


Stable isotope analysis confirms substantial differences between subtropical and temperate shallow lake food webs

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Abstract Differences in trophic web structure in otherwise similar ecosystems as a consequence of direct or indirect effects of ambient temperature differences can lead to changes in ecosystem functioning. Based on nitrogen and carbon stable isotope analysis, we compared the food-web structure in a series of subtropical (Uruguay, 30–35°S) and temperate (Denmark, 55–57°N) shallow lakes. The food-web length was on average one trophic position shorter in the subtropical shallow lakes compared with their temperate counterparts. This may reflect the fact that the large majority of subtropical fish species are omnivores (i.e., feed on more than one trophic level) and have a strong degree of feeding niche overlap. The shapes of the food webs of the subtropical lakes

(truncated and trapezoidal) suggest that they are fuelled by a combination of different energy pathways. In contrast, temperate lake food webs tended to be more triangular, likely as a result of more simple pathways with a top predator integrating different carbon sources. The effects of such differences on ecosystem functioning and stability, and the connection with ambient temperature as a major underlying factor, are, however, still incipiently known.

Keywords Food-web structure · Food-web length · Omnivory · Ecosystem functioning

Introduction

The height and shape of trophic webs may potentially affect the entire ecosystem functioning (Post et al.,

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2000; Woodward, 2009; Emmerson, 2012). Particularly, the number of steps involved in the transfer of energy from primary producers to top predators, i.e., the food-web length (hereafter FWL), seems at least partly determined by ecosystem productivity and size, ambient temperature, habitat heterogeneity, and changes in species richness (including arrival and loss of species). These variations may occur among, but also within, ecosystems across temporal and spatial scales [as described in the pioneer works by Elton (1927) and Lindeman (1942) and reviews by Doi et al. (2012), Pimm (1991), and Post (2002a)]. However, a global-scale analysis showed that lake and stream FWL exhibited no direct or, at most, a weak relationship with ecosystem size, mean annual air temperature, or latitude; however, there was a tendency for FWL to be longer at high latitudes than in the tropics (Vander Zanden & Fetzer, 2007).

Theoretical analyses (Arim et al., 2007a, b; Post & Takimoto, 2007) and modeling exercises (Takimoto et al., 2012) suggest that the length and also the connection strength within a food web may be explained, at least in part, by the degree of omnivory of intraguild predators (IGP) (Post & Takimoto, 2007; Takimoto et al., 2012). Widespread feeding on lower trophic positions would result in shorter FWL (Layman et al., 2005; Post & Takimoto, 2007), a phenomenon termed the “omnivory mechanism” (Post & Takimoto, 2007). In contrast, addition of species with potentially different diets, as expected in subtropical regions where fish richness and specific and functional diversity are far higher than in similar temperate shallow lakes (Teixeira-de Mello et al., 2009), could result in longer FWL [the “addition mechanism” sensu Post & Takimoto (2007)]. Thus, contrasting scenarios, indirectly linked to the climate regime, could emerge depending on the predominance of each mechanism. However, empirical evidence of the relationships between omnivory and specific richness and FWL, and the relative importance of the underlying mechanisms, is still scarce (Glazier, 2012).

The shape of food webs also responds to the occurrence of different types of primary producers. When several resources co-occur, more complex pathways for the transfer of energy and matter may exist (Polis & Strong, 1996; Vadeboncoeur et al., 2005). Such multiple pathways may occur in shallow lakes where both pelagic primary production (by

phytoplankton) and littoral or benthic primary production (by periphyton) can be important sources of energy (Vadeboncoeur et al., 2003; Vander Zanden et al., 2011). However, the extent to which the different basal resources are exploited might be indirectly linked to differences in ambient temperature (Meerhoff et al., 2007). Changes in the width of the carbon resources (carbon range, sensu Layman et al. (2007)), together with the occurrence of complex pathways, could thus be found in different lake types and under different climate regimes.

To elucidate variations in the length and shape of trophic webs from shallow lakes in two regions with distinct climates, we analyzed stable isotopes ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) of key biological communities of the food web in five subtropical (Uruguay, 30–35°S) and four temperate (Denmark, 55–57°N) shallow lakes. Considering the greater fish species richness of subtropical systems, we expected that longer FWL and a wider use of carbon sources would occur, if the “addition mechanism” prevails. Alternatively, FWL might be shorter in the subtropics, if the expected higher degree of omnivory and its consequent effects at the community level predominate in the set of studied lakes.

Methods

Study sites

We selected five shallow lakes located along the east coast of Uruguay covering a wide range in trophic states and water transparency (Table 1). The lakes represent the typical variability in the trophic state of Uruguayan shallow lakes (Kruk et al., 2009; Pacheco et al., 2010). For the comparison, we used data from four lakes representative of shallow lakes in Denmark, selected to ensure, to as high an extent as possible, comparability with the Uruguayan lakes regarding trophic state and key limnological characteristics, an exception being size that was somewhat smaller in the temperate region.

