Trophic relationships between primary producers and associated fauna in a pristine Cerrado pond

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ABSTRACT

Ponds arising from groundwater in Cerrado of Central Brazil are oligotrophic but highly biodiverse environments. In this study, we used stable isotope ratios to test if there are variations in carbon and nitrogen acquisition by different primary food sources and if they are influenced by seasonality in a well-preserved pond. We hypothesized that periphyton is the main food source for macrophyte-associated fauna. We found that $\delta^{13}C$, but not $\delta^{15}N$, can distinguish between primary food sources; however, the isotopic signatures of food sources did not differ significantly between seasons. The $\delta^{15}N$ values of macrophyte-associated fauna were significantly higher for predators compared with other trophic groups, but $\delta^{13}C$ values did not differ significantly between groups. Emergent macrophytes and periphyton were the main food sources for scrapers (contribution of 42.5%) and collector-gatherers (contribution of 41.6%), respectively. Compared with emergent macrophytes, submerged macrophytes were associated with a greater abundance of fauna and algal biomass but were not a significant food source for associated fauna. Our findings demonstrate that in this small shallow oligotrophic pond in the Cerrado, the stable isotope ratios of carbon and nitrogen of food sources did not vary between seasons but did differ between aquatic macrophyte life forms. We point out the different functional roles of macrophyte life forms, with emergent macrophytes serving as an important food resource, while submerged macrophytes mainly provide physical structure

INTRODUCTION

.Trophic webs are macrodescriptors of nutritional interactions within a community and can be used to map the flow of energy and matter (Jepsen and Winemiller, 2002). Identifying the basis of trophic webs is fundamental for understanding the dynamics of populations, communities, and ecosystems. According to Lopes and Benedito-Cecílio (2002), the understanding of energy flow within ecosystems starts with investigating processes that occur with primary producers and exploring how nutritional variability at this level influences higher trophic levels. In tropical lentic environments, the structure of the trophic web may be influenced by changes in the availability of carbon sources due to precipitation patterns and variations in primary productivity (Reis *et al*., 2016; Saigo *et al*., 2016). Furthermore, trophic dynamics may be affected by the presence of different types of primary producers. The co-occurrence of various food resources may result in more complex pathways for energy transfer (Vadeboncoeur *et al*., 2005; Vander Zanden *et al*., 2011). According to Doi (2009), autochthonous and allochthonous resource availability in lakes is influenced by light, nutrient level, and terrestrial subsidies. These factors also contribute substantially to variation in trophic structure across ecosystems.

Stable isotopes of carbon and nitrogen are important tools to study trophic webs in aquatic ecosystems (Arcagni *et al*., 2013; Mendonça *et al*., 2013; Lopes *et al*., 2015; Iglesias *et al*., 2017). Stable isotope analysis plus understanding of natural history of organisms and of species interactions lead to the most thorough understanding of food webs (Layman *et al*., 2007). The use of stable isotopes is based on the premise that isotopic ratios are conservative tracers and that a combination of distinct sources determines the flow of nutrients between organisms (Fry, 2006). The isotopic signature of carbon ($\delta^{13}C$) can distinguish between autotrophic energy sources, whereas the isotopic signature of nitrogen ($\delta^{15}N$) can distinguish between trophic levels, with $\delta^{15}N$ is enriched, on average, 3-4‰ per trophic level (Peterson, 1999; Eggers and Jones, 2000). Moreover, seasonality can affect environmental factors and modify $\delta^{13}C$ and $\delta^{15}N$ of the basal resources. This has been described by Alves *et al*. (2017) for pH and nutrient concentrations in a Neotropical floodplain and by Mao *et al*. (2014) for temperature, irradiance, and terrestrial input of carbon and nitrogen in a shallow eutrophic lake in China.

Because lentic systems are resistant to gas diffusion and

there is the formation of boundary layers, aquatic plants are adapted to the lower availability of CO_2 and oxygen (Chambers *et al.*, 2008). The available carbon source is the main factor responsible for differences in the $\delta^{13}C$ values of macrophytes (Finlay and Kendall, 2007). Primary producers in aquatic systems have more than one source of inorganic carbon (CO_2 and/or HCO_3^-); therefore, a large range of $\delta^{13}C$ signals is possible within the ecosystem, complicating carbon source tracing (Mendonça *et al.*, 2013). Compared with pelagic producers, littoral producers experience less turbulence and have a thicker boundary layer, and are therefore enriched in $\delta^{13}C$ (France, 1995). According to Chappuis *et al.* (2017), the assimilation of HCO_3^- in addition to CO_2, results in $\delta^{13}C$ signals more enriched compared with plants exclusively using CO_2. The functional group of aquatic plants is an important intrinsic factor determining $\delta^{13}C$ variability.

