


ANALYSIS OF RADIOCARBON DISTRIBUTION IN THE EUTROPHIC LAKE FISH ASSEMBLAGE USING STABLE C, N, S ISOTOPES

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ABSTRACT. The carbon isotope distribution and its relationship with stable N and S isotope ratio values were investigated within a fish assemblage from the shallow lake Tapeliai, which is constantly affected by inflows of ¹⁴C depleted water from the surrounding watershed mires. The “conventional” radiocarbon age within the fish from this lake varied from 119 to 693 yr. The ¹⁴C/¹²C and δ¹³C values correlated significantly ($r=0.85$ $p<0.001$), which is not typical in lakes of the temperate zone. There were no observed statistical differences (Kruskal–Wallis ANOVA tests) in the ¹⁴C/¹²C values among different fish species. The radiocarbon dating values and ¹⁵N/¹⁴N measurements did not correlate. The radiocarbon measurement values also did not correlate with δ³⁴S, however, the distribution of these isotopes in carp (119 yr and 1.3‰, respectively) and roach (344 yr and 4.5‰, respectively) indicated that fish may include allochthonous food sources in their diet.

KEYWORDS: fishes, lakes, nitrogen, radiocarbon, sulfur.

INTRODUCTION

During the last decade, there has been an increase in the number of studies aimed at determining the reservoir effect on radiocarbon dating of remains from ancient settlements near water bodies. In addition, stable isotopes are used, since they could be useful in determining the contribution of proteins from this water ecosystem to the diet of these individuals (Sayle et al. 2014, 2016; Schulting et al. 2014). It has been shown that the freshwater reservoir effect in the same water ecosystem varies between and even within different fish species, although they possess similar diets (Keaveney et al. 2012; Philippssen 2013). This is not surprising, as the carbon isotope composition in DIC (dissolved inorganic carbon), as well as aquatic primary producers, varies within the lake (Li et al. 2018). DIC concentration and its carbon isotope composition is determined by the air-water CO₂ exchange rate, which depends on the organic matter production and decomposition rates, organic and inorganic matter import/export, and water residence time in that particular place (Hou et al. 2012; Mischke et al. 2013; Wang et al. 2019). These processes also affect the content and composition of organic matter in the water column and sediments, thereby impacting on the diet of benthic suspension and primary pelagic feeders (Keaveney et al. 2015).

Stable isotope analysis is widely used to reconstruct diet and characterize trophic relationships in food webs and can be helpful in interpreting radiocarbon dating results. Nitrogen isotope ratios have been the main tool for determining trophic levels of consumers (Vander et al. 2001; Post 2002). The stable carbon isotope method allows separating littoral and pelagic habitat feeders/consumers, as littoral macrophytes have higher ¹³C concentrations (France 1995). However, the δ¹³C signal in DIC, which is the main carbon source for phytoplankton and submerged water plants, depends on CO₂ concentration in the water column, since its deficiency causes changes in fractionation during carbon fixation. During the bloom period, the carbon isotope composition in phytoplankton can vary by several ‰ (Savoye et al. 2003). In areas where C3 plants predominate, it is difficult to distinguish between autochthonous and allochthonous origin organic matter in freshwater ecosystems based on stable carbon isotope

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measurements alone, since the $^{13}\text{C}/^{12}\text{C}$ values overlap (Maksymowska et al. 2000). Meanwhile, $^{34}\text{S}/^{32}\text{S}$ ratio measurements can be used to separate terrestrial and freshwater origin food sources in diet (Richards et al. 2003; Bocherens et al. 2011), as well as in separation of benthic and pelagic habitat feeders (Fry 1986a). Phytoplankton utilizes S from the water column and reflects its sulfate (with fractionation of 0–2‰ (Kaplan et al. 1964)) isotope composition. In anoxic sediments, sulfate-reducing bacteria discriminate against the heavier sulfur isotope resulting its depletion (Donahue et al. 2008), which in marine environments reaches up to 50–60‰ (Thode 1991). However, in lakes, especially those with low sulfate concentration in the water column, fractionation due to sulfate reduction is significantly lower (6–8‰) (Croisetière et al. 2009; Karube et al. 2012; Proulx et al. 2014). Sulfur isotope composition in sediments is more related with the origin of sediment forming fractions.

The present study focuses on the $^{14}\text{C}/^{12}\text{C}$ ratio variation within the fish assemblage of the shallow lake Tapeliai. During spring flood periods or after heavy rains, this lake is fed by an inflow of colored water from the surrounding watershed mires (Moisejenkova et al. 2012). Repetitive inflows of such water which is highly enriched in organic compounds not only affect photosynthetic activity, but also cause short-term changes in the content and composition of organic and inorganic matter that may impact radiocarbon distribution in the lake ecosystem.