Field sampling

The samples were collected at the end of the growing season (late summer) in both countries (March in Uruguay, August in Denmark). A similar sampling protocol was used in both countries and included an

Table 1 Main limnological features of the subtropical and temperate study lakes measured simultaneously with the stable isotope sampling or according to the already published results (Pacheco et al., 2010)

	Vaeng	Tranevig	Gammellose	Denderup	Cisne	Diario	Garcia	Clotilde	Blanca
Area	15	2.7	1.3	4.6	127	101	13.5	29	60
Z _{max}	1.7	0.9	1.6	1.9	3.2	1.7	2	3.1	2.5
SD	0.9	0.75	1.3	1.8	0.4	1.05	1.7	1.8	0.64
Temp	17	16.6	16.4	16.3	13.1	19.2	16.3	17.6	19.6
pH	7.9	7.6	8.1	8.1	7.1	7.3	6.32	7	7.4
Cond	268	71	595	171	210	348	142	360	320
Chl-a	65.8	37.6	78.5	7.2	6	10	2	2.3	38.6
PVI	0	44	3	27	0	40	5	28	13
TP	113	60.5	157	54	413	75.8	29.8	27.7	51.9
TN	1018	1040	2212	664	1048	825	332	451	1017

Lake area (ha), maximum depth, Z_{max} (m), Secchi depth, SD (m), summer values of temperature, Temp (°C), conductivity, Cond ($\mu\text{S cm}^{-1}$), percentage of lake volume inhabited by submerged plants, PVI (%), phytoplankton biomass as chlorophyll-a concentration, Chl-a ($\mu\text{g l}^{-1}$), and water concentrations of total phosphorus, TP ($\mu\text{g l}^{-1}$), and total nitrogen, TN ($\mu\text{g l}^{-1}$). Lakes are ordered by decreasing fish richness in both regions (Table 2)

intensive sampling of the pelagic and littoral habitats to obtain taxa representing all trophic levels and carbon sources. For stable isotope analyses (SIA), we collected samples of the principal consumers in both pelagic and littoral areas. To ensure a sufficient amount of organisms for the analysis, lake water was pumped through conical plankton nets (20 and 65 μm for phytoplankton and zooplankton, respectively), macrophyte-associated macroinvertebrates and benthic macroinvertebrates were sampled by intensively swiping a hand net and by integrating several dredges covering the entire bottom of each lake, respectively. Fish were captured with multimesh-size gillnets and electrofishing; the sampling effort used included the deployment of several gillnets which were set overnight. Electrofishing was conducted in the littoral areas at sunset to capture small specimens and littoral sit-and-wait predators. This combined sampling method can appropriately capture the structure of the target community in both studied regions (Teixeira-de Mello et al., 2009), fact confirmed as we found species a priori unknown to be in the studied systems. All samples were rapidly frozen and transported to the laboratory.

Following recommendations by Post (2002b), principal carbon source signals from the pelagic and littoral areas were indirectly estimated from the two well-known primary consumers (as substitution for primary producers), namely filter-feeding bivalves

and grazing snails (Post, 2002b). The selection of the right baseline individuals is essential for the estimation of an “average” food-web length in the community, and the selection of gastropods and bivalves seems the best strategy as they are long-living and low dispersion organisms representing two contrasting energy uptake pathways (Post, 2002b; Jardine et al., 2014). Macrophyte leaves and periphyton washed from the predominant macrophytes were also sampled.

Sample processing for isotopic analysis and data analysis

In the laboratory, samples of plants, periphyton, phytoplankton, zooplankton, macroinvertebrates, fish flank muscle, and snail and bivalve soft tissue were freeze dried and ground to a fine powder for stable isotope analysis (SIA). Each sample (1–3 mg, weighed to 0.01 mg precision) was transferred to tin capsules and analyzed at the UC Davis Stable Isotope Facility (University of California, USA) for carbon and nitrogen stable isotopes. The food-web structure of each lake was visualized by plotting the trophic position (based on $\delta^{15}\text{N}$ isotopic signature values) against $\delta^{13}\text{C}$ values for all available organisms (Fry, 1991).

We estimated the trophic position of each individual according to Post (2002b):

$$\text{Trophic position (TrPo)} = [(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}})/2.98] + 2,$$

where $\delta^{15}\text{N}_{\text{consumer}}$ is the isotopic signature of each individual analyzed and $\delta^{15}\text{N}_{\text{base}}$ is the averaged baseline organisms (bivalves and snails), 2.98 is the expected $\delta^{15}\text{N}$ fractionation per trophic level (Vanderkluft & Ponsard, 2003), and 2 is the theoretical trophic level of baseline organisms (Post, 2002b). We estimated the FWL as the maximum trophic position for each lake.

