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# Food-web structure in a subtropical coastal lake: how phylogenetic constraints may affect species linkages

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**Abstract.** To describe a food-web structure is a main goal in any attempt to understand ecosystem functioning. In the present study, we analysed the isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of primary producers and consumers in the large subtropical Mangueira Lake in southern Brazil. On the basis of the $\delta^{13}C$ and $\delta^{15}N$ values and analysis of stomach contents of several fish species, we provide a description of the food-web structure and trophic positions of the dominant fish species. Analysis of nitrogen isotope ratios indicated the existence of two consumer trophic levels. Isotopic signatures of primary consumers were compatible with those of producers, indicating a food web sustained by autochthonous carbon. Nevertheless, when food items were classified in larger groups by relative size and source, the combined analysis of isotopic signature and feeding preferences revealed a phylogenetically structured arrangement for the fish species of Mangueira Lake. This indicates that the main feeding niches are shared by taxonomically related species.

**Additional keywords:** energy flow, matter flow, shallow lake, trophic linkage.

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## Introduction

The roles of fish in aquatic food webs are usually complex, because they may occupy multiple trophic levels and consume a variety of food sources (Christensen and Moore 2009). Also, ontogenetic shifts in diet preferences increase intraspecific variability at the same time as spatial and temporal shifts in food abundance will also increase both intraspecific and interspecific variability (Minagawa and Wada 1984; Winemiller 1989; Carpenter and Kitchell 1993; Vander Zanden *et al.* 2000; Post 2003).

Most frequently, characterisation of trophic levels and food-web structure has been based on gut-content analysis (GCA). Gut content gives a 'snapshot' of immediate feeding trends but may fail to notice variation between sampling periods (Vander Zanden *et al.* 2000; Power *et al.* 2002; Clarke *et al.* 2005). Although dietary analysis provides valuable information on the taxonomic composition of fish diets, it can be complemented by the analysis of stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) to characterise the food-web structure or trophic interactions (Peterson and Fry 1987).

Heavier isotopes of carbon and nitrogen ($^{13}C$ and $^{15}N$) are not so abundant when compared to lighter ones ($^{12}C$ and $^{14}N$). Although the ratio of carbon isotopes depends on the photosynthetic metabolism of producers (e.g. C3 and C4), because carbon isotopic ratio typically increases ~1‰ by trophic level (De Niro and Epstein 1981; Peterson and Fry 1987; Pinnegar and Polunin 2000; Vander Zanden and Rasmussen 2001; Post 2002; Vanderklift and Ponsard 2003; Caut *et al.* 2009), it can be used to trace primary food sources of species. By contrast, nitrogen isotopic ratios tend to increase from 2.5‰ to 3.5‰ between successive trophic levels, and have been used to estimate trophic position (De Niro and Epstein 1981; Peterson and Fry 1987; Pinnegar and Polunin 2000; Vander Zanden and Rasmussen 2001; Post 2002; Vanderklift and Ponsard 2003; Caut *et al.* 2009).

The combined analysis of carbon and nitrogen stable isotopes is a powerful tool to identify the ultimate organic-matter sources and trophic position of consumers (Michener and Schell 1994; Froneman 2001), because it integrates the diet over long time periods, depending on the selected tissue and its turnover ratio (Perga and Gerdeaux 2005), whereas stomach contents are indicative only of what the fish had eaten shortly before their capture. For example, Vinson and Budy (2011) identified high diet overlap between brown trout, rainbow trout and mountain whitefish when analysing gut content, but distinct feeding niche when analysing $^{13}C$ and $^{15}N$, suggesting that the three species consume the same basic items, but in different proportions.

In wetlands and lake littoral zones, there are generally four major groups of food sources, namely, biofim (periphyton), macrophytes, suspended particulate matter (SPM) and sediment

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**Fig. 1.** Mangueira Lake, a large coastal lake in the state of Rio Grande do Sul, southern Brazil (33°31′22″S, 53°07′48″W).

organic matter (Lamberti and Moore 1984; Frost *et al.* 2002). Concerning previous studies analysing isotopic signatures in the Mangueira Lake and Taim Hydrological System (THS; Garcia *et al.* 2006), primary consumers reflect autochthonous organic-matter production.

The use of resource and resource partitioning is also a key aspect of ecosystem functioning. Phylogenetically related species share not only a common ancestral, and same basic morphological design, but also a common ancient use of resources. At the community level, evolutive pressures force closely related species to diverge as the result of resource partitioning, at the same time that adaptive convergence can joint unrelated species in a same functional guild (Casatti and Castro 2006).

A relationship between morphology and phylogenetic distance was identified by Winemiller (1991) on the basis of the analysis of the ecomorphological patterns of several fish species in communities distributed worldwide. A strong correlation between morphology and phylogenetic distance was also identified by Oliveira *et al.* (2010), on the basis of the analysis of 35 fish species from the Paraná basin in Brazil. The same authors found a strong correlation between morphology and trophic guild. But, if there is a correlation between morphology and phylogenetic distance (Winemiller 1991; Oliveira *et al.* 2010), and between morphology and trophic guild (Oliveira *et al.* 2010), the presence of a phylogenetic driving pressure in the functional structure of a community can be hypothesised, a pattern already identified by Douglas and Matthews (1992) for 17 fish species in Roanoke River (VA, USA).

In the present study, we (1) analyse the isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of primary producers and numerically dominant consumers from Mangueira Lake in southern Brazil. On the basis of $\delta^{13}C$ and $\delta^{15}N$ values and analysis of stomach contents of several fish species, we (2) provide a classification of fish trophic positions and (3) describe the structure of the food web. Considering that phylogenetically related species share an ancient common use of resources, we (4) try to identify whether the species functional arrangement can emulate in some way the species phylogenetic structure. Also, (5) a new methodological approach is proposed to highlight species proximity in terms of isotopic composition.

## Materials and methods

### *Study site*

Mangueira is a large shallow lake (90 km long, 3–10 km wide, ~820 $km^2$ in area, and 3 m deep on average) located parallel to the Atlantic coast in southern Brazil (33°31′22″S, 53°07′48″W; Fig. 1). The regional climate is subtropical, with a mean annual temperature of 16°C and annual precipitation between 1800 and

2200 mm (Cfa type; Kottek *et al.* 2006). The lake was formed after the last postglacial marine regression (Holocene, ~5000 before present), when the ocean level fell 5 m to the present level (Tomazelli *et al.* 2000). In the northern part, the lake merges with wetlands, where large areas are covered with macrophytes, forming the Taim Hydrological System (THS), a federal conservation area.

### Sampling

Producers (macrophytes, biofilm) and primary consumers (the shrimp *Palaemonetes argentinus* and the gastropod *Pomacea* sp.) were sampled once (autumn 2007). The sampling was carried out in the littoral zone of the northern part of the lake. The emergent macrophytes *Zizaniopsis bonariensis*, *Scirpus californicus* and *Sagittaria montevidensis* and the submerged macrophytes *Egeria densa*, *Cabomba caroliniana* and *Ceratophyllum demersum* were collected. Biofilm was removed from *Z. bonariensis* and *S. californicus*. Each species and biofilm comprised four independent samples.

Zooplankton and seston were sampled once in each season, from the winter of 2006 to autumn of 2007, by using a depth-integrated water sampler, a tube 2 m long and 20 cm in diameter. Sampling proceeded from the surface down to ~20 cm above the bottom. Seston was retained by vacuum-filtering 1 L of water (45-μm-mesh membrane). Zooplankton was collected by filtering 50 L of water through a 65-μm-mesh plankton net.