Natural shallow ponds are widely distributed in Cerrado biome, presenting numerous plant species with different morphologies whose physical structure promotes considerable habitat heterogeneity in such ecosystems (Sousa *et al.*, 2014). The dominant *taxa* in these ecosystems are algae (periphyton and metaphyton), aquatic macrophytes, protozoa, and an impressive diversity of invertebrates, mainly Cladocera species (Bini *et al.*, 2007; Sousa *et al.*, 2014; Fonseca and Estrela, 2015; Fonseca *et al.*, 2017). Pond size is related to the length and width of the littoral zone, which displays the best ecological conditions in terms of oxygen and temperature (de Marco Jr *et al.*, 2014). In these natural shallow ponds of the Cerrado, water levels vary from 20 to around 80 cm (Fonseca *et al.*, 2017), it is not possible to delimit a pelagic zone.

In this study we investigated trophic relationships in a natural shallow pond in the Cerrado that is colonized by aquatic macrophytes (submerged and emergent). The following hypotheses were tested: i) submerged macrophytes are enriched in $\delta^{13}C$ compared with other food resources because of differential fractionation during the uptake of dissolved inorganic carbon; ii) food resources (periphyton and submerged/emergent macrophytes) have similar $\delta^{15}N$ values because nitrogen sources do not show a high degree of variability; iii) seasonality affects the carbon and nitrogen stable isotope ratios of food resources because of changes in water temperature and pH; and iv) periphyton is the main food source for macrophyte-associated fauna because of its lower carbon:nitrogen (C:N) ratio.

METHODS

Study area

The study was carried out at Henrique Pond (15°41'17"S 47°56'25"W), which is a shallow oligotrophic pond of approximately 2.9 ha (total phosphorus: 13.0 µg/L;

Fonseca *et al.*, 2017). The pond is located in the Brasilia National Park of the Federal District of Brazil, which is the largest conservation unit (42,389 ha) in the Federal District in the category of total protection. The park's aquatic ecosystems are part of the Upper Paraná River Basin. More than 80% of the pond's surface is covered by aquatic macrophytes (Sousa *et al.*, 2014). The main water sources are groundwater outcropping and precipitation. The terrain slope permits water accumulation, reaching a depth of 1.2 m. According to the National Meteorological Institute (http://www.inmet.gov.br/portal/), total annual precipitation for the year 2015 was 1252.7 mm. In this study we obtained samples during two seasons of 2015: the rainy season (October to March, total precipitation of 975.2 mm) and the dry season (April to September, total precipitation of 277.5 mm).

Limnological variables

An *in situ* evaluation was performed in each collection site in both seasons to determine water temperature (Oximeter YSI 55), water depth, dissolved oxygen concentration (Oximeter YSI 55), pH (Digimed MD 20), and electrical conductivity (Minipa MCD-2000). To analyze turbidity and alkalinity, 1 L water was collected and transported to the laboratory in an insulated container. Turbidity analysis (Hach 2100 AN) was carried out the day of sampling while, and alkalinity was analyzed the following day using the Gram method (Carmouze, 1994).

Macrophyte survey

To determine which macrophyte species would be used to evaluate trophic relationships, samples were collected in two perpendicular zones (1 m wide), extending across the longest and the widest parts of the pond, totaling 410 m². All macrophytes within these zones were collected, stored in plastic bags, and kept in an insulated box until transport to the laboratory on the same day. In the laboratory the macrophytes were washed with distilled water and a soft brush to remove adherent periphyton and then separated for identification. The macrophytes were lyophilized for 72 h and ground with a pulverizing mill (Planetary Mill Pulverisette 5, Fritsch) to produce a fine powder for isotopic analysis.

Food sources and sediment for evaluation of isotopic signatures

After we surveyed all macrophytes in the two collection zones, we selected one morphospecies of the emergent macrophytes belonging to the *Cyperus* L. genus (Cyperaceae), and one morphospecies of the submerged macrophytes belong to the *Cabomba* Aubl. genus (Cabombaceae) to evaluate trophic relationships. These macrophytes were chosen because they have different life

patterns and are abundant in the pond throughout the year (personal observation). Emergent and submerged macrophytes were sampled in five different sites, resulting in 10 plots for season. The plots were at least 3 m apart from each other and were selected based on the presence of macrophytes. A 20 cm ´ 20 cm square frame was used in order to standardize the sampling. This procedure was repeated during both rainy and dry seasons.