In addition to FWL, we also calculated community-wide metrics (Layman et al., 2007; Jackson et al., 2011) to identify key features of the specific food webs: (i) carbon range (CR), which is the difference between the most $\delta^{13}\text{C}$ -enriched and the most $\delta^{13}\text{C}$ -depleted values, for both the total consumer food web (excluding basal resources) and per trophic level (CR2, CR3); (ii) total area of the web (TA), measured as the convex hull area given by all species in the $\delta^{13}\text{C}$ -TrPo biplot and by the adjustment of the standard ellipse areas (SEA) in the biplot; and (iii) the mean nearest neighbor distance (NND), as the mean of the Euclidean distances to the nearest neighbor of each species in the biplot. CR indicates the amplitude of the carbon resources being used; TA and SEA represent a measure of the total amount of niche space occupied by the trophic web, whereas smaller NND values indicate redundancy of species with similar trophic ecology. Although both TA and SEA represent the trophic niche space occupied by communities, ellipse-based SEA are developed in a Bayesian framework, rendering this method unbiased with respect to sample size and thus more robust than the convex hull area-based TA metrics (Jackson et al., 2011). Despite that the estimation of these metrics is usually made using raw $\delta^{15}\text{N}$ (Layman et al., 2007), they have also been estimated by standardizing $\delta^{15}\text{N}$ to trophic web length. We used the latter method in our study as it shows reduced variability in $\delta^{15}\text{N}$ due to factors other than trophic fractionation (e.g., González-Bergonzoni et al., 2014).

We calculated these parameters using SIAR and SIBER packages in R software and PAST software (Hammer et al., 2001) and tested for differences between climate zones in the measured trophic web attributes (i.e., FWL, CR, TA, SEAb, and NND) using the Mann–Whitney nonparametric test. Spearman correlations among FWL, fish richness, CR,

ecosystem size (i.e., lake surface area), and lake pelagic productivity (using phytoplankton Chl-a concentration as a proxy) were also calculated.

Results

FWL was, on average, one trophic position shorter in the subtropical lakes than in the temperate lakes (Table 2; Fig. 1). There was no significant correlation between FWL and ecosystem size and pelagic productivity (inferred using Chl-a concentration as proxy). Mean fish richness was greater in the subtropical than in the temperate lakes (9.5 ± 1.5 and 5.3 ± 1.1 SE species per lake, respectively) and was significantly correlated with both ecosystem size ($r^2 = 0.82$) and pelagic productivity ($r^2 = 0.67$).

The subtropical fish assemblages included several relatively small-sized omnivorous species of which *Jenynsia multidentata* Jenyns, 1842 and *Cnesterodon decemmaculatus* Jenyns, 1842 were the most abundant (Table 3). Several potentially piscivorous species (Teixeira-de Mello et al., 2009) like *Australoheros facetus* Jenyns, 1842, *Hoplias malabaricus* Bloch, 1794, *Oligosarcus jenynsii* Günther, 1864, *Rhamdia quelen* Quoy & Gaymard, 1824, and *Synbranchus marmoratus* Bloch, 1795 were also frequently observed (Table 3). Among the piscivores, *H. malabaricus* did not reach the top of the food web but held the same trophic position as small-sized omnivores (Table 3). In contrast, *O. jenynsii* always occurred at the highest trophic level. Remarkably, the small-sized *J. multidentata*, usually classified as omni-planktivore (Goyenola et al., 2011), exhibited high mean $\delta^{15}\text{N}$ values in all systems (Table 3). Shrimps, in particular *Palaemonetes argentinus* Nobili, 1901, occurred in all the subtropical lakes and, was abundant in four of the lakes where they occupied the 3rd trophic position along with predatory macroinvertebrates and several omnivorous fish species.

Notwithstanding their relative paucity of species, temperate fish assemblages (Table 4) consisted roughly of the same trophic groups that characterized the subtropical communities caught during this study. Potential piscivores were abundant, including *Esox lucius* L., *Perca fluviatilis* L., and *Anguilla anguilla* L. (Table 4). Several fish species held higher trophic positions (around 4th trophic position) than observed in the subtropical lakes. *Esox lucius* was the apical

Table 2 Fish species richness (FR) and community-wide metrics from subtropical (above) and temperate (bottom) lakes, calculated based on the distribution of species in the $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplots (Fig. 1)