Fish samples were taken seasonally, from the winter of 2006 to the autumn of 2007. Fish were caught with multiple-mesh gill-nets (5-, 6.25-, 8-, 10-, 12.5-, 15-, 20-, 25-, 30-, 35-, 40-, 45-, 50-, 55-, 60-, 65- and 70-mm mesh size; Appelberg 2000) deployed overnight (15 h). All fish caught were preserved in 4% formalin, except samples for isotopic analysis, which were frozen. In laboratory, fish were identified, counted, weighed (to the nearest 0.1 g), measured (to 1.0 mm), and dissected for digestive-tract analysis. Stomach content was identified in a Petri dish under stereomicroscope ($n = 8$–404). To analyse the functional aspects of feeding guilds, main food items were classified by relative size (small, large) and source (bottom, water column) as sediment-detritus, large-benthos (e.g. *Palaemonetes argentinus*, aquatic insects), small-pelagic (e.g. Cladocera, Copepoda) and large-pelagic (e.g. fish, terrestrial insects). Fresh macrophyte fragments were not observed in fish-gut content, and fragments of macrophytes and algae were classified as being sediment-detritus. Frequency of occurrence of each feeding category (Hyslop 1980) was applied to evaluate the general trophic structure of fish.

To quantify stable isotopes, 4–16 specimens of each fish species were analysed, with samples of the same species taken in different seasons. The fish species were selected to include the most frequent species (in terms of biomass). The selected species were *Odontesthes humensis* ($n = 43$), *O.* aff. *perugiae* ($n = 29$), *Astyanax jacuhiensis* ($n = 153$), *A. eigenmanniorum* ($n = 260$), *A.* sp. ($n = 217$), *Hyphessobrycon luetkenii* ($n = 135$), *Bryconamericus iheringii* ($n = 264$), *Oligosarcus jenynsii* ($n = 937$) and *Cyphocharax voga* ($n = 277$).

### Sample processing and data analysis

Biological samples (muscle tissue of fish and invertebrates) were dried at 60°C (>48 h) and stored in a desiccator before isotopic analysis. Dried samples were ground to a fine powder (weighed to $10^{-6}$ g) and placed in Ultra Pure tin capsules (Costech, Valencia, CA, USA). Samples were sent to the UC Davis Stable Isotope Facility, Department of Plant Sciences at the University of California, for determination of stable isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) as follows. Samples were analysed for $^{13}C$ and $^{15}N$ by using a PDZ Europa ANCA–GSL elemental analyser interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). A preliminary isotope ratio was measured relative to reference gases of included laboratory standards. The long-term standard deviation was 0.2‰ for $^{13}C$ and 0.3‰ $^{15}N$. The final delta values are expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and Air for carbon and nitrogen, respectively. Results are reported as parts per thousand (‰) differences from a corresponding standard, as follows: $\delta X = [(R\text{sample}/R\text{standard}) - 1] \times 10^3$, where $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$.

Trophic position of fish was estimated assuming an increase in $\delta^{15}N$ values of 2.98‰ between successive trophic levels, following the recommendations of Vanderklift and Ponsard (2003) for freshwater organisms. The choice of a baseline organism followed Post (2002), who proposed the use of snails and bivalves (Trophic level 2) to estimate the littoral and pelagic bases of a food web. Trophic levels ($TL_f$) were estimated as follows:

$$TL_f = [(\delta^{15}N_f - \delta^{15}N_{ref})/2.98] + 2,$$

where $\delta^{15}N_f$ and $\delta^{15}N_{ref}$ are respectively the nitrogen isotope signature of a fish and a baseline reference organism (the snail *Pomacea* sp.); 2.98 is the $\delta^{15}N$ expected isotopic discrimination per trophic level; and 2 is the trophic level of the baseline organism (primary consumer).

The food-web structure was graphically represented by plotting $\delta^{15}N$ against $\delta^{13}C$ for all sampled organisms. In the present study, we propose a new proximity index to highlight linkage between elements or species along a matter–flow vector. The proximity index for $\delta^{15}N$ and $\delta^{13}C$ were calculated as follows:

$$P^{15}N = [(\delta\,\text{sp_2} - \delta\,\text{sp_1})/2.98 - 1]^2, \text{ and}$$

$$P^{13}C = (\delta\,\text{sp_2} - \delta\,\text{sp_1} - 1)^2,$$

where $P^{15}N$ is the nitrogen proximity index, $P^{13}C$ is the carbon proximity index, δ sp_1 and δ sp_2 are the mean δ values for nitrogen or carbon for the lower (1) and upper (2) elements of a food web.

In the proposed index, the direct linkage of elements (similar isotopic composition) will be more probable when the estimated proximity index is zero. To achieve this objective, the difference between $\delta^{15}N$ signatures is divided by 2.98, considering an enrichment of 2.98 as a general average for freshwater organisms (Vanderklift and Ponsard 2003). By subtracting this ratio from one, the index goes to zero if there is direct consumption, and by taking the square, negative values are eliminated. For carbon, an enrichment of 1‰ from one to another trophic level is generally accepted (Peterson and Fry 1987; Vander Zanden and

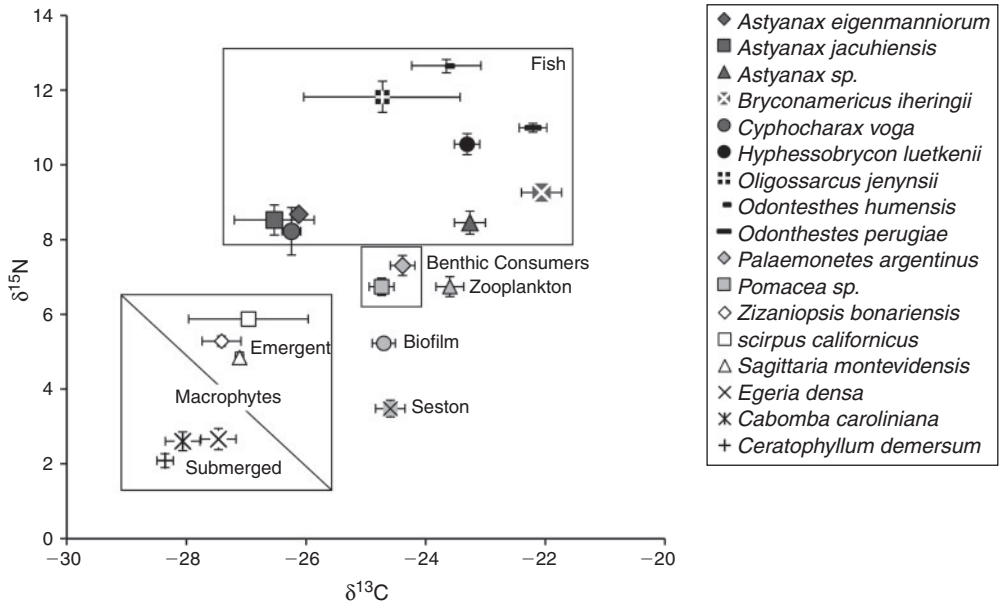


**Fig. 2.** Plot of $\delta^{15}N$ and $\delta^{13}C$ values (mean ± s.d.) for fishes in Mangueira Lake. Sources of carbon assimilated by consumers are indicated by the relative positions of taxa on the *x*-axis; trophic level is indicated by the relative position on the *y*-axis.