Mature vegetative parts that were submerged were collected and stored in plastic bags. Young and senescent plants were avoided to focus on a single successional stage of the periphyton community. The macrophytes were frozen after washed with 300 mL deionized water and softly brushed to collect the associated periphyton. The 300 mL water was filtered using a 250-µm mesh to remove large detritus and macroinvertebrates; 100 mL of the filtered water was used to analyze periphyton chlorophyll-*a* (Marker *et al.*, 1980; Sartory and Grobblelar, 1984), 100 mL was used to determine ash-free dry mass (AFDM) (APHA, 2005), and 100 mL was used for isotopic analysis of the periphyton.

When present, metaphyton, here represented by filamentous algae, was collected by filtering the water through a steel sieve with a 20-µm mesh. The collected material was frozen in Falcon tubes for further analysis.

Additionally, sediment was collected using a Kajak-type gravity core sampler at a depth of 0-20 cm. In the laboratory, macrophytes and roots were removed before homogenizing the sediment, which was then frozen in 50-mL Falcon tubes.

Macrophyte-associated fauna

Macrophyte-associated fauna was collected by dragging a plankton net (100-µm mesh) through the aquatic vegetation within a 2-m radius at each collection site (n=5 for each type of macrophyte [submerged (*Cabomba*) *vs* emergent (*Cyperus*)], for a total of 10 samples in each season). The samples were fixed in alcohol (final concentration, 70%). Organisms within each sample were identified at the level of the family for insect larvae, order for microcrustaceans, and class for other organisms. Organisms were then classified according to functional trophic group, as previously described (Elmoor-Loureiro, 1997; Ramírez and Gutiérrez-Fonseca, 2014). The individual organisms were frozen in Eppendorf tubes.

Isotope analysis of food sources

Frozen macrophyte and periphyton samples were lyophilized for 72 h. The macrophyte samples were ground as described in the "*Macrophyte survey*" section, and the periphyton samples were macerated in Eppendorf tubes with glass beads using a high-velocity benchtop homogenizer (FastPrep-24). The frozen macrophyte-associated fauna

samples were dried at 60ºC; organisms larger than 1 cm were ground in a mortar and pestle, and organisms smaller than 1 cm were used intact for isotope analysis.

It was necessary to combine samples according to family and season to obtain the minimum mass necessary for isotopic analysis. The fauna associated with macrophytes and periphyton were weighed (minimum of 1.5 mg and 0.9 mg, respectively) in an analytical balance and placed in tin capsules. Isotopic analyses were carried out by the Isotopic Ecology Laboratory in the Center Nuclear Energy in Agriculture (University of São Paulo, Piracicaba, Brazil). Samples were processed by combustion under a continuous flow of ultrapure helium in an elemental analyzer (Carlo Erba CHN 1110) coupled to a Thermo Finnigan Delta Plus mass spectrometer.

The results were expressed in delta notation (δ), in parts per thousand (‰) relative to standard international references (Vienna Pee Dee Belemnite from the Grand Canyon, USA, for C; atmospheric air for N) and were calculated using the following equation: $\delta X = (R \text{ sample} / R \text{ standard} - 1) \times 1000$, where X is ^{13}C or ^{15}N, and R represents the isotopic ratios $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample or the standard. The acceptable analytic error was ± 0.3% and 0.3‰ for C and $\delta^{13}C$, respectively.

Statistical analysis

Normality of the abiotic data was evaluated by using the Shapiro-Wilk test. Descriptive analysis of the abiotic data used the arithmetic mean as a measure of central tendency, and standard error (SE) as a measure of dispersion of the data. Student's *t*-test was used to compare limnological data between seasons when the data were normally distributed. Because electrical conductivity, dissolved oxygen, and periphyton AFDM data showed non-normal distribution, we used the Wilcoxon test.

Seasonal differences between food sources and differences between emergent and submerged macrophytes and the associated periphyton were evaluated using Student's t-test when the dataset was homoscedastic and the residuals were normally distributed, otherwise the Mann–Whitney test was used.

Differences in $\delta^{13}C$, $\delta^{15}N$, and C:N values between the food sources were evaluated by 1-factor analysis of variance (ANOVA) when the premises of dataset homoscedasticity and normal distribution of residuals were accepted. Welch's test was used when the residuals were normally distributed, but the dataset was not homoscedastic, and the Kruskal test was used when neither premise was accepted. Seasonal differences in stable isotopes of food sources were evaluated using Tukey HSD (honest significance difference) test for normally distributed datasets, otherwise the Kruskal multiple comparison was used. Differences in $\delta^{13}C$ and $\delta^{15}N$ values between primary consumers (collector-

gatherers and scraper) and secondary consumers (predators) were evaluated by Student's *t*-test. Differences in associated fauna abundance between macrophyte type and between seasons were evaluated by 2-way ANOVA. For this study, P<0.05 was considered significant. Normality was evaluated using the Shapiro-Wilk test, and homoscedasticity was evaluated using Levene's test. Statistical analyses were carried out using R software ver. 3.2.2 (R Core Team, 2015).