Lake	FR*	FWL*	CR	CR 2	CR 3*	SEAb*	TA	NND*
Cisne	13	4.0	7.7	4.5	3.5	5.2	35.2	0.4
Diario	11	3.4	9.7	9.7	5.9	4.2	27.6	0.4
Garcia	11	3.9	9.9	9.4	7.6	5.8	29.7	0.6
Clotilde	9	4.1	8.5	7.7	5.1	5.4	30.1	0.6
Blanca	4	4.4	7	7	3.4	5.2	17.2	0.7
UY Median	11	4.0	8.5	7.7	5.1	5.2	29.7	0.6
Range	4–13	3.4–4.4	7.0–9.9	4.5–9.7	3.4–7.6	4.2–5.8	17.2–35.2	0.4–0.7
Vaeng	8	5.1	4.9	4.3	2.6	6.8	58.7	0.6
Tranevig	6	5.8	10.5	7.3	0.6	6.8	45.7	0.9
Gammellose	4	4.6	4.6	4.4	3.0	6.7	18.3	1.2
Denderup	3	4.4	9.9	9.9	4.1	6.6	36.7	0.6
DK Median	5	4.8	7.4	5.9	2.8	6.75	41.2	0.8
Range	3–8	4.4–5.8	4.6–10.5	4.3–9.9	0.6–4.1	6.6–6.8	18.3–58.7	0.6–1.2
Z_{value}	1.85	2.2	0.1	0.7	1.96	2.32	1.35	1.98
P	0.06	0.02	0.9	0.5	0.05	0.02	0.18	0.05

Food web length (FWL), maximum trophic position for each lake, carbon range (CR), standard ellipse areas (SEAb), total area (TA), convex hull area encompassed by all species, and mean nearest neighbor distance (NND) values are shown. Median and range for each climate area are provided together with the results of statistical analyses, indicating significant ($P < 0.05$) or marginally significant ($0.05 < P < 0.10$) differences (*) between locations (Mann–Whitney non parametric tests). Lakes are ordered by decreasing FR in both the regions

species in the food web in two out of the four lakes, with values close to the 6th trophic position (corresponding to one individual; Table 4), and was not lower than the 4th position in any of the lakes. In one lake, both *P. fluviatilis* and *Tinca tinca* L. occupied higher trophic positions than *E. lucius*, likely reflecting the overall small body size of the latter (Table 4).

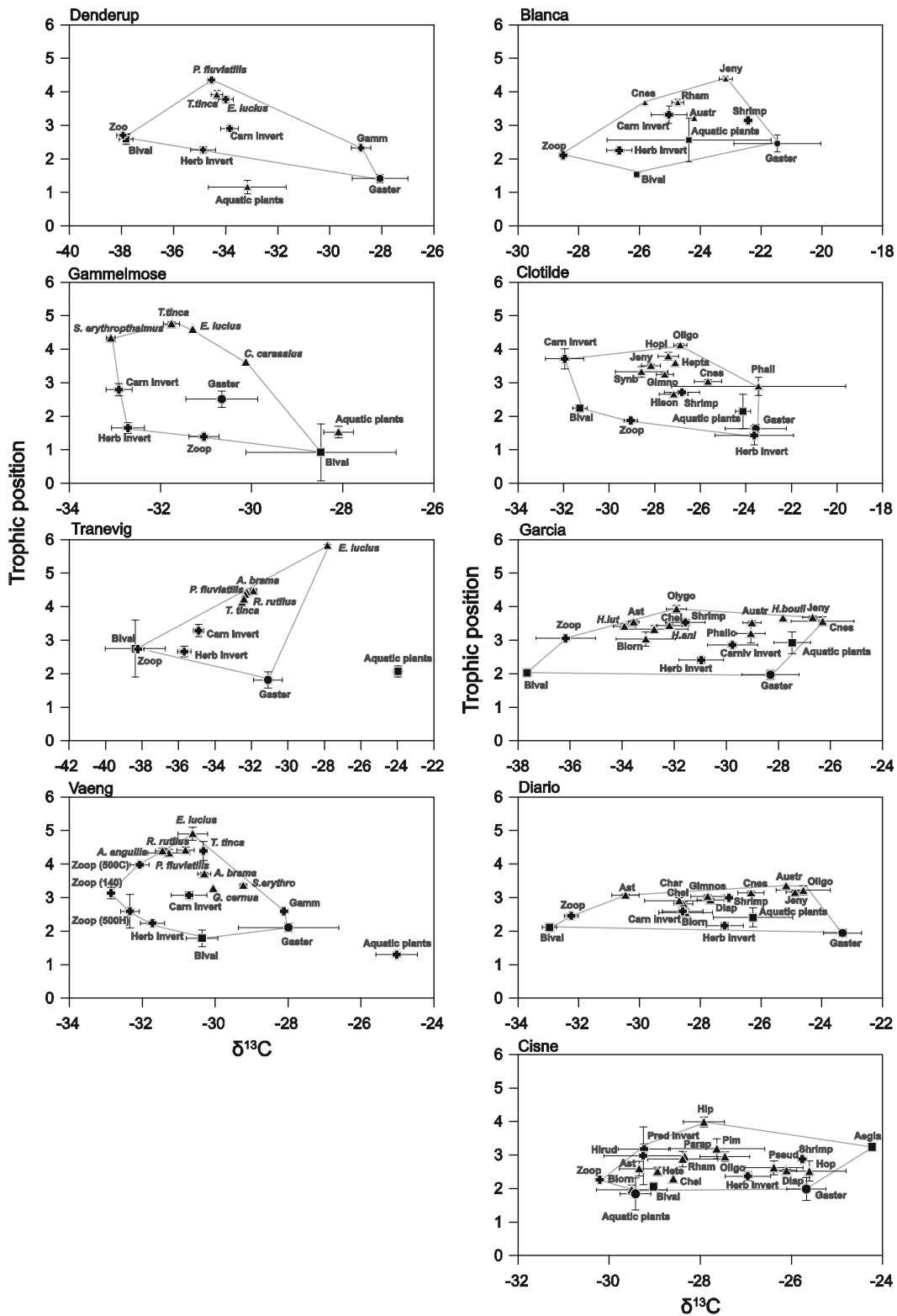
Taking the food webs as a whole, the overall mean $\delta^{13}\text{C}$ carbon range (CR) was slightly (though not statistically significant) wider in the warmer lakes, being 8.7 in the subtropical and 7.5 in the temperate lakes (Table 2). The carbon range per distinct trophic level showed some variation between regions: no significant differences appeared at trophic position 2 (primary consumers), but CR was twice as wide at position 3 (secondary consumers) in the subtropical lakes ($Z = 1.96$, $P = 0.05$; Table 2).

