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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

species, and benthic primary production is severely light limited (Nõges et al. 2004b). In Võrtsjärv, most of the primary producer biomass is constituted by planktonic cyanobacteria (Nõges and Nõges 2012). Diatoms are present, but they are mostly planktonic (Heinsalu et al. 2008), so over most of the lake bottom, epipelic and epipsammic primary production is assumed to be very low. Thus, benthic food webs in Võrtsjärv are probably heterotrophic, sustained by C from the microbial loop, sedimenting phytoplankton, and particulate organic matter (POM) originating from the littoral and inflow rivers (Nõges et al. 2004a). A study of Võrtsjärv fish communities revealed that, in 2009–2011, benthivorous fish composed ~50% of the species and 70% of the fish total biomass (Järvalt et al. 2011). These findings demonstrate the importance of the benthic food web in transferring OM from phytoplankton to fish in this lake. However, pelagic and littoral components of Võrtsjärv have received extensive attention, but detailed knowledge of the benthic foodweb structure is lacking (Kangur et al. 2004).

Benthic primary production is very low in Võrtsjärv because the compensation depth is well above the bottom of the lake, so benthic invertebrate consumers are mostly sustained by a limited number of sources: POM derived from sedimented phytoplankton and detrital material originating from macrophyte decay (Nõges et al. 2004a). Thus, markers of consumer food source and trophic level, like C and N stable isotopes (SI) signatures, respectively ($\delta^{13}C$ and $\delta^{15}N$; Peterson and Fry 1987), are expected to reflect these patterns. In general, $\delta^{13}C$ varies little with trophic level and constitutes a good tracer for organic-matter source (Peterson and Fry 1987, Cremona et al. 2009). On the other hand, $\delta^{15}N$ increases 1 to 4‰ between food source and consumer (Post 2002, Cremona et al. 2010) and is a good indicator of the trophic level of an organism. Therefore, we used C and N SI analysis to assess the structure of Lake Võrtsjärv benthic food webs. Our working hypotheses were that: 1) $\delta^{13}C$ signatures of benthic consumer

taxa at the same site would show little variation because of their similar dependence on homogeneous C sources; and 2) $\delta^{13}C$ and $\delta^{15}N$ signatures would show a shift between sites along a south–north, on-shore/off-shore axis as the contribution of sedimented phytoplankton to the total OM increases relative to the littoral or riverine contributions.

METHODS

Study site

Lake Võrtsjärv (surface area at mean water level ca 270 km^2) is a eutrophic, polymictic lake in southern Estonia. The lake is shallow (mean depth = 2.8 m) and, because of poor outflow conditions and unregulated flow, experiences important water-level fluctuations (Nõges et al. 2003) that affect its surface area and volume (Järvet 2004). The lake has well buffered water (pH = 8.1) with medium hardness (335 µS/cm; Tuvikene et al. 2004). Two 4th-order rivers (Õhne and Väike Emajõgi) are the largest inflows to the lake. The pH in their lower reaches (8.0 and 8.1, respectively) is similar to that in the lake (Järvekülg 2001). Sapropel (lake mud) forms about ⅔ of the upper part of the bottom sediments, dominating particularly in the southern part of the lake (Raukas 2004). The distribution of aquatic vegetation is uneven. The southern tip of the lake is heavily vegetated, but vegetation becomes gradually sparser as the lake widens and is more wind exposed. The northern part of the lake is essentially vegetation free, except along the shores, because of unfavorable conditions (Fig. 1). Tondisaar islet can be regarded as a boundary point, south of which conditions are better for macrophyte colonization (Feldmann and Mäemets 2004).