The aim of this study was to investigate the radiocarbon distribution within fish species of different diet and trophic levels, and the possibility to relate radiocarbon distribution in fishes of this constantly changing ecosystem with their stable C, N, and especially S isotope ratio values.

MATERIALS AND METHODS

Sampling Area

Lake Tapeliai (54°46'28"N, 25°26'45"E) is located 17 km northeast of the city of Vilnius, Lithuania. The areal of the lake lies in the deepened up to 15–20 m tunnel valley with the glaciofluvial deposits (gravel with sand and other deposits containing up to 40 % of carbonates) of the Late Weichselian glaciation, which is a part of the plain of the Neris River (Bitinas et al. 2004; Bitinas 2012). This lake belongs to a chain of small hydrologically connected lakes. In the north, Lake Tapeliai is connected by a brook with Lakes Juodis (54°46'49"N, 25°26'29"E) and was connected by an artificial ditch with the small humic Lake Lydekis in the south. The ditch was opened between 1879 and 1923 causing an increase in sediment reservoir age of about 1175 ± 111 yr and closed in the 1960s (Tarasiuk et al. 2009; Barisevičiūtė et al. 2019). The surface area of the lake is ~ 0.126 km², the drainage basin area exceeds 0.7 km² (Tarasiuk et al. 2009; Moisejenkova et al. 2012). The lake is surrounded by peat bogs. Thus, during spring flood periods or after long-term rains, the lake is additionally fed by colored water (Moisejenkova et al. 2012).

According to the maximal and average depth measurements the lake is identified as a terminally shallow (polimictic) lake. The fish community is primarily composed of lentic water fish such as *Esox lucius* (Linnaeus 1758), *Perca fluviatilis* (Linnaeus 1758), *Rutilus rutilus* (Linnaeus 1758) and *Abramis brama* (Linnaeus 1758). In addition to these dominant species, the lake is inhabited by introduced *Cyprinus carpio* (Linnaeus 1758) which is not native and cannot breed under local thermal conditions (Virbickas 2018). It is worth noting that the lake is under very intensive recreational use (city beaches, water sports, and angling) as it is close to the city of Vilnius.

Field Sampling

All samples were collected in September 2018. The fish were trapped using multi-mesh benthic gillnets, each of which was 40 m long and 3 m high. The mesh size (bar length) varied every 5 m and was 14, 18, 22, 25, 30, 40, 50, and 60 mm. Four such benthic gillnets, clustered into 2 sets for stability and convenience, were used. The nets were positioned randomly to cover different parts (western, eastern) and depths (2–7 m) of the lake, and left for 12 hr overnight, including sunset and sunrise. All trapped fish were identified to species level, measured to the nearest 1 mm (total body length—TL), weighed to the nearest 0.1 g, and their age was determined from scales (Thoreson 1993). The standard catch per unit of effort (CPUE) was estimated as fish biomass trapped per 40-m-long multi-mesh benthic gillnet per sampling occasion. The fish taxonomy used in the present paper follows the taxonomy provided in FishBase (<http://www.fishbase.org>, accessed 2022.01.06).

Littoral snail was collected for distinguishing the second trophic level in the lake. Due to the constantly changing environmental conditions in the ecosystem, it was not appropriate to make measurements in DIC, water column sulfates, dissolved and particulate organic matter. The aquatic moss *Fontinalis antipyretica* Hedv. was collected instead. The absence of roots and other similar systems in this plant excludes the substrate influence on the uptake dynamics (Maberly 1985). The isotope composition in the tissues of this plant should reflect the average isotopic values in DIC and sulfates during its growth season.

Stable Isotope Analysis (SIA)

Fish for SIA were obtained from the standardized catches of autumn 2018. A sample of white dorsal muscle was cut out from each fish subjected to SIA. Up to three individuals of the same species and size were used for SIA. Perch were divided into two length groups (see Table 1) as it is known that this fish species undergoes ontogenetic niche shifts (Froese and Pauly 2022). The description of fish specimens' size, age, and number of replicates are presented in Table 1. Additionally, gastropods *Bithynia tentaculata* were sampled as an integrator of the SIA signature of the benthic or littoral primary producers (Post 2002). The sampled mollusks were separated from shells, and only their soft tissues (mantle muscle, if possible) were used. Nine individuals of *B. tentaculata* were used to form one replicate for SIA. All samples were then oven dried to constant weight for 48 hr at 60°C, ground to fine powder in an agate mortar, and placed into foil cups.