**Table 1. $\delta^{13}C$ and $\delta^{15}N$ values (mean ± s.d.) for primary producers and primary consumers of Mangueira Lake**

Zooplankton and seston were sampled once each season from the winter of 2006 to autumn of 2007. All other values represent the mean of three samples; data were collected in autumn 2007

| Taxon | Species | $\delta^{13}C$ | $\delta^{15}N$ |
|---|---|---|---|
| Emergent macrophyte | *Scirpus californicus* | −26.96 ± 1.28 | 5.88 ± 0.15 |
| | *Zizaniopsis bonariensis* | −27.41 ± 0.33 | 5.28 ± 0.14 |
| | *Sagittaria montevidensis* | −27.11 ± 0.07 | 4.85 ± 0.14 |
| Submerged macrophyte | *Egeria densa* | −27.47 ± 0.29 | 2.66 ± 0.28 |
| | *Cabomba caroliniana* | −28.06 ± 0.29 | 2.61 ± 0.25 |
| | *Ceratophyllum demersum* | −28.36 ± 0.14 | 2.09 ± 0.18 |
| Periphyton | | −24.70 ± 0.19 | 5.23 ± 0.11 |
| Seston | | −24.59 ± 0.25 | 3.48 ± 0.22 |
| Zooplankton | | −23.60 ± 0.23 | 6.74 ± 0.27 |
| Crustacean decapod | *Palaemonetes argentinus* | −24.39 ± 0.21 | 7.31 ± 0.27 |
| Mollusk | *Pomacea* sp | −24.74 ± 0.21 | 6.74 ± 0.23 |

Rasmussen 2001). So, the difference δ sp_2 - δ sp_1 – 1 tends toward zero if there is an isotopic composition linkage. Again, by taking squares, negative values are eliminated. A combined proximity index is proposed as the simple mean of $P^{15}N$ and $P^{13}C$ and combines both carbon and nitrogen signatures to identify possible linkage among food-web elements. Limitations of the method concerning the species signature as the result of mass balance of different items are presented in discussion.

The structure of the fish assemblage was inspected using cluster analysis (Euclidian distance, within-group linkage; SPSS , IBM SPSS Statistics for Windows, Version 17.0. IBM Corp., Armonk, NY). Clustering was applied using the data for $\delta^{13}C$, $\delta^{15}N$ and stomach contents; all values were rescaled to 0–1 by species to assure the same relative discriminative power. Functional-group comparisons in terms of isotopic signatures were made by using Student's *t*-test and ANOVA.

## Results

Plotting the $\delta^{15}N$ against $\delta^{13}C$ values provided a visual characterisation of the food-web structure of Mangueira Lake (Fig. 2, Tables 1, 2). Although seasonal variation was not computed, relatively small standard deviations for most consumers, especially for nitrogen signature, suggested little temporal variation.

Macrophytes as a functional group showed high intra-group variation in both the $\delta^{15}N$ and $\delta^{13}C$ signatures. Emergent macrophytes (*Zizaniopsis bonariensis*, *Scirpus californicus* and *Sagittaria montevidensis*) were more enriched in both

**Table 2. $\delta^{13}C$ and $\delta^{15}N$ values (mean ± s.d.) and total length (TL, mm) of fishes sampled in Mangueira Lake**

| Family | Species | *n* | TL (mm) | $\delta^{13}C$ | $\delta^{15}N$ |
|---|---|---|---|---|---|
| Atherinopsidae | *Odontesthes humensis* | 8 | 279.2 ± 19.6 | −23.7 ± 0.6 | 12.6 ± 0.2 |
| | *Odontesthes perugiae* | 7 | 176.0 ± 11.3 | −22.2 ± 0.2 | 11.0 ± 0.1 |
| Characidae | *Astyanax* sp. | 15 | 51.0 ± 7.1 | −23.3 ± 0.3 | 8.5 ± 0.3 |
| | *Astyanax jacuhiensis* | 8 | 119.1 ± 4.4 | −26.5 ± 0.7 | 8.5 ± 0.4 |
| | *Astyanax eigenmanniorum* | 4 | 104.0 ± 8.8 | −26.1 ± 0.0 | 8.7 ± 0.1 |
| | *Bryconamericus iheringii* | 4 | 76.5 ± 4.9 | −22.1 ± 0.3 | 9.3 ± 0.2 |
| | *Hyphessobrycon luetkenii* | 6 | 94.0 ± 9.0 | −23.3 ± 0.2 | 10. ± 0.3 |
| | *Oligosarcus jenynsii* | 16 | 142.75 ± 34.9 | −24.7 ± 1.3 | 11.8 ± 0.4 |
| Curimatidae | *Cyphocharax voga* | 8 | 146.5 ± 62.9 | −26.2 ± 0.2 | 8.2 ± 0.6 |

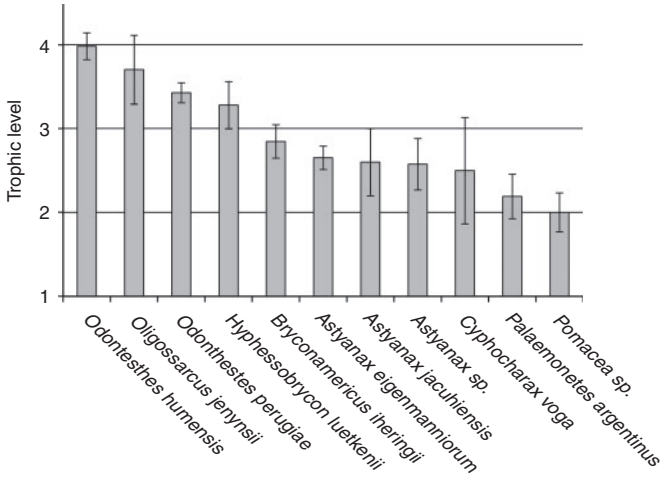


**Fig. 3.** Trophic position estimates of fishes collected in Mangueira Lake, according to the $\delta^{15}N$ signature.

$^{13}C$ ($t = 2.75$; $P = 0.05$) and $^{15}N$ ($t = 8.24$; $P = 0.00$) than were submerged macrophytes (*Egeria densa*, *Cabomba caroliniana* and *Ceratophyllum demersum*) (Table 1, Fig. 2).

The biofilm showed $\delta^{15}N$ levels similar to those of the emergent macrophytes ($t = 0.02$; $P = 0.98$), and enriched $\delta^{13}C$ signature ($t = -6.52$; $P = 0.00$). By contrast, the measured signatures of $\delta^{13}C$ for biofilm were close to those for the seston ($t = 0.70$; $P = 0.51$), but with increased $\delta^{15}N$ values ($t = -14.14$; $P = 0.00$). Zooplankton and the large gastropod *Pomacea* sp. had similar $\delta^{15}N$ values ($t = -0.01$; $P = 0.99$) but contrasting $\delta^{13}C$ signatures ($t = -7.33$; $P = 0.00$), corroborating different carbon sources for these primary consumers (Table 1, Fig. 2). The shrimp *Palaemonetes argentinus* was more nitrogen enriched than were *Pomacea* sp. and zooplankton ($F = 6.56$; $P = 0.02$), although having carbon values between them.

Fish had $\delta^{13}C$ values ranging from −22.00‰ to −27.00‰, whereas $\delta^{15}N$ values ranged from 7.7‰ to 12.7‰ (Table 2, Fig. 2). On the basis of the analysis of nitrogen isotope ratios, we were able to distinguish two trophic levels among the fish species in Mangueira Lake (Figs 2, 3). *Cyphocharax voga*, species of *Astyanax* and *Bryconamericus iheringii* were in the second trophic level, whereas the characids *Hyphessobrycon luetkenii* and *Oligosarcus jenynsii* and the silversides *Odontesthes perugiae* and *O. humensis* were in the third trophic level.