Sample collection

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

Eight lake (L1–8) and 3 inflow river (S1–3) sites (Table S1) were selected for invertebrate sampling (Estonian State Environmental Monitoring 2011). The lake sites stretched along a south–north gradient from the southernmost part of the lake to north of Tondisaar islet (Fig. 1). The river sites comprised downstream reaches of the 2 main inflow rivers Väike Emajõgi and Õhne. The main substratum in the rivers and in the lake is fine sand and silt. Sites S1–3 and L1–5 were covered with macrophytes, with the 2 dominant species being emergent *Phragmites australis* (Cav.) Trin. ex Steud. and submerged *Myriophyllum spicatum* L. 1753. Sites L6 and 7 did not have noticeable macrophyte presence, whereas site L8 had very low macrophyte density. Flow at the river sites was very low or absent (0–0.5 m/s; Estonian Institute of Hydrology and Meteorology). Invertebrate samples were collected with a 0.5-mm-mesh hand net with different handle lengths (2–6 m) and by Borutski and Zabolotski grabs. In the laboratory, taxa were identified to the lowest practical taxonomical unit, which was often genus for Chironomidae (*Chironomus* in offshore areas, *Stictochironomus* in nearshore areas). The following taxonomic keys were used for identifying invertebrates: Killeen et al. (2004) for mussels, Timm (2009) for annelids, and Nilsson (1996, 1997) for insects. Individual taxa were isolated with steel forceps under a microscope. Chironomid larvae and unionid mussels formed most of the material.

Sediment samples were collected only at L1 and L6 with a Willner sediment corer. In the laboratory the 25-cm-long sediment core was sectioned into 2-cm (top layer), 3-cm, and three 5-cm-thick slices, which were freeze-dried for the later analyses. POM from rivers and Võrtsjärv was sampled once a month by sieving a depth-integrated water sample through a 100-µm net and then collecting material onto precombusted (550°C, 3 h) GF/F filters. The 2 dominant macrophytes (*P. australis* and *M. spicatum*) were collected manually 3 times during the vegetated period (May, August, and October) in 2008 from L1.

SI analyses

Because of the high inorganic C content in Võrtsjärv lake water, $\delta^{13}C$ and $\delta^{15}N$ values of sediment, *M. spicatum* (not *P. australis* because this species has aerial leaves), and POM filters were measured in separate analyses. These samples were all acidified prior to the $\delta^{13}C$ analyses. The freeze-dried sediment was treated with 12% HCl to remove inorganic C. Approximately 3 mg of dried, acidified (only for $\delta^{13}C$), and pure material was used for $\delta^{15}N$ and $\delta^{13}C$ analyses. For sediments, average stable N and C signatures of the sliced 25-cm sediment core were used. For macrophytes, the whole aboveground plant (leaves and stems) was used for SI analyses. POM filters and macrophytes were maintained for 24 h in HCl vapors and then dried to constant mass at 60°C. The collected material was dried, pulverized, and stored as a ground powder.

An average of 10 individuals from each invertebrate sample was used for SI analyses. The shells of snails and mussels do not reflect the SI signature of their diets (McConnaughey et al. 1997), so they were removed manually with forceps and only the soft tissue was used. Prior to analysis, invertebrate samples were dried to constant mass at 60°C. Failure to allow organisms clear their gut content could bias SI analyses done on whole individuals (Junger and Planas 1994), but the need to clear gut content has been questioned (Kaehler and Pakhomov 2001, Jardine et al. 2005, Cremona et al. 2009). Therefore, we tested the extent to which gut clearance would influence SI signatures of Lake Võrtsjärv Chironomidae because they made up the overwhelming majority of the macroinvertebrate samples. Three groups of animals were kept alive for 12 h before drying to permit clearance of their gut contents. The SI values for these groups were compared with those of control groups dried immediately without gut clearance. Comparison made with a nonparametric Mann–Whitney *U* test revealed no significant difference

between the treated and untreated groups ($p > 0.05$, $n = 10–24$; Table S2). Therefore, subsequent samples were processed without gut clearance.