Carbon, nitrogen and sulfur stable isotope ratio measurements were performed using a Thermo Flash EA 1112 elemental analyser interfaced to a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer (IRMS). All the results of the stable isotope ratio measurements were expressed relative to a standard using delta notation in units of permil (‰):

$$\delta X = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000, \quad (1)$$

where X are ¹³C, ¹⁵N or ³⁴S, respectively; R=¹³C/¹²C, ¹⁵N/¹⁴N or ³⁴S/³²S in the sample or in the standard. The stable carbon, nitrogen and sulfur isotope ratios were expressed relative to the Vienna Pee Dee Belamnite (V-PDB), atmospheric nitrogen and Vienna Canyon Diablo troilite (VCDT), respectively. The laboratory standards were calibrated using NIST Standard Reference Material IAEA600, IAES-N-2, IAEA-S-1, and IAEA-S-2. The long-term reference material measurements were performed with a precision of <0.15‰ for δ¹³C, <0.2‰ for δ¹⁵N and <0.6‰ for δ³⁴S.

Table 1 Summary of SIA of different consumer species from Lake Tapeliai in 2018. Values are mean \pm standard deviation, total length of fish (TL), fish age determined from scales, number of replicates (N), stable isotope values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$, and ^{14}C ‰), the radiocarbon age determined from ^{14}C values. Perch size categories: small (S), large (L).

Species	TL (cm)	True age (yr)	N	C/N	$\delta^{13}\text{C}$ (‰) untreated	$\delta^{13}\text{C}$ (‰) lipid corrected	$\delta^{15}\text{N}$ (‰)	$\delta^{34}\text{S}$ (‰)	pMC	Conventional age (yr BP)
Mollusks										
<i>Bithynia tentaculata</i>			5	3.8	-34.3	-33.8	2.0	—	95.17	398
Aquatic plants										
<i>Fontinalis antipyretica</i>			6	13.3	-37.9		1.7	6.5	89.97	849
Benthivorous fishes										
Bream, <i>Abramis brama</i>	19.6	3	3	3.3	-33.2	-33.2	7.1	4.6	93.48	542
	18.6	3		3.3	-33.7	-33.7	7.8	5.2	91.8	687
	60.0 (L)	13		3.3	-33.1	-33.1	8.4	7.1	93.64	529
Carp, <i>Cyprinus carpio</i>	70.2	9	1	5.0	-32.2	-30.6	9.7	1.3	98.53	119
Ruffe, <i>Gymnocephalus cernua</i>	10.5	6	3	3.4	-35.6	-35.6	7.5	4.5	91.17	743
	10.9	6		3.3	-34.9	-34.9	6.9	6.8	92.44	631
	9.8	5		3.3	-34.7	-34.7	7.7	5.8	91.61	704
Perch, <i>Perca fluviatilis</i> S	10.7	2	2	3.4	-32.7	-32.7	9.5	7.4	92.3	644
	11.6	2		3.4	-32.2	-32.2	8.7	6.9	93.13	571
Roach, <i>Rutilus rutilus</i>	18.5	6	3	3.3	-29.8	-29.9	7.8	5.1	97.22	226
	19.3	6		3.3	-27.9	-27.9	7.5	5.2	96.84	258
	17.3	5		3.3	-31.0	-31.0	6.8	3.3	93.42	547
Rudd, <i>Sardinus erythrophthalmus</i>	15.9	5	1	3.3	-28.7	-28.7	8.4	7.7	97.59	196
Piscivorous fishes										
Pike, <i>Esox lucius</i>	63.0	6	1	3.2	-31.2	-31.2	9.9	6.9	94.32	470
Perch, <i>Perca fluviatilis</i> L	24.5	8	3	3.4	-34.2	-34.2	10.5	7.4	91.69	697
	20.0	6		3.3	-34.7	-34.7	10.0	7.2	92.49	627
	21.0	7		3.4	-33.0	-33.0	10.2	7.7	91.28	733

As lipids are depleted in ¹³C (DeNiro et al. 1977), any variation in lipid concentrations between consumer species could influence comparisons of δ¹³C. However, lipid removal in the consumer samples was not performed in order to keep the δ¹⁵N values unaffected by treatment (Post et al. 2007). The C:N ratios in some of the samples were higher than the recommended limit for aquatic organisms (C:N>3.5), at which a lipid correction should be performed (Table 1). Therefore, consumer δ¹³C data were arithmetically lipid-normalised according to Post et al. (2007): δ¹³C = δ¹³C_{untreated} -3.32 + 0.99 × C:N. We used lipid-normalised consumer δ¹³C data for all statistical analyses due to significant variation in the C:N ratios between different consumer species (see Table 1).