The proposed proximity index for fish species in Mangueira Lake is presented in Tables 3–5 for nitrogen, carbon and combined data respectively. Considering the combined proximity of both carbon and nitrogen (Table 5), it is interesting that the values for emergent macrophytes (*Zizaniopsis bonariensis, Scirpus californicus* and *Sagittaria montevidensis*), and macrophytes in general, are close to those of most of the fish species in the second trophic level, except *Astyanax* sp. and *Bryconamericus iheringii*, but with no identified relationship with the gastropod *Pomacea* sp. and the shrimp *Palaemonetes argentinus.* However, in the second trophic level, *Astyanax* sp. showed an apparent isotopic relationship that can include biofilm and seston, but also zooplankton, the shrimp *P. argentinus* and the gastropod *Pomacea* sp., depending on mass balance. *B. iheringii*, classified on the borderline between the second and third trophic levels, showed an apparent isotopic relationship to zooplankton, but also with the fish species *Astyanax* sp.

There was no apparent relationship of the submerged macrophytes (*Cabomba caroliniana* and *Ceratophyllum demersun*) to all the elements of the food web. *Egeria densa*, also a submerged macrophyte, presents a carbon signature signalising possible

**Table 3. Proximity matrix estimated from $\delta^{15}N$ ($P^{15}N = ((\delta sp_2 - \delta sp_1)/2.98 - 1)^2$) for selected producers, primary consumers and frequent fish species from northern Mangueira Lake**

Proximity coefficient suggests isotopic relationship when close to zero. Values larger than 0.5 are not presented to emphasise only species with close potential proximity

| | Zooplankton | *Pomacea* sp. | *Palaemonetes argentinus* | *Cyphocharax voga* | *Astyanax* sp. | *Astyanax jacuhiensis* | *Astyanax eigenmanniorum* | *Bryconamericus iheringii* | *Hyphessobrycon luetkenii* | *Odonthestes perugiae* | *Oligossarcus jenynsii* | *Odontesthes humensis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Second trophic level | | | | | | Third trophic level | | |
| *Ceratophyllum demersum* | 0.32 | 0.32 | | | | | | | | | | |
| *Cabomba caroliniana* | 0.15 | 0.15 | 0.33 | | | | | | | | | |
| *Egeria densa* | 0.14 | 0.14 | 0.31 | | | | | | | | | |
| *Zizaniopsis bonariensis* | 0.26 | 0.26 | 0.10 | 0.00 | 0.00 | 0.01 | 0.02 | 0.11 | | | | |
| *Sagittaria montevidensis* | 0.13 | 0.13 | 0.03 | 0.02 | 0.04 | 0.06 | 0.08 | 0.23 | | | | |
| *Scirpus californicus* | 0.50 | 0.50 | 0.27 | 0.04 | 0.02 | 0.01 | 0.00 | 0.02 | 0.33 | | | |
| Macroph. average | 0.00 | 0.00 | 0.02 | 0.21 | 0.28 | 0.31 | 0.37 | | | | | |
| Seston | 0.01 | 0.01 | 0.08 | 0.35 | 0.45 | 0.48 | | | | | | |
| Biofilm | 0.24 | 0.24 | 0.09 | 0.00 | 0.01 | 0.01 | 0.03 | 0.13 | | | | |
| Zooplankton | | | | 0.25 | 0.18 | 0.16 | 0.12 | 0.02 | 0.08 | 0.18 | 0.49 | |
| *Pomacea* sp. | | | | 0.25 | 0.18 | 0.16 | 0.12 | 0.02 | 0.08 | 0.18 | 0.49 | |
| *Palaemonetes argentinus* | | | | 0.48 | 0.38 | 0.35 | 0.29 | 0.12 | 0.01 | 0.06 | 0.26 | |
| *Cyphocharax voga* | | | | | | | | 0.42 | 0.05 | 0.00 | 0.04 | 0.23 |
| *Astyanax* sp. | | | | | | | | | 0.09 | 0.02 | 0.02 | 0.17 |
| *Astyanax jacuhiensis* | | | | | | | | | 0.10 | 0.03 | 0.01 | 0.15 |
| *Astyanax eigenmanniorum* | | | | | | | | | 0.14 | 0.05 | 0.00 | 0.11 |
| *Bryconamericus iheringii* | | | | | | | | | 0.32 | 0.18 | 0.02 | 0.02 |
| *Hyphessobrycon luetkenii* | | | | | | | | | | | 0.33 | 0.09 |
| *Odonthestes perugiae* | | | | | | | | | | | | 0.20 |
| *Oligossarcus jenynsii* | | | | | | | | | | | | |
| *Odontesthes humensis* | | | | | | | | | | | | |

**Table 4. Proximity matrix estimated from $\delta^{13}C$ ($P^{13}C = (\delta sp_2 - \delta sp_1 - 1)^2$) for selected producers, primary consumers and frequent fish species from northern Mangueira Lake**

Proximity coefficient suggests isotopic relationship when close to zero. Values larger than 0.5 are not presented to emphasise only species with close potential proximity

| | Zooplankton | *Pomacea* sp. | *Palaemonetes argentinus* | *Cyphocharax voga* | *Astyanax* sp. | *Astyanax jacuhiensis* | *Astyanax eigenmanniorum* | *Bryconamericus iheringii* | *Hyphessobrycon luetkenii* | *Odonthestes perugiae* | *Oligossarcus jenynsii* | *Odontesthes humensis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Second trophic level | | | | | | | Third trophic level | | | | |
| *Ceratophyllum demersum* | | | | | | | | | | | | |
| *Cabomba caroliniana* | | | | | | 0.28 | | | | | | |
| *Egeria densa* | | | | 0.05 | | 0.00 | 0.12 | | | | | |
| *Zizaniopsis bonariensis* | | | | 0.03 | | 0.01 | 0.08 | | | | | |
| *Sagittaria montevidensis* | | | | 0.02 | | 0.17 | 0.00 | | | | | |
| *Scirpus californicus* | | | | 0.08 | | 0.32 | 0.03 | | | | | |
| Macroph. average | | | | 0.10 | | 0.00 | 0.19 | | | | | |
| Seston | 0.00 | | | | 0.11 | | | | 0.08 | | | 0.00 |
| Biofilm | 0.01 | | 0.47 | | 0.19 | | | | 0.15 | | | 0.00 |
| Zooplankton | | | | | 0.44 | | | 0.28 | | 0.15 | | |
| *Pomacea* sp. | | | 0.42 | | 0.23 | | | | 0.18 | | | 0.01 |
| *Palaemonetes argentinus* | | | | | 0.02 | | | | 0.01 | | | 0.07 |
| *Cyphocharax voga* | | | | | | | | | | | 0.27 | |
| *Astyanax* sp. | | | | | | | | 0.04 | | 0.00 | | |
| *Astyanax jacuhiensis* | | | | | | | 0.35 | | | | | |
| *Astyanax eigenmanniorum* | | | | | | | | | | | 0.16 | |
| *Bryconamericus iheringii* | | | | | | | | | | | | |
| *Hyphessobrycon luetkenii* | | | | | | | | 0.06 | | 0.01 | | |
| *Odonthestes perugiae* | | | | | | | | | | | | |
| *Oligossarcus jenynsii* | | | | | 0.21 | | | | 0.17 | | | 0.00 |
| *Odontesthes humensis* | | | | | 0.37 | | | 0.35 | 0.43 | 0.20 | | |

**Table 5. Proximity index (PCN = ($P^{15}N + P^{13}C$)/2) estimated for selected producers, primary consumers and frequent fish species from northern Mangueira Lake**