All SI analyses were done at the University of Jyväskylä (Finland) using a Flash EA 1112 elemental analyzer connected to a DELTAplus Advantage IRMS (Thermo Finnigan, Bremen, Germany). SI ratios are expressed as δ values ($\delta^{13}C$, $\delta^{15}N$) in ‰ referenced to the international standards for C (PeeDee Belemnite) and N (atmospheric N_2). Internal laboratory standards were homogenized potato leaves for POM, macrophytes, and sediment and white muscle tissue of pike for invertebrates. Lipids have more negative $\delta^{13}C$ values relative to other major biochemical compounds, so the lipid-corrected value for C was used for statistical analyses (Logan et al. 2008). Both corrected and uncorrected data are presented in Table S3.

Data treatment

Benthic taxa were classified into 3 functional feeding groups (FFGs) according to their feeding mode (Kangur et al. 2004): collectors, filterers and predators. The collector group comprises organisms feeding on bottom detritus like POM and settled phytoplankton. Filterers are taxa that obtain their food from the water column. In this study, they were particle feeders like mussels. Predators feed on other live benthic invertebrates. Samples were not separated according to year because most (75%) of our samples were collected in 2010 and because the year effect on isotopic signature is weak (Cremona et al. 2010). Several taxa were not found either during all time periods or at all the sites, so a general linear model (GLM) with factorial test effect was used for data analysis. Adjusted values (i.e., Least Square Means, LSM) of $\delta^{13}C$ and $\delta^{15}N$ were used as the response variable. LSMs are predicted values from the model across 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 2nd 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.

C signatures of sediments were –30‰ for the southernmost site (L1) with no difference between depth of sampling (Table S4) and –33‰ in the northernmost site (L6). Sediment samples from L1 exhibited a slight depletion (~1.15‰) of the heavier isotope for $\delta^{15}N$ between the surface and 25 cm. River and lake POM SI signatures were nearly identical, which is indicative of similar environmental conditions between phytoplankton from tributaries and Võrtsjärv (Fig. 2). According to the signatures of foodweb components, the main food sources for invertebrate consumers appeared to be POM, a little sediment OM, and another, much more ^{13}C-depleted source that was not sampled. Macrophyte SI values indicated that vascular plants do not contribute substantially to the invertebrate dietary OM.

Influence of categorical variables on SI signatures

The GLM explained 60% and 69% of $\delta^{13}C$ and $\delta^{15}N$ variation, respectively (Table 1). For both $\delta^{13}C$ and $\delta^{15}N$, sampling site appeared as the most important variable, followed by month and FFG. No significant differences were observed between the center of the lake and the south shore or between on-shore and off-shore areas (Fig. 3A–D). Significant differences in isotopic signatures were observed among invertebrate samples from a few different sites (Fig. 4A, Table 2). These differences can be explained by the more negative $\delta^{13}C$ signature of *C. plumosus* and unidentified *Chironomus* at these sites. Thus, the spatial variations in $\delta^{13}C$ and $\delta^{15}N$ in Võrtsjärv appeared to correspond to local in-site peculiarities. River site (S1–3) isotopic signatures did not differ from those of most lake sites (L1, 4, 5, 7, 8). $\delta^{13}C$ signature tended to decrease from the eastern shore (L8) to the center (L6), but the differences were not statistically significant. For example, benthos collected at sites L2 and L3 had a significantly lower $\delta^{13}C$ signature than benthos collected from other sites, but samples collected from the most vegetated sites (L1–5)

did not differ significantly from samples collected at less-vegetated sites (L6–8). $\delta^{13}C$ signature varied little between months. September was the only month that differed from the others (Fig. 4B, Table 3). Thus, spatial homogeneity of $\delta^{13}C$ paralleled temporal stability of the same signature, a result implying reliance on the same C source by benthic consumers during most of the growing season.