Radiocarbon Dating

Before radiocarbon dating, all samples were graphitized using Automated Graphitization Equipment AGE-3 (IonPlus AG). The measurements were performed with a 250 kV single stage accelerator mass spectrometer (SSAMS, NEC, USA) at the Center for Physical Sciences and Technology in Vilnius, Lithuania. Typical SSAMS system parameters can be found in the paper by (Ežerinskis et al. 2018). The background of the measurements was estimated to be 2.45×10^{-3} F¹⁴C_m (fraction of modern carbon) using phthalic anhydride (Alfa Aesar). The IAEA-C3 standard was used as a reference material (the percent of a modern carbon (pMC) value of 129.41). The ¹⁴C/¹²C ratio was measured with an accuracy better than 0.3%. For the ¹⁴C isotopic fractionation correction, the ¹³C/¹²C ratio measured with SSAMS was used.

The “conventional” radiocarbon age (RA) was calculated (Eriksson Stenström et al. 2011):

$$T_{14C_{yr}} = -8033 \times \ln (F^{14}C_m); \quad (2)$$

where F¹⁴C_m is fraction of modern carbon in aquatic samples.

Calculations and Data Analysis

Kruskal–Wallis ANOVA tests were applied to test for species effects on the stable δ¹³C, δ¹⁵N and δ³⁴S isotope values of different sampled consumers. Spearman rank correlations were used to show connections between stable C and N isotopes. Non-parametric tests were used, as the data did not meet the normality assumption of parametric methods (Shapiro–Wilk’s W tests, P < 0.05). The analyses were performed using STATISTICA 12.0 software. The significance level of P < 0.05 was specified for all statistical analyses.

RESULTS

Fish Assemblage

Overall, seven species were encountered during this study, five species were considered as benthivorous, and one—piscivorous. Functional classification in Froese and Pauly (2022) was followed. However, European perch is known to undergo a substantial ontogenetic diet shift from benthivory to piscivory (Froese and Pauly 2022); therefore, small specimens of this species were considered benthivorous, while large specimens were regarded as piscivores (Table 1). The species threshold for the diet switch was defined as the TL at which the percentage of fish-prey exceeds 50% of the total gut content biomass in most individuals. Thus, the threshold for *P. fluviatilis* was TL 20 cm (Hjelm et al. 2000). An average CPUE (the catch per unit effort) contained 3.1 kg of fish per 40-m-long multi-mesh benthic gillnet. Standard

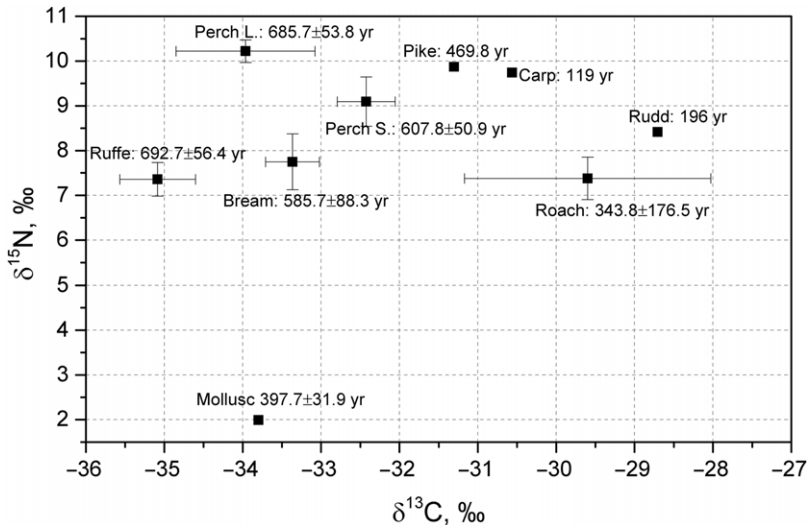


Figure 1 Isotopic bi-plot showing the mean (\pm SD) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in different fish species of Lake Tapeliai in 2018. The radiocarbon ages are shown adjacent to fish names.

catches showed roach to be the dominant fish species by the proportion of number (78.2%), while carp and bream dominated the fish assemblage by their biomass (43.2 and 30.4%, respectively). The main top predators in the fish assemblage are represented by large-sized predatory fish species such as pike and perch. All together, they constituted 13.6% of the biomass in the fish catch.