Temperate trophic webs typically had a triangular convex hull area, whereas the subtropical webs were typically trapezoid shaped (Figs. 1, 2), being shorter and wider at the 3rd trophic position (Table 3; Fig. 2). Also SEAb captured the differences in trophic niche space between the regional food webs, the minor and major axes being more similar in the temperate lakes,

whereas a larger area towards the major axis was occupied in the subtropical systems (Fig. 3). Surprisingly, TA did not show statistically significant differences between climate zones, in contrast to SEAb ($Z = 2.32$, $P = 0.02$; Table 2; Fig. 2). The nearest neighbor distance (NND) was significantly shorter in the subtropical lakes ($Z = 1.98$, $P = 0.05$; Table 2).

Discussion

Results based on SIA showed large differences in trophic web structure between comparable shallow lakes from regions with contrasting climates. In particular, food-web length was shorter in the subtropical than in temperate shallow lakes, supporting the second of the contrasting hypotheses. This finding cannot be ascribed to differently sized top predators in each climate region as large fish specimens, usually classified as piscivores or omni-benthic-piscivores (Teixeira-de Mello et al., 2009; Gelós et al., 2010), occurred in both the regions. However, in the subtropical lakes, the trophic position of larger fish was similar to that of smaller-sized species and in



◀ **Fig. 1** Stable isotope-based biplots showing the convex hull areas encompassing all fish species. *Left* temperate lakes, *right* subtropical lakes. The *diagrams* show trophic position (inferred from $\delta^{15}\text{N}$) against $\delta^{13}\text{C}$ signals. For fish, each point represents the mean value of 2–20 individuals of different sizes. Herb. Invert. and Carn. Inv. are the averages of all invertebrate specimens as assigned to each particular trophic group according to the literature (herbivorous, invertivorous, or carnivorous). Gaster and Bival are the averages of Gastropoda and Bivalvia in each lake (baseline signals of littoral and pelagic food webs in the calculations). *Error bars* represent \pm 1SE. Lakes are ordered by decreasing fish richness in both the regions (Table 2)

some cases similar to that of predatory macroinvertebrates. Such an apparent mismatch between measured and expected trophic positions for large predatory fish has previously been reported for some tropical rivers (Layman et al., 2005), where the wide variation in trophic position of tropical predatory fish was suggested to be due to multiple feeding strategies, which typically occur in low latitude species-rich systems (Layman et al., 2005). Large-sized tropical piscivores usually feed on the most abundant items of prey, typically detritivorous species, which gives them a short trophic position, only two trophic steps away from basal resources such as detritus (e.g., Watson et al., 2013; Jardine, 2016).

Concerns may arise regarding the application of nitrogen stable isotopes for estimation of trophic web length using a single average trophic fractionation value, as trophic fractionation is not truly constant throughout the whole food web (Bunn et al., 2013). In streams and rivers, it has been shown that trophic steps between algae and grazing macroinvertebrates can produce average trophic enrichment values as low as 0.6‰, and 1.6‰ enrichment between grazing and predator macroinvertebrates, whereas the trophic enrichment between invertebrate and fish compartments can range from approximately 2.2–3.9‰. By using an average trophic enrichment of 2.98‰ from a meta-analysis specifically arrayed for lake systems (Post, 2002b), we assumed that there were no differences in trophic enrichment created by climate regions and that the number of trophic steps between invertebrate and fish compartments was the same in both. This seems reasonable as there is no evidence for differential trophic fractionation in different regions of the world (e.g., Bunn et al., 2013) and as we found both grazing and predatory macroinvertebrates in both the regions. Thus, we have no reason to suppose that the observed differences can be caused by factors

other than the higher average number of trophic steps in the temperate than in the subtropical lakes. In fact, our study might overestimate the maximum trophic position in some Uruguayan lakes as herbivorous and omnivorous fish usually enrich their N signature by 4‰ with respect to algae (Bunn et al., 2013). This probably explains the surprisingly elevated trophic position observed here when using the lower average trophic enrichment value of Post,(2002b). Another potential methodological limitation in the use of stable isotopes in trophic position estimates is the fact that stable isotopes reflect dietary assimilation in the last sampling weeks/months (Heady & Moore, 2012), whereas there are well-known seasonal changes in feeding strategies of fish in both subtropical and temperate regions, for example, towards higher vegetal consumption by several omnivores in summer (Persson, 1986; González-Bergonzoni et al., 2016). We aimed to avoid the bias of different time frames in the fish stable isotopes by conducting the sampling during the same season (the end of the growing/reproductive season) in both the regions.

