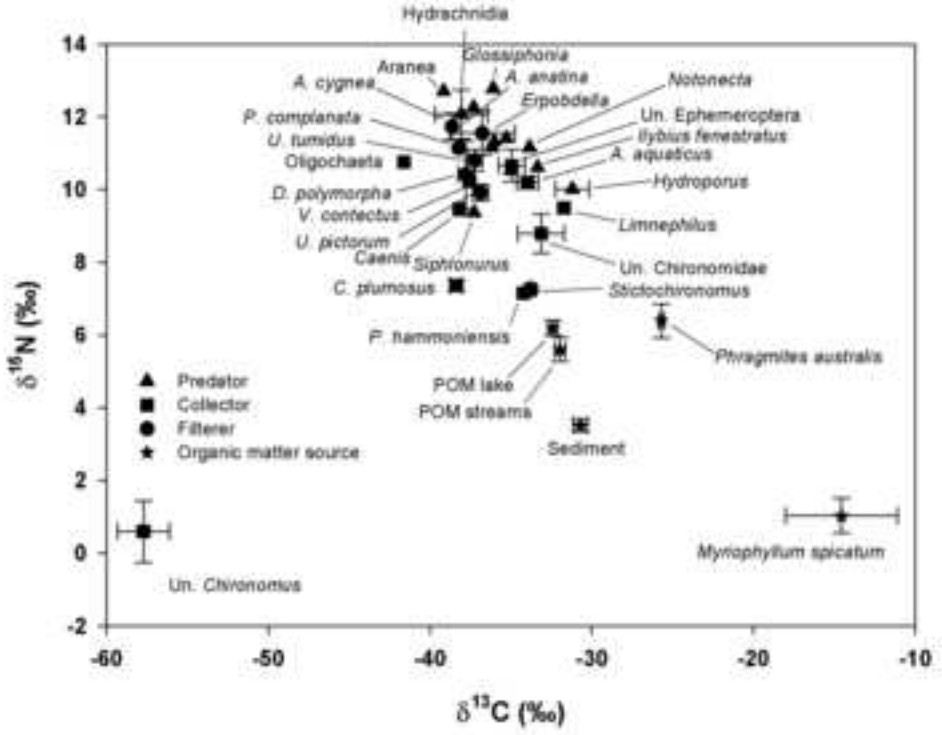

Hydrachnidia
Glossiphonia
Aranea
A. anatina
A. cygnea
Notonecta
Erpobdella
P. complanata
Un. Ephemeroptera
U. tumidus
Ilybius fenestratus
Oligochaeta
A. aquaticus
Hydroporus
D. polymorpha
V. contectus
Limnephilus
U. pictorum
Caenis
Siphlonurus
Un. Chironomidae
C. plumosus
Stictochironomus
P. hammoniensis
Phragmites australis
POM lake
POM streams
Sediment
Myriophyllum spicatum
Un. Chironomus
Predator
Collector
Filterer
Organic matter source
δ15N (‰)
δ13C (‰)
14
12
10
8
6
4
2
0
-2
-60
-50
-40
-30
-20
-10