Stable Isotopes ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$)

In total, 18 replicates of seven consumer species were analysed for stable $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ isotope values in Lake Tapeliai (Table 1). $\delta^{13}\text{C}$ values ranged between -35.1‰ and -28.7‰ ($\text{range}_{\delta^{13}\text{C}} = 6.4\text{‰}$), and $\delta^{15}\text{N}$ values between -2.0 and 10.2‰ ($\text{range}_{\delta^{15}\text{N}} = 8.2\text{‰}$). $\delta^{34}\text{S}$ range was also low, from 1.3‰ to 7.7‰ ($\text{range}_{\delta^{34}\text{S}} = 6.4\text{‰}$). However, this follows the pattern typical of temperate freshwater lakes ranging from top predators (*Esox lucius*, large *P. fluviatilis*) to primary consumer (*B. tentaculata*) on the $\delta^{15}\text{N}$ axis and from profundal dwelling fish (*G. cernua*) to littoral fitophagous fish (*S. erythrophthalmus*) on the $\delta^{13}\text{C}$ axis (Figure 1, Table 1).

The mean stable isotope values differed among consumer species (Figure 1). Although comparisons were limited due to differences in sample sizes, there was clear statistical evidence of differences in the mean $\delta^{13}\text{C}$ among different consumers (Kruskal–Wallis ANOVA test: $H_{8,18} = 15.84$, $P = 0.04$). In the sampled consumers as a whole, the mean $\delta^{13}\text{C}$ values varied between $-35.1 \pm 0.5\text{‰}$ (*G. cernua*) and -28.7‰ (*S. erythrophthalmus*), with a difference of 6.4‰ . Of all the consumers studied, *G. cernua* and *S. erythrophthalmus* were most isotopically distinct (Figure 1). *G. cernua* was more ^{13}C -depleted suggesting their foraging for benthic-profundal food sources; while *S. erythrophthalmus* was ^{13}C -enriched, indicating their reliance on vegetated-littoral food sources.

The mean $\delta^{15}\text{N}$ values ranged between 2.0‰ (*B. tentaculata*) and 10.2‰ (large *P. fluviatilis*), and also varied significantly among species (Kruskal–Wallis ANOVA test: $H_{8,18} = 15.25$, $P = 0.04$). To illustrate isotopic variation in size, *P. fluviatilis* specimens were subsequently

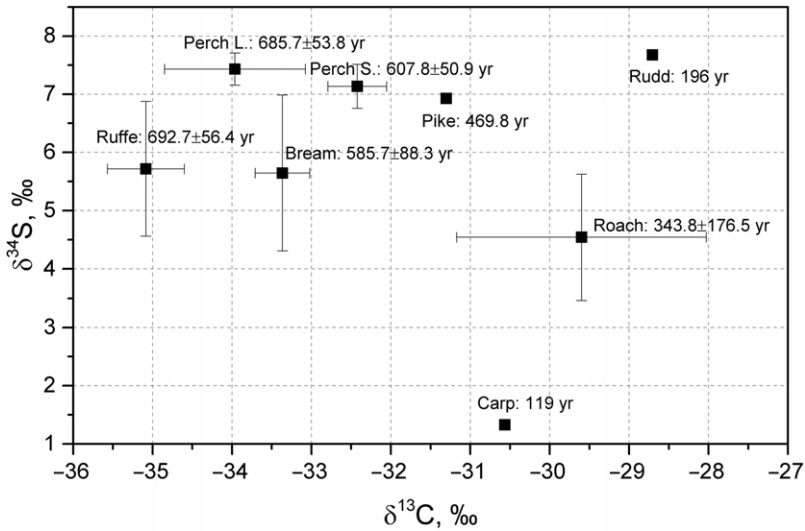


Figure 2 Isotopic bi-plot showing the mean (\pm SD) $\delta^{34}\text{S}$ and $\delta^{13}\text{C}$ values in different fish species of Lake Tapeliai in 2018. The radiocarbon ages are shown adjacent to fish names.

classified into small (S) and large (L) size groups (Figure 1 and Table 1). As expected, the most enriched in ^{15}N in comparison with other fishes was the large *P. fluviatilis* and *E. lucius* indicating these fish species as top predators in the food chain of Lake Tapeliai. It is worth noting that *C. carpio* has also ^{15}N -enriched $\delta^{15}\text{N}$ value (Figure 1). Littoral primary consumer, snail *B. tentaculata* possess the most depleted $\delta^{15}\text{N}$ values. There was no significant correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in sampled consumer specimens (Spearman rank order correlation: $\rho = 0.14$, $p = 0.59$).