Output values from the GLM revealed that FFG did not affect $\delta^{13}C$ ($p = 0.49$), and LSM (adjusted) values were within the range of standard error (Fig. 4C, Table 4). LSM $\delta^{13}C$ values were –36.5 ± 0.9‰ for collectors, –37.2 ± 1.26‰ for filterers, and –34.8 ± 1.95‰ for predators. This result implies that the Võrtsjärv benthic food web is sustained primarily by 1 C source or by a fixed proportion of sources. On the other hand, FFG did affect $\delta^{15}N$ ($p < 0.0001$) for. There was a significant difference between collector and filterer LSM $\delta^{15}N$ values, but not between predators and the 2 other feeding functional groups (Fig. 2). LSM $\delta^{15}N$ values were 7.11 ± 0.36‰ for collectors, 10.28 ± 0.49‰ for filterers and 8.81 ± 0.76‰ for predators. The average $\delta^{15}N$ enrichment between the collector group and the next trophic level represented by the invertebrate predator group was therefore 1.7‰.

DISCUSSION

Our 1st hypothesis was supported because no difference was detected among $\delta^{13}C$ signatures of consumers within sampling sites. Samples collected from in-shore sites and rivers generally had $\delta^{13}C$ values between –30‰ and –40‰, which would correspond to a primary uptake of POM (~–32‰). In a long-term study of a shallow Danish lake, benthic macroinvertebrates had relatively lower $\delta^{13}C$ signatures and taxonomic richness during years 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

Võrtsjärv. Kangur et al. (2004) estimated that Chironomidae represent 84% of bottom animal abundance in the lake with *C. plumosus* alone constituting 73%. The invertebrate predators we analyzed are not capable of consuming the bivalve mollusks that constitute the only other significant component of the benthic biomass (Kangur et al. 2004), so chironomid larvae are likely to be the principle prey for the invertebrate predators. We obtained a mean $\delta^{15}N$ value of 7.4‰ for *C. plumosus* and a 3.4‰ trophic enrichment above this value would give a $\delta^{15}N$ value of 10.8‰, which is actually slightly lower than most of the predator $\delta^{15}N$ values we obtained. Furthermore, the average $\delta^{15}N$ of *C. plumosus* is exactly 3.22‰ enriched relative to the upper layer of sediments that constitute its natural food input. Thus, N trophic fractionation among benthic invertebrates in Võrtsjärv is consistent with widely adopted values when considered in the context of probable prey–predator interactions rather than as community-wide averages.

Among the 3 FFGs categorized in our study, the filterers exhibited the highest adjusted $\delta^{15}N$ signature. In Lake Võrtsjärv, the filterer FFG essentially comprises freshwater mussels, and some physiological and ecological characteristics specific to these taxa might explain their high $\delta^{15}N$ signatures. Mussels are larger bodied and longer lived organisms than chironomids. They have a very efficient rate of retention for particles >5 µm, which is the size of the large flagellates and ciliates (Jørgensen et al. 1984, Thorp and Casper 2002) that constitute a high proportion of the zooplankton biomass in Võrtsjärv (Zingel and Nõges 2010). The $\delta^{15}N$ signature of some Võrtsjärv zooplankton is ~8.5‰ (H. Agasild, Estonian University of Life Sciences, unpublished data), ~1.5 to 3.0‰ lower than that of the mussels and at the same level as that of the chironomids, which suggests that mussels in Võrtsjärv might feed extensively on the abundant protozoan zooplankton.

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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FIGURE CAPTIONS

Fig. 1. Location of sampling sites on the main river inflows (S1–3, left) and in Võrtsjärv (L1-L8, right). Dashed line represents lower limit of macrophyte distribution.

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

Fig. 3. Adjusted values of sampling site mean $\delta^{13}C$ (A, B) and $\delta^{15}N$ (C, D) of benthic macroinvertebrates calculated by the test-effect model plotted against distance from the southern end of the lake (A, C) and distance from nearest shore (B, D). Only data from the in-lake sites (L1–8) are shown in the plots.

Fig. 4. Mean (±1 SE) Least Square Means (LSM) adjusted values of isotopic signatures of macroinvertebrates among sampling sites (see Fig. 1 for locations) (A), months (B), functional feeding groups (C). The adjusted values were calculated by the test effect model.