Bayesian mixing models using Stable Isotope Analysis in R (SIAR v. 4.0, Parnell *et al.*, 2010) were used to estimate isotope incorporation by the associated fauna. The SIAR model is fit *via* Markov Chain Monte Carlo (MCMC) methods and takes the variability in source and consumers isotopic signatures. This approach estimates the proportional contribution of potential sources within the tissue of the consumer and we consider the carbon and nitrogen composition of the sources (Parnell *et al.*, 2010).

Emergent macrophytes, submerged macrophytes, and periphyton were selected as potential food sources for primary consumers (collector-gatherers and scraper invertebrates). The collector-gatherers and scraper invertebrates were selected as potential food sources for predators. The food sources used in each calculation were selected with the consideration of the feeding mode of the consumers and availability in habitat. In our study, constant TEF values of +0.4 ±1.23 SD δ13C and +2.3 ±1.61 SD δ15N were used. These values corresponded to the mean TEF estimates in aquatic ecosystems (McCutchan *et al.*, 2003).

RESULTS

Limnological variables

In Henrique Pond, water temperature (23.9±0.2°C) and water turbidity (2.55± .2 NTU) were significantly greater in the dry season, but the temperature difference from the rainy season (22.7±0.1°C) was much less pronounced than the seasonal difference found for turbidity (rainy season -0.9±0.1 NTU). The dissolved oxygen concentration (6.6±0.1 mg L^{-1}) and depth (0.9±0.04 m) were greater in the rainy season but seasonal difference of dissolved oxygen was subtle (Tab. 1). Water depth in the dry season was 0.2 m lower. The other variables (pH, electrical conductivity, alkalinity, periphyton chlorophyll-*a*, and AFDM) did not differ between seasons.

Analysis of food sources, metaphyton and sediment

Of the 12 species collected in the macrophyte survey, six were emergent and six were submerged (Tab. 2). Mean δ^{13}C values differed significantly between emergent and submerged macrophytes (-27.73 and -23.32‰, respectively; t= -4.32, P=0.001), but δ^{15}N values did not differ significantly (t= -0.65, P=0.533). These results were used to select the aquatic macrophyte species for the experiment.

Our results show that carbon and nitrogen stable isotope ratios of the food sources did not differ significantly between seasons (Tab. 3). Because of this lack of seasonal variation, samples collected in the dry and rainy seasons were combined to evaluate the stable isotope ratios of the food sources. We evaluated 10 samples of *Cyperus* sp. (five samples for each season), 9 samples of *Cabomba* sp. (five samples for rainy season plus four samples for dry season), 16 samples of periphyton (some samples presented enough mass for the analysis), five samples of metaphyton (collected when present), and six samples of sediment (three samples for each season).

Our results showed that food sources differed significantly with regard to δ^{13}C values (H$_4$=28.2, P<0.001; Fig. 1a). Food sources did not show significant

Tab. 1. Limnological variables of Henrique Pond, Brasilia National Park, Federal District, during the rainy and dry seasons of 2015. P values in bold represent significant differences.

Variable	Rainy season		Dry season		P
	Mean value±SE	CV	Mean value±SE	CV	
Water temperature (°C)	22.7±0.1	0.02	23.9±0.2	0.03	**<0.001**
Depth (m)	0.9±0.04	0.13	0.7±0.06	0.27	**0.030**
pH	5.6±0.04	0.02	5.5±0.05	0.03	0.085
Electrical conductivity (μS cm^{-1})	3.7±0.5	0.43	3.9±0.2	0.19	0.140
Dissolved oxygen (mg L^{-1})*	6.6±0.1	0.04	5.8±0.1	0.07	**0.001**
Turbidity (NTU)	0.9±0.1	0.46	2.5±0.2	0.27	**<0.001**
Alkalinity (mg CaCO$_3$ L^{-1})	1.6±0.1	0.13	1.7±0.1	0.14	0.460
Periphyton chlorophyll-*a* (g m^{-2})	16.1±4.4	0.86	12.2±2.9	0.72	0.480
Periphyton ash-free dry mass (g m^{-2})*	0.006±0.002	1.34	0.002± 0.001	0.91	0.722