The mean $\delta^{34}\text{S}$ values ranged between 1.3‰ (*C. carpio*) and 7.7‰ (*S. erythrophthalmus*). Of all the consumers studied, *C. carpio* was the most isotopically distinct (Figure 2). There was no clear statistical evidence of differences in the mean $\delta^{34}\text{S}$ among different consumers (Kruskal–Wallis ANOVA test: $H_{8,17} = 12.74$, $P = 0.08$). However, a significant correlation between $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$ values in sampled fish specimens was observed (Spearman rank order correlation: $r < 0.65$, $P = 0.004$; and even more significant if carp was excluded: $r < 0.83$, $P < 0.001$). $\delta^{34}\text{S}$ value the aquatic moss *Fontinalis antipyretica Hedv.* was 6.5‰ (Table 1).

Radiocarbon Dating

The results of radiocarbon dating and stable isotope analysis for Lake Tapeliai are shown in Table 1 and Figures 1–4. The $T_{14\text{C}_{\text{yr}}}$ value in the aquatic moss *Fontinalis antipyretica Hedv.* was 849 yr. The studied fish species present a wide range in radiocarbon ages, from 119 years for *C. carpio* (true age nine years) up to 693 years for *G. cernua* (true age of six years). Furthermore, there was also a wide range in the ^{14}C ages within the same fish species. The results showed a difference of more than 300 years in $T_{14\text{C}_{\text{yr}}}$ for the same age (true age of five years) of the roach specimens. However, there was no statistical evidence of differences in the mean $T_{14\text{C}_{\text{yr}}}$ among different fish species (Kruskal–Wallis ANOVA test: $H_{8,18} = 13.80$, $P = 0.09$). Thus, $^{14}\text{C}/^{12}\text{C}$ values did not correlate with $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$. Only $\delta^{13}\text{C}$ values were associated significantly with radiocarbon measurements (Table 2).

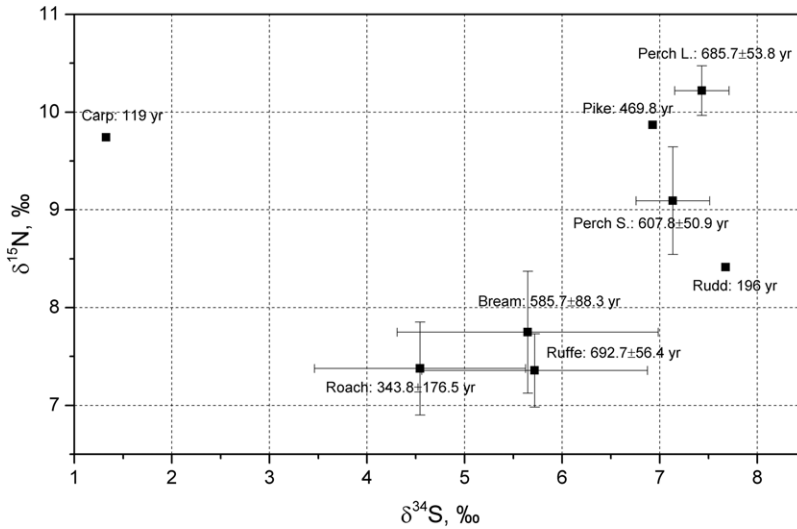


Figure 3 Isotopic bi-plot showing the mean (\pm SD) $\delta^{34}\text{S}$ and $\delta^{15}\text{N}$ values in different fish species of Lake Tapeliai in 2018. The radiocarbon ages are shown adjacent to fish names.

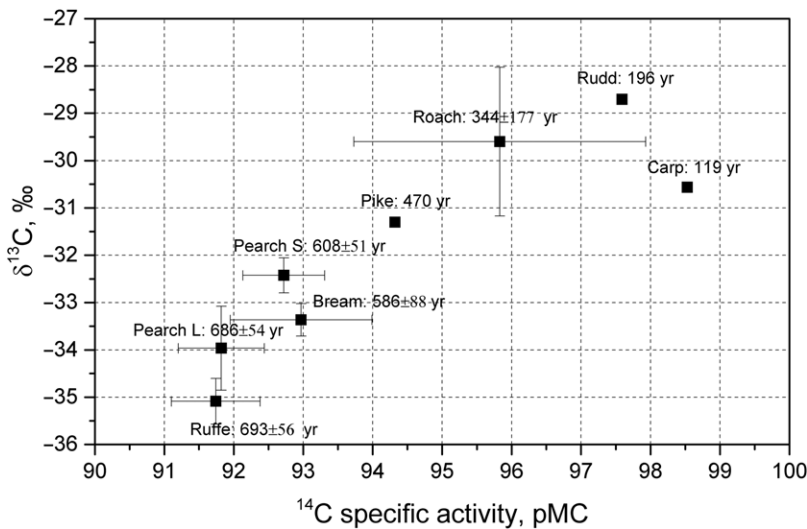


Figure 4 Isotopic bi-plot showing the mean (\pm SD) $\delta^{13}\text{C}$ and ^{14}C specific activity values in different fish species of Lake Tapeliai in 2018. The ^{14}C ages are shown adjacent to fish names.