As expected from earlier studies (e.g., Lazzaro et al., 2003; Meerhoff et al., 2007; González-Bergonzoni et al., 2012), we also observed higher fish species richness in the subtropical lakes. Fish richness was positively correlated with both lake surface area and pelagic primary producer biomass as expected from the richness–productivity and richness–ecosystem size relationships (Rosenzweig, 1995; Lawton, 1999; Dodson et al., 2000). According to the proposed insertion and addition mechanisms (Post, 2002a), additional (including higher) trophic levels might be expected as more fish species occur in the subtropical food webs. However, we observed shorter FWLs in the subtropical lakes, suggesting that other mechanisms prevailed. One such mechanism could be a different degree of omnivory, which is a predominant characteristic of subtropical and tropical fish assemblages (e.g., Jepsen & Winemiller, 2002; Lazzaro et al., 2003; Meerhoff et al., 2007). An increase in the proportion of herbivorous fish species has been observed with the decreasing latitude and increasing water temperature in a variety of aquatic ecosystems worldwide (González-Bergonzoni et al., 2012), concurring at community level with predictions of the Metabolic Theory of Ecology (Brown et al., 2004) suggesting that energy limitation may lead to enhanced omnivory to satisfy the boosted metabolic needs (Brown et al., 2004; Arim

Table 3 Fish species from the Uruguayan (subtropical) lakes used for the stable isotope analyses

Species	Cisne			Diario			García			Clotilde			Blanca		
	n	Mean	TrPo	n	Mean	TrPo	n	Mean	TrPo	n	Mean	TrPo	n	Mean	TrPo
<i>Australoheros facetus</i> * Jenyns, 1842				1	3.1	3.69	10	5.8	3.62	1	2.6	3.34			
<i>Hoplias malabaricus</i> * Bloch, 1794	3	5.2	3.23							5	30.2	4.31			
<i>Oligosarcus jenynsii</i> * Günther, 1864	2	12	3.66	10	12.7	3.55	10	8.6	4.02	9	16.9	4.65			
<i>Rhamdia quelen</i> * Baird & Girard, 1854	4	4	3.59							6	24.3	3.85	6	3.9	3.69
<i>Synbranchium marmoratus</i> * Bloch, 1795															
<i>Characidium rachovii</i> Regan, 1913				6	2.7	3.24									
<i>Corydoras paleatus</i> Jenyns, 1842	1	3.5	4.03												
<i>Asyanax</i> sp. Baird & Girard, 1854	8	5.8	3.30	3	2	3.4	10	3.5	3.63						
<i>Cheirodon interruptus</i> Jenyns, 1842	1		2.76	10	4	3.16	6	4.7	3.53						
<i>Cnesterodon decemmaculatus</i> Jenyns, 1842				10	2.6	3.47	3	2.5	3.66	10	2.4	3.57	1	1.8	3.79
<i>Diapoma terofali</i> Géry, 1964	10	4.6	3.25	10	5.6	3.26									
<i>Gymnogobagus</i> cf. <i>meridionalis</i>				8	3.4	3.37				15	3.7	3.78			
<i>Hyphessobrycon anisitsi</i> Eigenmann, 1907							2	3.7	3.53						
<i>Hyphessobrycon boulengeri</i> Eigenmann, 1907							1	3.7	3.76						
<i>Heptapterus mustelinus</i> Valenciennes, 1835										1	2.6	3.9			
<i>Heterocheirodon yatai</i> Casciotta, Miquelarena & Protogino, 1992	2	4.1	3.22												
<i>Hyphessobrycon luetkenii</i> Boulenger, 1887							8	5	3.51						
<i>Jenynsia multidentata</i> Jenyns, 1842				10	2.8	3.49	10	4.3	3.78	9	3.6	4.06	11	3.6	4.38
<i>Phalloceros caudimaculatus</i> Hensel, 1868							3	2.2	3.21	2	2.2	3.42			
<i>Pimelodella australis</i> Eigenmann, 1917	2	4	3.89												
<i>Pseudocorynopoma doriae</i> Perugia, 1891	6	4.5	3.29												
<i>Steindachnerina biornata</i> Braga & Azpelicueta, 1987	10	9.9	2.67	9	11.7	2.55	6	10.1	3.13						
<i>Hypostomus commersoni</i> Valenciennes, 1836	2	4.1	4.69												
<i>Hisonotus</i> sp. Eigenmann, 1889										5	5.2	3.2			
<i>Parapimelodus valenciennis</i> Lütken, 1874	1	15.1	2.95												