Proximity coefficient suggests isotopic relationship when close to zero. Values larger than 0.5 are not presented to emphasise only species with close potential proximity

| | Zooplankton | *Pomacea* sp. | *Palaemonetes argentinus* | *Cyphocharax voga* | *Astyanax* sp. | *Astyanax jacuhiensis* | *Astyanax eigenmanniorum* | *Bryconamericus iheringii* | *Hyphessobrycon luetkenii* | *Odonthestes perugiae* | *Oligossarcus jenynsii* | *Odontesthes humensis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Second trophic level | | | | | | | Third trophic level | | | | |
| *Ceratophyllum demersum* | | | | | | | | | | | | |
| *Cabomba caroliniana* | | | | | | | | | | | | |
| *Egeria densa* | | | | 0.40 | | 0.47 | | | | | | |
| *Zizaniopsis bonariensis* | | | | 0.01 | | 0.01 | 0.05 | | | | | |
| *Sagittaria montevidensis* | | | | 0.02 | | 0.11 | 0.04 | | | | | |
| *Scirpus californicus* | | | | 0.06 | | 0.17 | 0.01 | | | | | |
| Macroph. average | | | | 0.15 | | 0.15 | 0.28 | | | | | |
| Seston | 0.00 | | 0.36 | | 0.28 | | | | | | | |
| Biofilm | 0.13 | | 0.28 | | 0.10 | | | | 0.39 | | | |
| Zooplankton | | | | | 0.31 | | | 0.15 | 0.29 | 0.17 | | |
| *Pomacea* sp. | | | | | 0.20 | | | | 0.13 | | | 0.49 |
| *Palaemonetes argentinus* | | | | | 0.20 | | | | 0.01 | | | 0.35 |
| *Cyphocharax voga* | | | | | | | | | | | 0.16 | |
| *Astyanax* sp. | | | | | | | | 0.29 | | 0.01 | | |
| *Astyanax jacuhiensis* | | | | | | | | | | | 0.33 | |
| *Astyanax eigenmanniorum* | | | | | | | | | | | 0.08 | |
| *Bryconamericus iheringii* | | | | | | | | | | | | |
| *Hyphessobrycon luetkenii* | | | | | | | | | | 0.37 | | |
| *Odonthestes perugiae* | | | | | | | | | | | | |
| *Oligossarcus jenynsii* | | | | | | | | | | | | 0.26 |
| *Odontesthes humensis* | | | | | | | | | | | | |

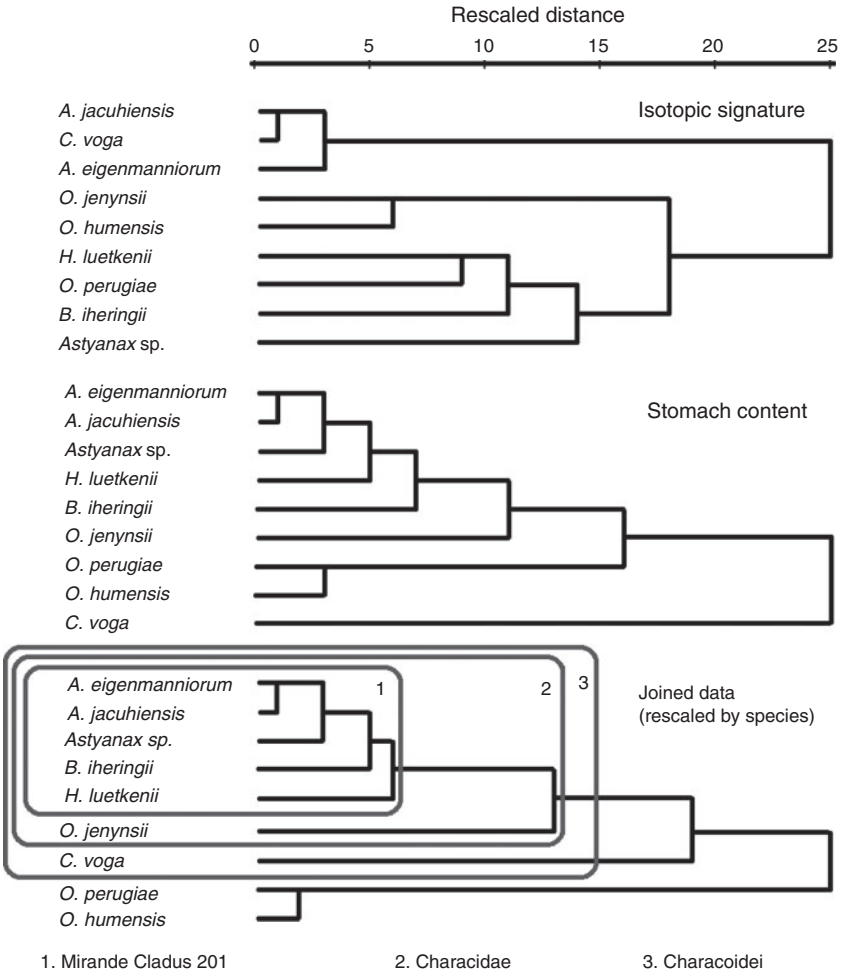


**Fig. 4.** Dendrogram of similarity (Euclidian distance, within-group linkage) applied to fish species of Mangueira Lake by using $\delta^{13}C$ and $\delta^{15}N$ values (upper); stomach contents (middle) and combined data (lower).

relationship with *Cyphocharx voga* and *Astyanax jacuhiensis*, but incompatible nitrogen values, although the combined proximity coefficient is near the 0.5, the upper cutting threshold for presenting possible linkage.

Although very different in nitrogen isotopic values, biofilm and seston showed a similar pattern as food sources. Both presented close proximity to zooplankton, *Palaemonetes argentinus* and *Astyanax* sp., all in the second trophic level. Zooplankton showed a close relationship with *Astyanax* sp. and *Bryconamericus iheringii,* both being classified in the second trophic level, and with *Hyphessobrycon luetkenii* and *Odontesthes perugiae* in the third trophic level. The shrimp *Palaemonetes argentinus* and the gastropod *Pomacea* sp. were identified as potential food source related mainly to *Astyanax* sp. and the third-level species *Hyphessobrycon luetkenii* and *Odontesthes humesis.* For the gastropod *Pomacea* sp., although presenting nitrogen signatures compatible with macrophytes, both carbon and the combined index suggested no isotopic relationship to primary producers. In the third trophic level, *Hyphessobrycon luetkenii* was related to biofilm, zooplankton and benthic consumers, but all other species presented some apparent relationship with fish species.

By constructing a dendrogram based on the fish isotopic signatures, three major groups were identified (Fig. 4, upper tree). The first group clustered the detritivorous *Cyphocharax voga* with *Astyanax jacuhiensis* and *A. eigenmanniorum* (macrophyte linkage). The second group joined two larger predator species, *Odontesthes humensis* and *Oligosacus jeninsii* (higher trophic position). The third group loosely joined *Odontesthes perugiae* and small characids, which are mainly known as generalist feeders (*Hyphessobrycon luetkenii, Bryconamericus iheringii* and Astyanax sp.; Table 5, zooplankton linkage).