DISCUSSION

The lake fish assemblage is formed from a very low number of fish species. Only four native fish species represent the benthivorous fish guild in the lake. Additionally, introduced non-native carp enlarge the number of benthivorous fish species within the lake. Two species represent top predator’s guild, and one species—phytophagous. Overall, the observed fish assemblage is typical for such small, eutrophic lakes, where benthivorous species form most of the fish biomass, while piscivorous constitute 1/10 part of the overall fish assemblage.

Table 2 Partial correlations for all explanatory variables for the RA value variation in fish muscle.

Explanatory variable	Beta	t (13)	p
δ ¹³ C	0.81	6.33	< 0.001
δ ¹⁵ N	0.08	0.61	0.55
δ ³⁴ S	-0.26	-1.91	0.08

The trophic chain of Lake Tapeliai appears to be rather short. For instance, the total difference in the mean δ¹⁵N of consumers ranging from the primary littoral consumer mollusk (*B. tentaculate*) to the top predators of the lake food chain such as large perch or pike is 8‰. If we use the typically quoted fractionation factor of 3.4‰ per trophic level (Vander et al. 2001; Post 2002), it turns out that the large perch or pike has the mean trophic level of 4.4, with only 2.4 trophic levels separating these top predators from the primary littoral consumers (Figure 1).

The main goal of this work was to determine the distribution of radiocarbon in the fish of a constantly changing lake ecosystem, and to investigate the possibility of linking the distribution of radiocarbon in those fish to their stable isotope values. A strong correlation ($r=0.85$ $p<0.001$, Table 2) between ¹⁴C/¹²C and δ¹³C values in fish tissues is not typical for the water ecosystems of the temperate zone, where C3 plants predominate. Carbon stable isotope values of modern (fresh plants), “old” (terrestrial soil, peat) terrestrial organic matter, as well as plants of the fresh water ecosystem overlap (Maksymowska et al. 2000; Xie et al. 2004; Esmeijer-Liu et al. 2012). Thus, stable carbon isotope ratio values and radiocarbon dating values revealing the modern/terrestrial carbon fraction usually do not correlate in organic and inorganic matter of aquatic ecosystems.

As a result of constantly changing environmental conditions, it was not appropriate to perform sulfate and DIC (or phytoplankton/zooplankton) measurements in the water column. δ³⁴S and radiocarbon measurements were performed in the aquatic moss *Fontinalis antipyretica* Hedv. instead, as the absence of roots excludes the nutrient uptake from the sediments (Maberly 1985). Thus, ¹⁴C/¹²C and δ³⁴S values in the moss should reflect the averaged stable carbon and sulfur isotope distribution in DIC and water column sulfates, respectively during its growth season. All the investigated fishes were significantly younger than the moss (849 yr), even rudd (196 yr) known from its feeding preferences for littoral macrophytes (Table 1) which theoretically should correspond averaged over several months (time period tissues undergo biochemical change) radiocarbon distribution in DIC. The high ¹⁴C/¹²C value in rudd could be explained that shallow helophytes fixing atmospheric CO₂ (not in water dissolved inorganic carbon) were the important food source in their diet.

The top predator large perch had the same highest T_{14C_{yr}} value (686 ± 54 yr) as a bottom dwelling fish ruffe mostly relying on profundal organic source such as chironomids, ostracods, and mollusks. Their isotope ratio value differences Δδ¹³C = 1.1 ± 0.9‰, Δδ¹⁵N = 2.8 ± 0.4‰, and Δδ³⁴S = 1.7 ± 1.2 ‰ (typical for high-protein diet: 2.0 ± 0.65‰, McCutchan et al. 2003) showed that ruff was the main/important food source for large perch. The second most ¹⁴C depleted values (of 586 ± 88 yr) had bream and small perch (Table 1). Both species feed on benthic invertebrates, small perch also may rely on zooplankton. High δ³⁴S value of 7.1 ± 0.4‰ in small perch (S) indicated that its diet was probably based on zooplankton feeding that reflects phytoplankton

production rather than bottom invertebrate fauna affected by sulfate reduction. Meanwhile, lower $\delta^{34}\text{S}$ value in bream revealed it feeding more on bottom invertebrate fauna.