The number of analyzed specimens (*n*), mean body length, and estimated trophic position (TrPo) are shown. * Potentially piscivorous species. Lakes are ordered by decreasing fish richness (Table 2)

Table 4 Fish from the Danish (temperate) lakes used for the stable isotope analyses

Species	Vaeng			Tranevig			Gammellose			Denderup		
	<i>n</i>	Mean	TrPo	<i>n</i>	Mean	TrPo	<i>n</i>	Mean	TrPo	<i>n</i>	Mean	TrPo
<i>nAbramis brama</i> L.	28	9.2	3.68	11	24.2	4.47						
<i>Exox lucius</i> * L.	7	33.8	4.87	1	50.8	5.82	1	38	4.59	4	14.4	3.37
<i>Perca fluviatilis</i> * L.	43	12.7	4.36	17	13.5	4.35				32	16.2	3.95
<i>Rutilus rutilus</i> L.	60	15	4.38	12	14.7	4.34						
<i>Scardinius erythrophthalmus</i> L.	31	9	3.33	21	13.5	4.13	6	12.9	4.33			
<i>Carassius carassius</i> L.							1	15	3.61			
<i>Gymnocephalus cernua</i> L.	1	7	3.26	4	34.6	4.23						
<i>Tinca tinca</i> L.	2	41	4.36				8	45.4	4.76	7	45.4	3.52
<i>Anguilla anguilla</i> * L.	9	44.3	4.3									

The number of analyzed specimens (*n*), mean body length, and estimated trophic position (TrPo) are shown. * Potentially piscivorous species. Lakes are ordered by decreasing fish richness (Table 2)

et al., 2007a). A diverse diet that incorporates a higher amount of different items (Arim et al., 2007a), and enhanced feeding on lower trophic positions (Beisner et al., 1997; Petchey et al., 1999), could potentially satisfy the greater energy demands of organisms at a given trophic position under higher ambient temperatures.

Regarding the carbon range, we found similar values at the base of the trophic web in the two climatic zones, indicating a similar use of carbon sources (i.e., phytoplankton and periphyton) by primary consumers. However, at the secondary consumer level (CR3), the carbon range was significantly broader in the subtropical lakes, pointing to a mixture of simultaneously occurring strategies where some taxa have a lower integration of carbon sources, while other co-occurring taxa integrate several carbon sources (Fig. 3). Fish reliance on periphyton as a major carbon source has previously been demonstrated in shallow temperate lakes; its importance depends, however, on water clarity and the consequent relative importance of benthic primary and secondary production (Vander Zanden & Vadeboncoeur, 2002; Jones & Waldron, 2003). In our subtropical systems, many species occupied an intermediate position (secondary consumers) in the food web (i.e., several fish species and shrimps) and may act as additional pathways for the different carbon sources (Post & Takimoto, 2007). Therefore, intermediate consumers could enhance the transfer of basal carbon to higher trophic positions without adding more trophic links to the web. In addition, higher functional redundancy in warmer lakes was evidenced here by a closer nearest neighbor distance (NDD), meaning that more species occupied similar trophic web positions in the subtropical compared to the temperate lakes.

As a consequence of the shorter FWL in the subtropical lakes and the suggested differences in energy pathways in the different climate zones, the shapes of the food webs (depicted by the convex hull shapes and community-wide metrics) differed between the two climatic regions studied (see Fig. 2). Our results suggest that temperate trophic webs are characterized by multichain omnivory [IGP module, sensu Vadeboncoeur et al. (2005)], with one top predator integrating the different carbon sources fuelling the web (mainly represented here by phytoplankton and periphyton and with an intermediate $\delta^{13}\text{C}$ value). Conversely, in the subtropical lakes, the