A completely different result was obtained by using stomach contents (Table 6, Fig. 4, middle tree). The detritivore Cy*phocharax voga* remained apart from all the other species. Nevertheless, for the remaining species, an almost phylogenetic structure was identified. The three *Astyanax* species were all combined together, and then with other small characids (*Hyphessobrycon luetkenii* and *Bryconamericus iheringii*). Next, *Oligosarcus jenynsii* joined the group, forming a

**Table 6. Frequency of occurrence (%) of main feeding items grouped by source and relative size**

| Species | *n* | Large–benthos | Sediment–detritus | Large–pelagic | Small–pelagic |
|---|---|---|---|---|---|
| *Astyanax eigenmanniorum* | 131 | 23 | 52 | 0 | 25 |
| *Astyanax jacuhiensis* | 43 | 33 | 48 | 0 | 19 |
| *Astyanax* sp. | 76 | 28 | 54 | 0 | 18 |
| *Bryconamericus iheringii* | 11 | 20 | 44 | 0 | 36 |
| *Cyphocharax voga* | 32 | 0 | 100 | 0 | 0 |
| *Hyphessobrycon luetkenii* | 19 | 32 | 43 | 0 | 25 |
| *Odontesthes perugiae* | 8 | 72 | 3 | 13 | 12 |
| *Odontesthes humensis* | 41 | 81 | 3 | 16 | 0 |
| *Oligosarcus jenynsii* | 404 | 79 | 5 | 16 | 0 |

monophyletic clade (Characidae). The Atheriniphormes *Odontesthes humensis* and *O. perugiae* grouped together and apart from the Characidae.

However, when all data (isotopic signatures and stomach contents) were used to identify the functional structure, a more perfect phylogenetic arrangement was achieved (Fig. 4, bottom tree), with *Cyphocharax voga* grouping with the characids and forming a Characoidei clade, apart from the Atherinomorphs (*Odontesthes humensis* and *O. perugiae*). The obtained tree emulates a recently proposed phylogenetic arrangement for Characidae (Mirande, 2010).

## Discussion

Mangueira Lake macrophytes showed a $\delta^{13}C$ range in accordance with reported values for terrestrial plants using the $C_3$ photosynthetic pathway (−27‰ to −29‰, Deegan and Garritt 1997; −25‰ to −27‰, Garcia *et al.* 2006). Garcia *et al.* (2006) for the Taim Hydrological System and Manetta *et al.* (2003) for the Paraná River floodplain, found a strong link between the $\delta^{13}C$ values of macrophytes and consumers, suggesting that macrophytes are an important carbon source supporting aquatic food webs. This linkage could function both by direct consumption and by an indirect pathway such as sediment organic matter (SOM) or particulate organic carbon (POC) in the water column (Marinho *et al.* 2010). Although not evaluated directly in the present study, the isotopic signature of primary consumers, except *Pomacea* sp., were compatible with autochthonous primary production from macrophytes. The apparently more enriched carbon signature of *Pomacea* sp. than that of macrophytes could be explained by a balance concerning macrophytes and biofilm consumption.

Nevertheless, important differences were identified among primary producers. Submerged macrophytes (*Egeria densa*, *Cabomba caroliniana* and *Ceratophyllum demersum*) showed very low values for both $\delta^{13}C$ and $\delta^{15}N$ signatures if compared with the primary consumers. Differences concerning $\delta^{13}C$ signatures could be related to the primary carbon source, e.g. air for emergent macrophytes and dissolved carbonates for submerged macrophytes (Aichner *et al.* 2010). However, although they did not show a direct relationship to consumers, submerged macrophytes could contribute to build-up sediment organic matter, because the average signature of macrophytes is in close relationship with that of *Cyphocharax voga*, *Astyanax eigenmanniorum* and. *A. jacuhiensis*.

Another carbon source, the biofilm, which covers a wide range of submerged surfaces, can also be a key factor for the Mangueira food web. Biofilm plays a fundamental role in nutrient cycling and storage (Azim and Wahab 2005), and freshwater fish can use it as a food source in either direct or indirect pathways (Petr 2000). The biofilm isotopic signature was within the range described for other freshwater environments ($\delta^{13}C$ signature range from −15‰ to −27‰, France 1999; −17‰ to −28‰, Trudeau and Rasmussen 2003; and $\delta^{15}N$ signature range of 1.5–7‰, MacLeod and Barton 1998; 2.3–7.2‰, Trudeau and Rasmussen 2003), and showed an possible direct linkage with zooplankton, *Palaemonetes argentinus* and *Astyanax* sp.

*Astyanax eigenmanniorum* and *A. jacuhienisis* were both considered to be in the third trophic level by Garcia *et al.* (2007), whereas here they were included in the second trophic level. Although the size ranges of the analysed individuals were quite different, being larger in the present study, the smaller fractionation value of nitrogen used by Garcia *et al.* (2007), namely, 2.54 per trophic level, contributed to an estimate of a higher trophic position. Although nitrogen isotopic ratios increase from 2.5‰ to 3.5‰ between successive trophic levels (De Niro and Epstein 1981; Peterson and Fry 1987; Pinnegar and Polunin 2000; Vander Zanden and Rasmussen 2001; Post 2002; Vanderklift and Ponsard 2003; Caut *et al*. 2009), we followed the general revision of Vanderklift and Ponsard (2003), who suggested for freshwater organisms an increase in $\delta^{15}N$ values of 2.98‰ between successive trophic levels.

*Astyanax* species are usually described as omnivorous fish (Teixeira 1989; Esteves and Galetti Jr 1995; Esteves 1996; Hartz *et al.* 1996; Vilella *et al.* 2002). However, detritus and sediment are commonly found in their gut contents, perhaps indicating accidental ingestion during food intake associated with aquatic invertebrates or plant fragments on the bottom (Abelha *et al.* 2006). Here, the isotopic signatures indicated that macrophytes could play an important role in the food intake of *Astyanax* species (Table 3), which has been underestimated by the literature.

*Bryconamericus iheringi* was the most enriched species in both $\delta^{13}C$ and $\delta^{15}N$ still classified in the second trophic level. Grosman *et al.* (1996), studying trophic niches of several fish species in an Argentinean lake, classified *B. iheringi* as a phytoplankton–periphytophagous species, whereas Oricolli and Bennemann (2006) found detritus as the main food item

for the species; neither of these results matched our findings with suggested linkage with zooplankton. Nevertheless, it should be remembered that some widely distributed characid species are in need of taxonomic revision, and may include distinct taxa within the same specific name. Also, the linkage of *B. iheringii* with *Astyanax* sp. could be attributed to the frequent consumption of fin tips or scales by small characids, although it was not identified in stomach contents.

The characids *Hyphessobrycon luetkenii* and *Oligossarcus jenynsii* and the silversides *Odontesthes perugiae* and *O. humensis* were classified in the third trophic level. *H. luetkenii* was classified a little above the threshold between the second and third trophic levels. Some studies have found that *H. luetkenii* feeds mainly on algae and detritus (Soneira *et al.* 2006), whereas others have categorised it as an omnivorous species (Sánchez-Botero *et al.* 2008) or as omnivore–benthi–planktivorous (Teixeira de Mello *et al.* 2009). In the present study, the observed consumption of large benthic species such as the shrimp *Palaemonetes argentinus* could explain the enhanced $\delta^{15}N$ signature of *H. luetkenii* in relation to other small characids.

In analysing data from Mangueira Lake, by combining information for carbon and nitrogen signatures and stomach contents, a phylogenetic arrangement of species rather than a strictly functional pattern was obtained. As proposed by Mirande (2010), *Astyanax* clade comprises most of the *Astyanax* species, although *Astyanax* sp. and *A. jacuhiensis* were not included in the proposed tree. All the *Bryconamericus* species, including *B. iheringii*, joined with the *Astyanax* clade and then with the *Hyphessobrycon luetkenii* clade, forming a node lacking the supraorbital bone, or Clade 201 *sensu* Mirande (2010). This node, together with the Tetragonopterinae clade, forms Node 202 and comprises most of the small characids known as piabas or lambarís. Clade 202 joins with *Oligossarcus jenynsii* in Node 203, and with *Cyphocharax* in Node 164.