A low $\delta^{34}\text{S}$ value of 1.3‰ (Figures 2 and 3, Table 1) in carp could indicate its preferences on benthic production influenced by sulfur reduction in anoxic sediments. Carp can tolerate anoxic conditions and is extremely well adapted for feeding on sediment dwelling chironomids—they have specialized feeding behavior that allow them to penetrate deep (up to one meter) into sediments as well as mouthparts, which enable them to sort and retain food from sediments, and it appears they constantly sucking sediments in order to locate aggregations of chironomids (Lammens et al. 1991). Strong discrimination of sulfate-reducing bacteria against ^{34}S isotope leads to lower $\delta^{34}\text{S}$ values in anoxic sediments comparing to sulfates in water column. This fractionation in some lakes was reported to vary from -3.7‰ to -14‰ (Fry 1986b; Donahue et al. 2008; Croisetière et al. 2009; Karube et al. 2012; Proulx et al. 2014) depending on sulfate concentration in water column. The difference between $\delta^{34}\text{S}$ values in aquatic moss (6.5‰) and carp just falls within the range of values for fractionation due to sulfate reduction in lakes' anoxic sediments. Another two fish species, ruffe and bream, are also benthivorous profundal dwelling fishes (Froese and Pauly 2022), thus they also may compete with carp for chironomid prey in such turbid waters as lake Tapeliai. $\delta^{34}\text{S}$ values for these species were 5.7‰ and 5.6‰, respectively, and were between the most depleted value for carp and the value of the moss (Table 1). These fish may feed chironomids from the upper oxic sediment layers having more positive $\delta^{34}\text{S}$ values than those from lower anoxic layers. Martin et al. 2008 showed that larvae of a *Chironomus* species feeding on oxic sediments had higher $\delta^{34}\text{S}$ values than those that fed on anoxic sediments. Carp also had very low $T_{14\text{C}_{\text{yr}}}$ value (119 yr) comparing to the other bottom dwelling fishes bream and ruffe (586 ± 88 yr and 693 ± 56 yr, respectively, Table 1). However, this difference in $T_{14\text{C}_{\text{yr}}}$ of several hundred years cannot be explained by the adaptation of the carp to feeding in deeper sediment layers that could be affected by radiocarbon due to the “bomb peak.” Our previous study (Barisevičiūtė et al. 2019) revealed that Tapeliai received ^{14}C depleted organic (and possible inorganic) carbon due to the opening of the ditch with Lake Lydekis at the beginning of the last century. Sediments at 25–30 cm (according ^{210}Pb dating this layer corresponds the period of 1960–1942) are ~ 1100 yr “older” than surface sediments. Thus, carp receiving a significant amount of their diet from chironomids from deeper layers would appear to be “older” than other fish species. Thus, only an additional allochthonous food source could be the reason for the low $\delta^{34}\text{S}$, unusually high $\delta^{15}\text{N}$ and $^{14}\text{C}/^{12}\text{C}$ values in carp tissues. To our knowledge (V. Rakauskas personal observation), carp were rather intensively fed by anglers in Lake Tapeliai, that could explain the trace of additional allochthonous food sources in their tissues. Low $\delta^{34}\text{S}$ values in general feeder roach tissues ($\delta^{34}\text{S}$ value of $4.5 \pm 0.5\text{‰}$ was lower than in other bottom dwelling fishes such as ruff and bream) also likely indicate that their diet, like that of carp, contained additional allochthonous food sources. On the other hand, low $\delta^{15}\text{N}$ values ($7.4 \pm 0.5\text{‰}$) in roach tissues indicates that the fish could also include terrestrial plants in their diet (Table 1, Figure 3).

CONCLUSIONS

The $^{14}\text{C}/^{12}\text{C}$ ratio distribution and its relationship with stable S, C, and N isotopes among the fish of Lake Tapeliai was studied. The radiocarbon age of the fish in this lake, which was constantly affected by the inflows of the ^{14}C depleted carbon sources from the surrounding watershed mires, ranged from 119 to 693 yr. No relationship of the carbon isotope ratio values with sulfur or nitrogen isotope ratios was observed in the studied ecosystem. However, $^{14}\text{C}/^{12}\text{C}$ measurements correlated significantly with $\delta^{13}\text{C}$ values in fish tissues.

¹⁴C analysis is essential to trace the pathways of modern terrestrial carbon through the food web in freshwater lakes. However, in reconstructing the diet of fish such as carp, adapted to penetrate deep (up to one meter) into sediments, it is necessary to examine the sediments themselves and how they have been affected by ¹⁴C from the bomb peak. It was our previous sediment studies that showed that the deeper sediment layers were not enriched in ¹⁴C due to the bomb peak, but depleted in ¹⁴C, and this helped determine that carp was being fed by allochthonous food sources provided by anglers.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit <https://doi.org/10.1017/RDC.2022.82>

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