Table 1. Analysis of variance for the test effect model of $\delta^{13}C$ and $\delta^{15}N$ of benthic macroinvertebrates with the variables site (L1–8), month (June, July, August, September, October, November), and functional feeding group (FFG; collector, filterer, predator). For $\delta^{13}C$, 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*	

Table 2. Results of Tukey's Honestly Significant Difference test ($\alpha = 0.05$) on the Least Square Means (LSM) of C and N isotopic signatures for site categorical variables. Sites with the same 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

Table 3. Results of Tukey's Honestly Significant Difference test ($\alpha = 0.05$) on the Least Square Means (LSM) of C and N isotopic signatures for month categorical variable. Months with the 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

Table 4. Results of Tukey Honestly Significant Difference test ($\alpha = 0.05$) on the Least Square Means (LSM) of C and N isotopic signatures for functional feeding groups (FFGs). FFGs with the same letter are not significantly different.

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

Figure 1

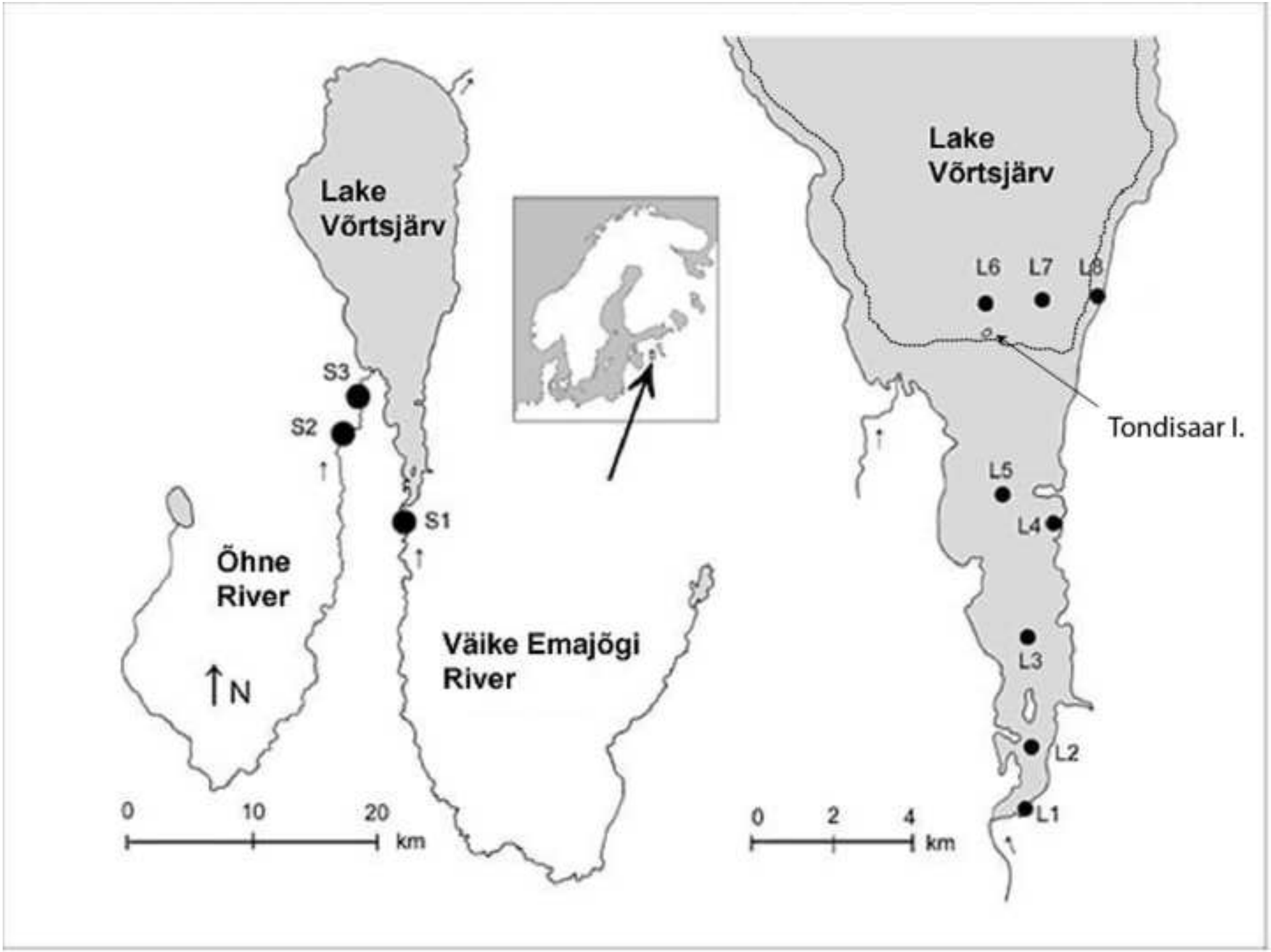

Lake
Võrtsjärv
S3
S2
S1
Õhne
River
Väike Emajõgi
River
N
0
10
20
km
Lake
Võrtsjärv
L6
L7
L8
Tondisaar I.
L5
L4
L3
L2
L1
0
2
4
km

Figure 2

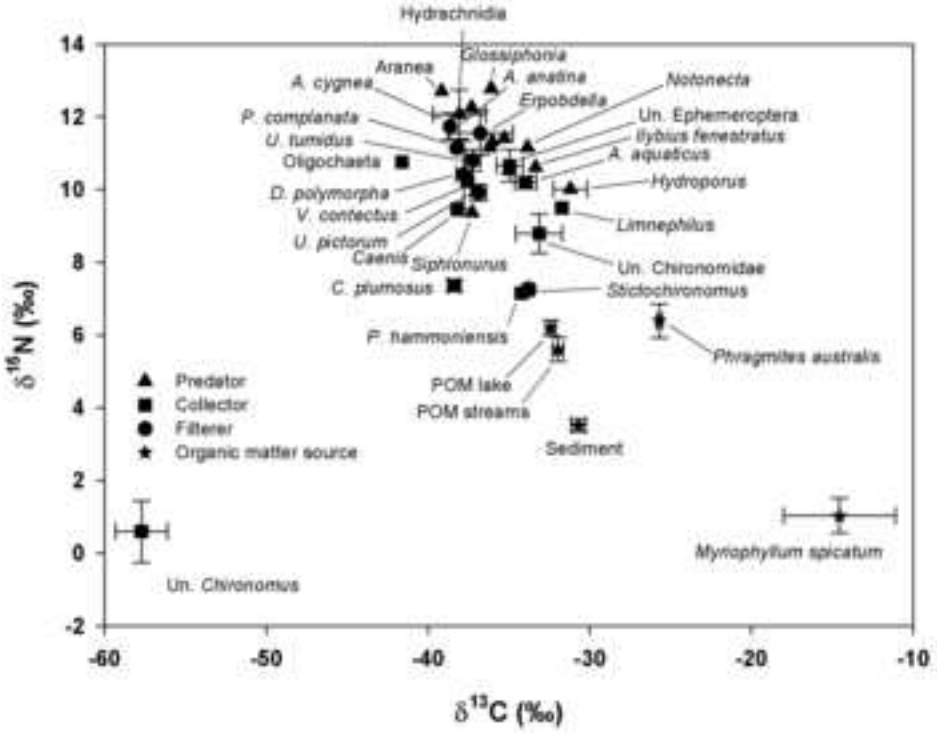

Hydrachnidia
Glossiphonia
Aranea
A. cygnea
A. anatina
Notonecta
Erpobdella
P. complanata
Un. Ephemeroptera
U. tumidus
Ilybius fenestratus
Oligochaeta
A. aquaticus
Hydroporus
D. polymorpha
V. contectus
Limnephilus
U. pictorum
Caenis
Siphlonurus
Un. Chironomidae
C. plumosus
Stictochironomus
P. hammoniensis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