As usually described in the literature, a trophic guild, the functional group of species inside a community that shares the same basic feeding strategy, can be the result of opposite evolutive driving forces (Ross 1986; Winemiller 1991; Douglas and Matthews 1992; Oliveira *et al.* 2010). The same feeding resource can be exploited by different species, from diverse orders and families. In this case, evolutive convergence can make some aspects of morphology become similar, such as mouth size and relative position, supporting the ecomorphological paradigm (Winemiller 1991).

In contrast, closely related species exploring the same feeding resource diverge because of competition and character displacement, a well documented process throughout the literature (for revision, see Krebs 2001). Of course, the ancient morphological and physiological solutions that constrain an evolving clade will limit the possibilities for specialisation and niche partitioning. In this respect, convergence of non-related species and character displacement of parental species are driving factors counteracting to model the structure and function of a food web.

In the present work, by combining food sources by size and source, the effects of specialisation and niche partitioning was minimised, allowing to group together species with the same broad feeding guild. Although the effect of the phylogenetic proximity as a driving factor modelling trophic arrangements was already described for fish (Winemiller 1991; Douglas and Matthews 1992; Oliveira *et al.* 2010), the novelty in the present work, as a case study, concerns the perfect match between the functional and the phylogenetical arrangement, a pattern still not documented.

Of course, the obtained phylogenetical/functional or phylo-functional arrangement was possible only because of filtering approaches. By combining feeding items in large general classes, all specialisation was lost, grouping together species that share the same basic strategy, even with different specialties. Nevertheless, as estimated by our proposed proximity index, an apparent isotopic relationship of a consumer to an item could be mismatched by a combination of other apparent unrelated feeding sources in different proportions. All these combinations could be quantified by more complex approaches as mixing models (*sensu* Phillips *et al.* 2005) and are neglected by the present proposal.

In this respect, the proposed proximity index is an interesting tool in showing, by simple procedures, candidate relationships, although not necessarily true ones. So, the simultaneous use of isotopic signatures and stomach content to construct functional trophic structures appear to be an attractive approach, as the direct identification of feeding items could reduce the effect of artificial trophic linkage as estimated by the proximity index.

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## References

Abelha, M. C. F., Goulart, E., Kashiwaqui, E. A. L., and Silva, M. R. (2006). *Astyanax paranae* Eigenmann, 1914 (Characiformes: Characidae) in the Alagados Reservoir, Paraná, Brazil: diet composition and variation. *Neotropical Ichthyology* **4**, 349–356. doi:10.1590/S1679-62252006000300006

Aichner, B., Herzschuh, U., and Wilkes, H. (2010). Influence of aquatic macrophytes on the stable carbon isotopic signatures of sedimentary organic matter in lakes on the Tibetan Plateau. *Organic Geochemistry* **41**, 706–718. doi:10.1016/J.ORGGEOCHEM.2010.02.002

Appelberg, M. (2000). Swedish standard methods for sampling freshwater fish with multi-mesh gillnets. *Fiskeriverket Information* **1**, 3–32.

Azim, M. E., and Wahab, M. A. (2005). Periphyton-based pond polyculture. In 'Periphyton: Ecology, Exploitation and Management'. (Eds M. E. Azim, M. C. J. Verdegem, A. A.van Dam and M. C. M. Beveridge.) pp. 207–222. (CABI Publishing, Wallingford, UK.)

Carpenter, S. R., and Kitchell, J. F. (1993). 'The Trophic Cascade in Lakes.' (Cambridge University Press: Cambridge, UK.)

Casatti, L., and Castro, R. C. M. (2006). Testing the ecomorphological hypothesis in a headwater riffles fish assemblage of the rio São Francisco, southeastern Brazil. *Neotropical Ichthyology* **4**, 203–214.

Caut, S., Angulo, E., and Courchamp, F. (2009). Variation in discrimination factors ($^{15}N$ and $^{13}C$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology* **46**, 443–453. doi:10.1111/J.1365-2664.2009.01620.X

Christensen, D. R., and Moore, B. C. (2009). Using stable isotopes and a multiple-source mixing model to evaluate fish dietary niches in a mesotrophic lake. *Lake and Reservoir Management* **25**, 167–175. doi:10.1080/07438140902905588

Clarke, L. R., Vidergar, D. T., and Bennett, D. H. (2005). Stable isotopes and gut content show diet overlap among native and introduced piscivores in a large oligotrophic lake. *Ecology Freshwater Fish* **14**, 267–277. doi:10.1111/J.1600-0633.2005.00103.X

De Niro, M. J., and Epstein, S. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta* **45**, 341–351. doi:10.1016/0016-7037(81)90244-1

Deegan, L. A., and Garritt, R. H. (1997). Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* **147**, 31–47. doi:10.3354/MEPS147031

Douglas, M. E., and Matthews, W. J. (1992). Does morphology predict ecology? Hypothesis testing within a freshwater stream fish assemblage. *Oikos* **65**, 213–224. doi:10.2307/3545012

Esteves, K. E. (1996). Feeding ecology of three *Astyanax* species (Characidae, Tetragonopterinae) from a floodplain lake of Mogi–Guaçú River, Paraná River Basin, Brazil. *Environmental Biology of Fishes* **46**, 83–101. doi:10.1007/BF00001701

Esteves, K. E., and Galetti Jr, M. P. (1995). Food partitioning among some characids of a small Brazilian floodplain lake from the Paraná River basin. *Environmental Biology of Fishes* **42**, 375–389. doi:10.1007/BF00001468

France, R. L. (1999). Relationship between DOC concentration and epilithon stable isotopes in boreal lakes. *Freshwater Biology* **41**, 101–105. doi:10.1046/J.1365-2427.1999.00391.X

Froneman, P. W. (2001). Stable isotope ($\delta^{13}C$) study of the food web of the temperate Kariega estuary (eastern Cape). *African Journal of Aquatic Science* **26**, 49–56. doi:10.2989/16085910109503724

Frost, P. C., Stelzer, R. S., Lamberti, G. A., and Elser, J. J. (2002). Ecological stoichiometry of trophic interactions in the benthos: understanding the role of C : N : P ratios in lentic and lotic habitats. *Journal of the North American Benthological Society* **21**, 515–528. doi:10.2307/1468427

Garcia, A. M., Hoeinghaus, D. J., Vieira, J. P., Winemiller, K. O., Motta-Marques, D. M. L., and Bemvenuti, M. A. (2006). Preliminary examination of food web structure of Nicola Lake (Taim Hydrological System, south Brazil) using dual C and N stable isotope analyses. *Neotropical Ichthyology* **4**, 279–284. doi:10.1590/S1679-62252006000200014

Garcia, A. M., Hoeinghaus, D. J., Vieira, J. P., and Winemiller, K. O. (2007). Isotopic variation of fishes in freshwater and estuarine zones of a large subtropical coastal lagoon. *Estuarine, Coastal and Shelf Science* **73**, 399–408. doi:10.1016/J.ECSS.2007.02.003

Grosman, M. F., Gonzalez-Castelain, J. R., and Usunoff, E. J. (1996). Trophic niches in an Argentine pond as a way to assess functional relationships between fishes and other communities. *Water S.A.* **22**, 345–350.

Hartz, S. M., Silveira, C. M., and Barbieri, G. (1996). Alimentação das espécies de *Astyanax* Baird and Girard, 1854 ocorrentes na lagoa Caconde, RS, Brasil (Teleostei, Characidae). *Revista Unimar* **18**, 269–281.

Hyslop, E. J. (1980). Stomach contents analysis - a review of methods and their application. *Journal of Fish Biology* **17**, 411–429.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., and Rubel, F. (2006). World Map of the Köppen–Geiger climate classification updated. *Meteorologische Zeitschrift* **15**, 259–263.