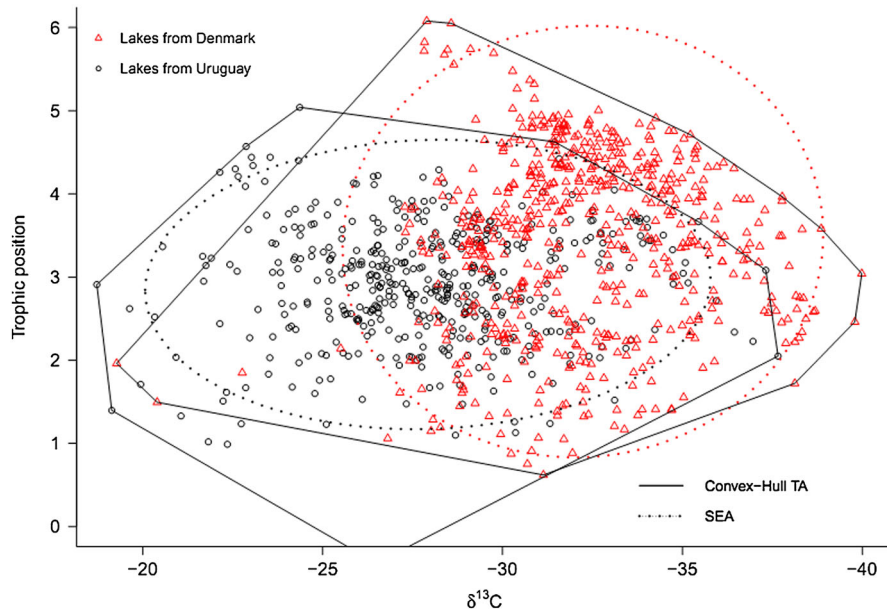


Fig. 2 Trophic diversity for the set of shallow lakes in Denmark (*triangles*) and Uruguay (*circles*), depicted by Total Convex Hull area (*full lines*) and Standard Bayesian Ellipses (SEA; *dotted lines*). Both representations graphically captured

the higher trophic positions in the temperate systems. However, only the SEA analysis was able to statistically express the differences (Table 2)

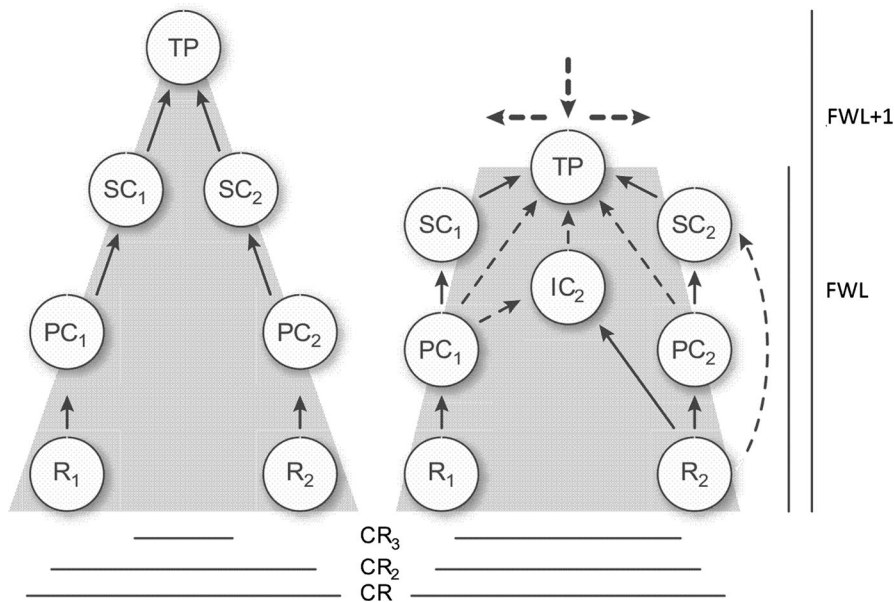


Fig. 3 Conceptual models of trophic web functioning in the temperate (*left*) and subtropical (*right*) lakes inferred from $\delta^{13}\text{C}$ -TP biplots and community-wide metrics. The arrows above the model indicate lowering of one trophic position occurring concomitantly with a widening of the carbon range at the third level of the chain. R1 and R2, phytoplankton and periphyton,

respectively; PC, primary consumers; SC, secondary consumers; IC, intermediate consumers; TP, top predators; CR, total carbon range; CR3 and CR2, the carbon range that reaches the trophic positions of primary and secondary consumers; FWL, food-web length

occurrence of a combination of multichain and single-chain omnivory, and the resultant more complex energy transfer pathways, might explain the community metrics (particularly CR3) and the shapes observed. The higher strength of the IGP module, together with a more reticulated topology of the trophic web in the warmer lakes (Meerhoff et al., 2007), may account for both the lower realized trophic web length and the wider CR in higher trophic positions (and the same basal range) in such lakes.

Our results should be interpreted with caution due to possible limitations of the applied methodology (for instance, the assumption of a constant fractionation rate or the appropriateness of baseline value calculations) or by excluding effects of fish foraging behavior (Lazzaro et al., 2009) and fish-induced stoichiometry alterations as those described by Danger et al. (2009). Nevertheless, they provide empirical evidence for previously raised hypotheses suggesting that the structure and interactions of the trophic webs in subtropical lakes are more complex than those in cold temperate ones (Lazzaro et al., 2003; Meerhoff et al., 2007; Jeppesen et al., 2012).

We are also proposing here a conceptual model rising the principal differences between trophic webs in both the regions and the underlying forcing mechanisms occurring (Fig. 3); however, we still lack complete understanding of how such differences in food-web shape affect, for instance, the biomass of particular communities and biotic interactions at given trophic levels as well as how lake ecosystem functioning, resilience, and stability (Post & Takimoto, 2007) are affected.

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