Figure 3

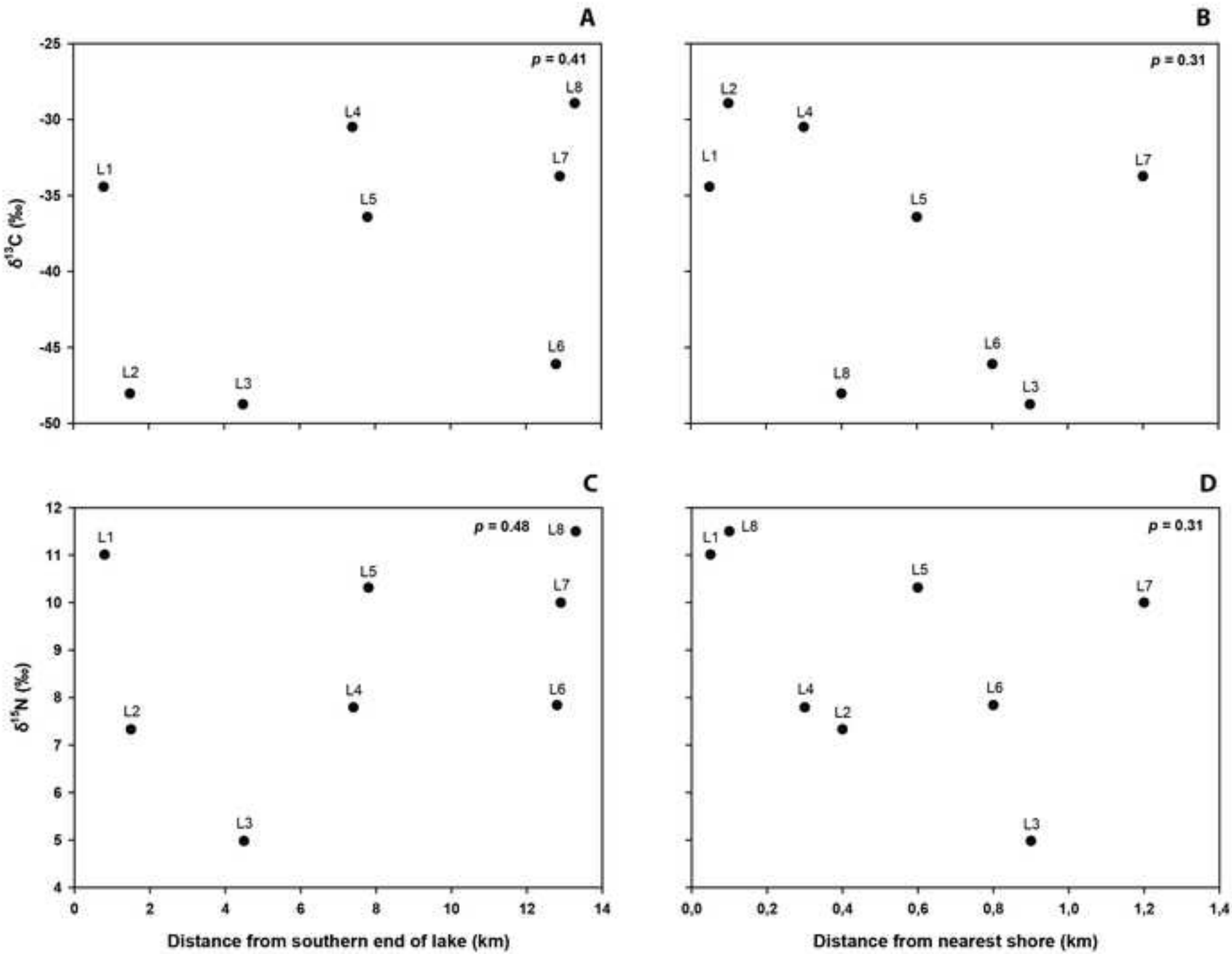
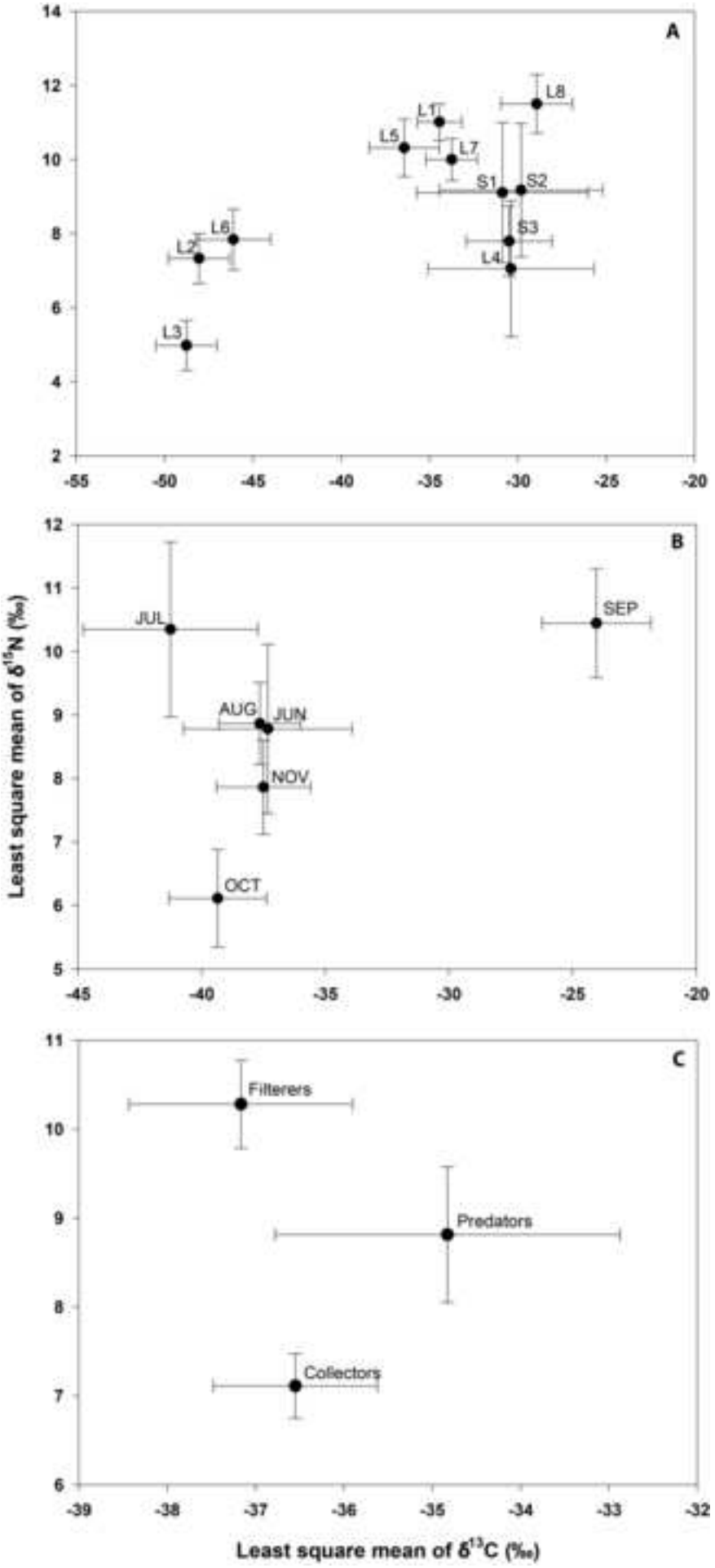

A
B
C
D
p = 0.41
p = 0.31
p = 0.48
p = 0.31
δ13C (‰)
δ15N (‰)
L1
L2
L3
L4
L5
L6
L7
L8
Distance from southern end of lake (km)
Distance from nearest shore (km)

Figure 4


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