Krebs, C. J. (2001). 'Ecology: the Experimental Analysis of Distribution and Abundance.' (Benjamin Cummings, San Francisco, CA.)

Lamberti, G. A., and Moore, J. W. (1984). Aquatic insects as primary consumers. In 'The Ecology of Aquatic Insects'. (Eds V. H. Resh and D. M. Rosenberg.) pp. 164–195. (Praeger Publishers, New York.)

MacLeod, N. A., and Barton, D. R. (1998). Effect of light intensity, water velocity, and species composition on carbon and nitrogen stable isotope ratios in periphyton. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 1919–1925. doi:10.1139/F98-075

Manetta, G. L., Benedito-Cecilio, E., and Martinelli, M. (2003). Carbon sources and trophic position of the main species of fishes of Baía River, Paraná River floodplain, Brazil. *Brazilian Journal of Biology* **63**, 283–290. doi:10.1590/S1519-69842003000200013

Marinho, C. C., Meirelles-Pereira, F., Gripp, A. R., Guimarães, C. C., Esteves, F. A., and Bozelli, R. L. (2010). Aquatic macrophytes drive sediment stoichiometry and the suspended particulate organic carbon composition of a tropical coastal lagoon. *Acta Limnologica Brasiliensia* **22**, 208–217.

Michener, R. H., and Schell, D. M. (1994). Stable isotope ratios as tracers in marine aquatic food webs, In 'Stable Isotopes in Ecology and Environmental Science'. (Eds K. Lajtha and R. Michener.) pp. 138–157. (Blackwell Scientific, Malden, MA.)

Minagawa, M., and Wada, E. (1984). Stepwise enrichment of N15 along food chains: further evidence and the relation between delta-n-15 and animal age. *Geochimica et Cosmochimica Acta* **48**, 1135–1140. doi:10.1016/0016-7037(84)90204-7

Mirande, J. M. (2010). Phylogeny of the family Characidae (Teleostei: Characiformes): from characters to taxonomy. *Neotropical Ichthyology* **8**, 385–568. doi:10.1590/S1679-62252010000300001

Oliveira, E. F., Goulart, E., Breda, L., Minte-Vera, C. V., Paiva, L. R. S., and Vismara, M. R. (2010). Ecomorphological patterns of the fish assemblage in a tropical floodplain: effects of trophic, spatial and phylogenetic structures. *Neotropical Ichthyology* **8**, 569–586.

Oricolli, M. C. G., and Bennemann, S. T. (2006). Dieta de *Bryconamericus iheringii* (Ostariophysi: Characidae) em riachos da bacia do rio Tibagi, Estado do Paraná. *Acta Scientiarum. Biological Sciences* **28**, 59–63.

Perga, M. E., and Gerdeaux, D. (2005). 'Are fish what they eat' all year round? *Oecologia* **144**, 598–606. doi:10.1007/S00442-005-0069-5

Peterson, B. J., and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology Evolution and Systematics* **18**, 293–320. doi:10.1146/ANNUREV.ES.18.110187.001453

Petr, T. (2000). Interactions between fish and aquatic macrophytes in inland waters. A review. *FAO Fisheries Technical Paper* **396**, 1–185.

Phillips, D. L., Newsome, S. D., and Gregg, J. W. (2005). Combining sources in stable isotope mixing models: alternative methods. *Oecologia* **144**, 520–527. doi:10.1007/S00442-004-1816-8

Pinnegar, J. K., and Polunin, N. V. C. (2000). Contributions of stable-isotope data to elucidating food webs of Mediterranean rocky littoral fishes. *Oecologia* **122**, 399–409. doi:10.1007/S004420050046

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* **83**, 703–718. doi:10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

Post, D. M. (2003). Individual variation in the timing of ontogenetic niche shifts in largemouth bass. *Ecology* **84**, 1298–1310. doi:10.1890/0012-9658(2003)084[1298:IVITTO]2.0.CO;2

Power, M., Power, G., Caron, F., Doucett, R. R., and Guiguer, K. R. A. (2002). Growth and dietary niche in *Salvelinus alpinus* and *Salvelinus fontinalis* as revealed by stable isotope analysis. *Environmental Biology of Fishes* **64**, 75–85. doi:10.1023/A:1016035509246

Ross, S. T. (1986). Resource partitioning in fish assemblages: a review of field studies. *Copeia* **1986**, 352–388. doi:10.2307/1444996

Sánchez-Botero, J. I., Caramaschi, E. P., and Garcez, D. S. (2008). Spatio-temporal variation in fish assemblage in a coastal lagoon without direct contact with the sea (southeastern Brazil). *Journal of Coastal Research* **24**, 181–194.

Soneira, P. A., Ruiz-Díaz, F. J., Bechara, J. A., Almirón, A. E., and Casciotta, J. R. (2006). Hábitos tróficos de las especies del genero Hyphessobrycon (characidae) en los Esteros del Iberá. Universidad Nacional del Nordeste, Comunicaciones Científicas y Tecnológicas, Corrientes, Argentina.

Teixeira, R. L. (1989). Aspectos da ecologia de alguns peixes do arroio Bom Jardim, Triunfo, RS. *Revista Brasileira de Biologia* **49**, 183–192.

Teixeira-de-Mello, T., Meerhoff, M., Pekcan-Hekim, Z., and Jeppesen, E. (2009). Substantial differences in littoral fish community structure and dynamics in subtropical and temperate shallow lakes. *Freshwater Biology* **54**, 1202–1215. doi:10.1111/J.1365-2427.2009.02167.X

Tomazelli, L. J., Dillenburg, S. R., and Villwock, J. A. (2000). Late Quaternary geological history of Rio Grande do Sul coastal plain, southern Brazil. *Revista Brasileira de Geociencias* **30**, 474–476.

Trudeau, V., and Rasmussen, J. B. (2003). The effect of water velocity on stable carbon and nitrogen isotope signatures of periphyton. *Limnology and Oceanography* **48**, 2194–2199. doi:10.4319/LO.2003.48.6.2194

Vander Zanden, M. J., and Rasmussen, J. B. (2001). Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. *Limnology and Oceanography* **46**, 2061–2066. doi:10.4319/LO.2001.46.8.2061

Vander Zanden, M. J., Shuter, B. J., Lester, N. P., and Rasmussen, J. B. (2000). Within and among population variation in the trophic position of the pelagic top predator, lake trout. *Canadian Journal of Fisheries and Aquatic Sciences* **57**, 725–731. doi:10.1139/F00-011

Vanderklift, M. A., and Ponsard, S. (2003). Sources of variation in consumer-diet δ15N enrichment: a meta-analysis. *Oecologia* **136**, 169–182. doi:10.1007/S00442-003-1270-Z

Vilella, F. S., Becker, F. G., and Hartz, S. M. (2002). Diet of *Astyanax* species (Teleostei, Characidae) in an Atlantic Forest river in southern Brazil. *Brazilian Archives of Biology and Technology* **45**, 223–232. doi:10.1590/S1516-89132002000200015

Vinson, M. R., and Budy, P. (2011). Sources of variability and comparability between salmonid stomach contents and isotopic analyses: study design lessons and recommendations. *Canadian Journal of Fisheries and Aquatic Sciences* **68**, 137–151. doi:10.1139/F10-117

Winemiller, K. O. (1989). Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan llanos. *Environmental Biology of Fishes* **26**, 177–199. doi:10.1007/BF00004815

Winemiller, K. O. (1991). Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. *Ecological Monographs* **61**, 343–365. doi:10.2307/2937046