Figure 3

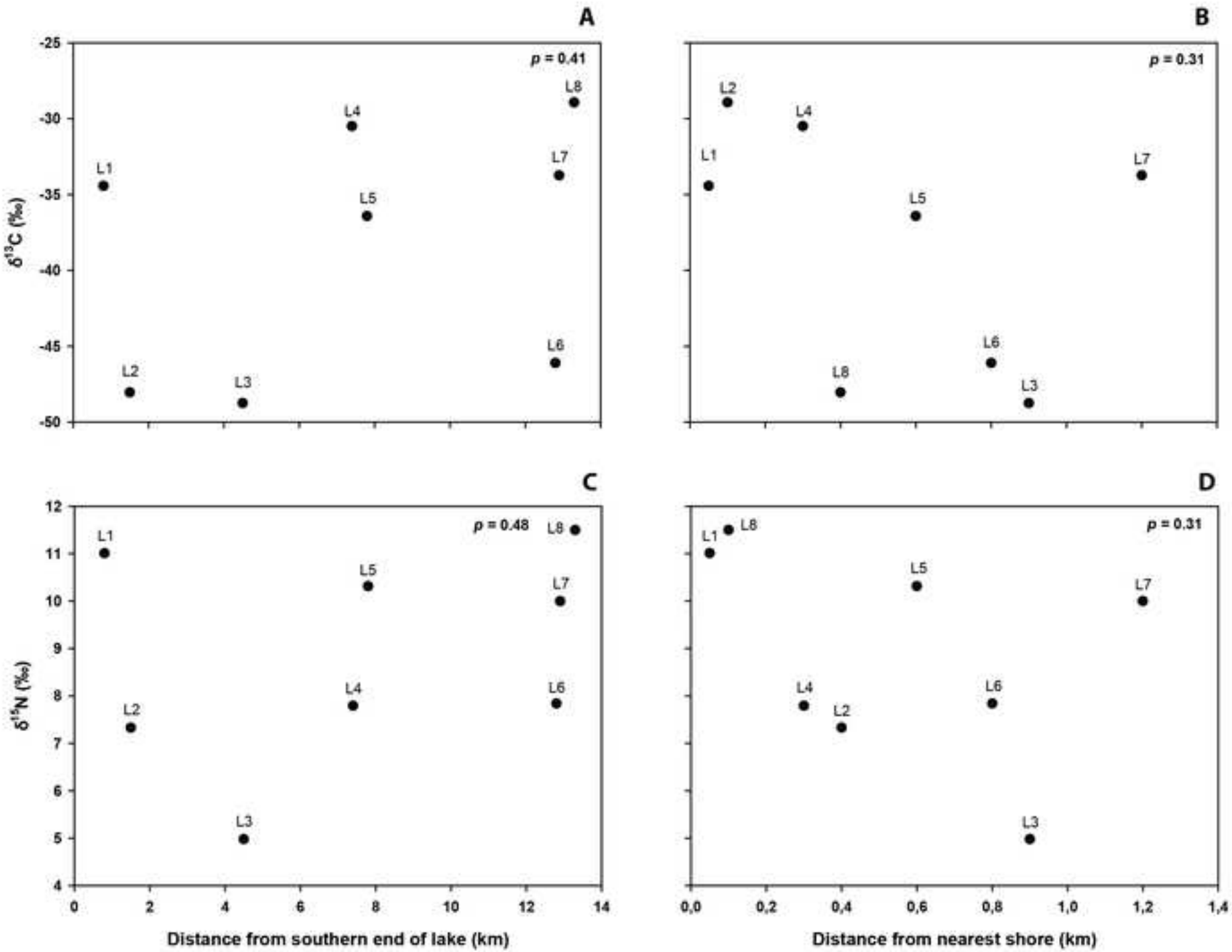
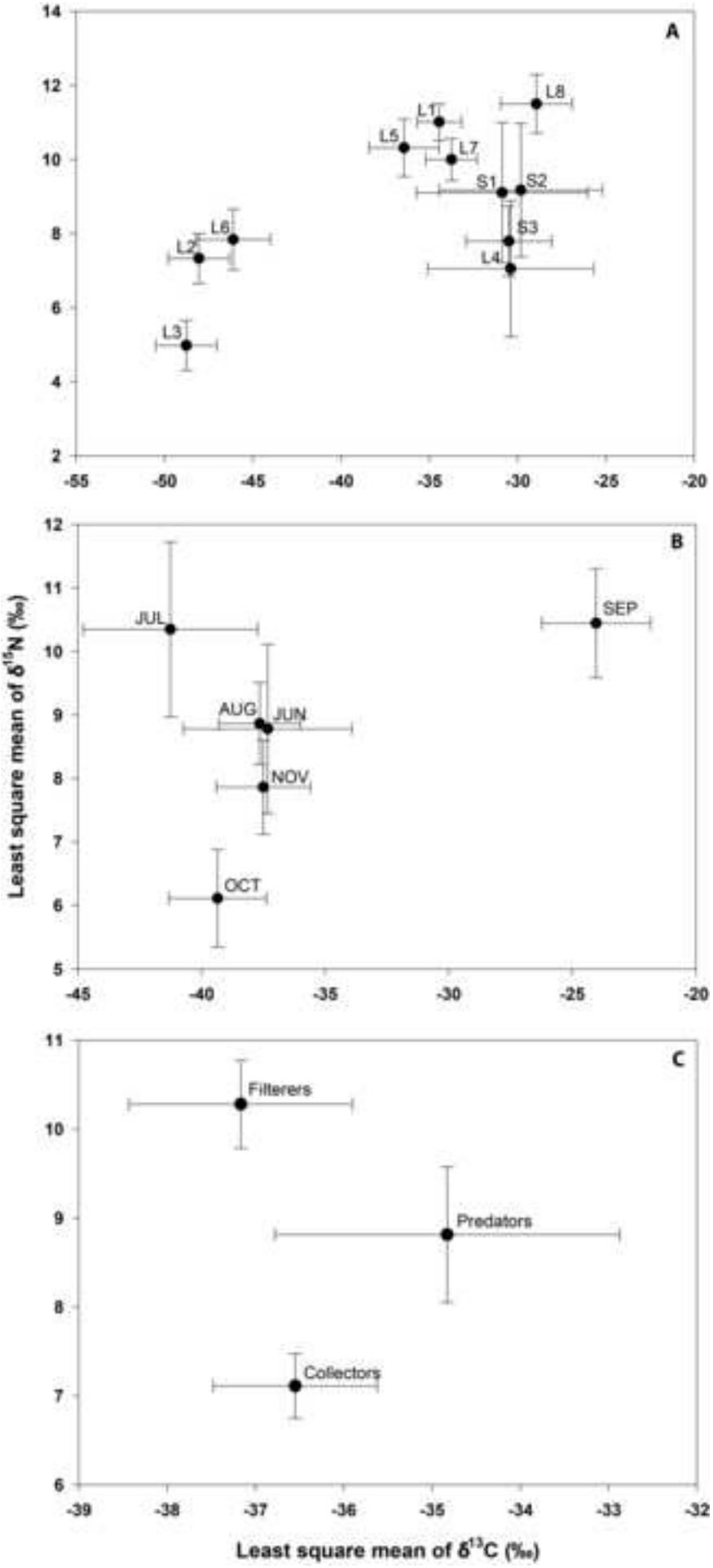

A
p = 0.41
L1
L2
L3
L4
L5
L6
L7
L8
δ13C (‰)
-25
-30
-35
-40
-45
-50
B
p = 0.31
L1
L2
L3
L4
L5
L6
L7
L8
C
p = 0.48
L1
L2
L3
L4
L5
L6
L7
L8
δ15N (‰)
12
11
10
9
8
7
6
5
4
0
2
4
6
8
10
12
14
Distance from southern end of lake (km)
D
p = 0.31
L1
L2
L3
L4
L5
L6
L7
L8
0,0
0,2
0,4
0,6
0,8
1,0
1,2
1,4
Distance from nearest shore (km)

Figure 4


A
L8
L1
L5
L7
S1
S2
L6
L2
S3
L4
L3
B
JUL
SEP
AUG
JUN
NOV
OCT
C
Filterers
Predators
Collectors
Least square mean of δ15N (‰)
Least square mean of δ13C (‰)