*CV, coefficient of variation; *Wilcoxon test; NTU, nephelometric turbidity unit.*

difference for $\delta^{15}N$ values ($F_{3,13}$=1.4, P=0.296), but differs significantly from sediment ($F_{4,15}$=5.6, P=0.005; Fig. 1b). Sediment and the submerged macrophyte were more ^{13}C-enriched than the other food sources. The mean $\delta^{13}C$ and $\delta^{15}N$ values were -25.59±0.20‰ and 0.84±0.68‰, respectively, for *Cyperus* sp.; -21.20±0.30‰ and 0.40±1.15‰, respectively, for *Cabomba* sp.; -25.23±0.62‰ and 1.99±0.34‰, respectively, for periphyton; -24.42±1.01‰ and 2.27±0.58‰, respectively, for metaphyton; and -22.50±0.20‰ and 4.23±0.46‰, respectively, for sediment.

The C:N ratios of the food sources also differed significantly (H_4= 7.2, P<0.001; Fig. 1c), with the lowest C:N ratios for sediment (11.9±0.2) and periphyton (12.1±0.5), and the highest C:N ratio for the emergent macrophyte *Cyperus* sp. (90.1±4.7). Intermediate C:N ratios were observed for metaphyton (18.2±2.3) and the submerged macrophyte *Cabomba* sp. (28.3±1.1).

The macrophytes differed significantly with regard to periphyton chlorophyll-*a* concentration (W=0, P<0.001) and AFDM (W=0, P<0.001), with greater values observed for *Cabomba* sp. (114.9 g/m² and 0.212 g/m², respectively) (Fig. 2 a,b), despite the higher biomass of *Cyperus* sp. compared to *Cabomba* sp. (W=64, P<0.001; Fig. 2c).

Macrophyte-associated fauna

The collection of macrophyte-associated fauna resulted in a total 3,983 individual organisms for analysis. Insects accounted for 78.2% of the macrophyte-associated fauna, and mites, microcrustaceans, and tadpoles were also present (Tab. S1). The sampled organisms were more often associated with the submerged macrophyte *Cabomba* sp. (in both seasons) and were more abundant in the dry season for both macrophytes studied ($F_{1,16}$=17.9, P<0.001).

Isotopic analysis of macrophyte-associated fauna

Despite the large number of individual organisms collected, it was not possible to carry out isotopic analysis for some *taxa* because of low overall mass. Isotopic analysis was carried out for the following 11 *taxa*: Coenagrionidae, Dicteriadidae, Lestidae, Libellulidae, Tanypodinae, Ceratopogonidae, Chironominae, Culicidae, Hydroptilidae, Cladocera, and Anura (Tab. 4). As there were no statistical differences between the isotopic signatures for associated fauna in

Tab. 2. Isotopic signatures of carbon ($\delta^{13}C$, ‰) and nitrogen ($\delta^{15}N$, ‰) of all aquatic macrophytes collected in Henrique Pond, Brasilia National Park, Federal District, Brazil. These results were used to select the aquatic macrophyte species for the experiment.

Habitat	Aquatic macrophyte	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Emergent	CYPERACEAE		
	Cyperus sp.	-27.37	5.75
	Rhynchospora sp.	-29.84	3.06
	Morphospecies 1	-25.97	1.65
	MELASTOMATACEAE		
	Morphospecies 1	-29.22	2.22
	POACEAE		
	Otachyrium sp.	-25.33	2.28
	Panicum sp.	-28.65	-0.96
Submerged	CABOMBACEAE		
	Cabomba sp.	-22.87	3.56
	MENYANTHACEAE		
	Nymphoides indica	-26.01	4.64
	Unidentified family		
	Morphospecies 1	-23.66	2.95
	Morphospecies 2	-23.63	1.00
	Morphospecies 3	-23.07	0.89
	Morphospecies 4	-20.67	5.49

Tab. 3. Isotopic signatures of food sources collected during the rainy and dry seasons in Henrique Pond, Brasilia National Park, Federal District, Brazil.

Food source		Rainy (‰)	Dry (‰)	df	P
Cyperus sp. (emergent macrophyte)*	$\delta^{15}N$	1.28	0.40	8	0.55
	$\delta^{13}C$	-25.95	-25.24	8	0.06
Cabomba sp. (submerged macrophyte)*	$\delta^{15}N$	1.32	-0.76	7	0.40
	$\delta^{13}C$	-21.55	-20.77	7	0.19
Periphyton	$\delta^{15}N$	1.77	2.21	14	0.52
	$\delta^{13}C$	-25.62	-24.84	14	0.55
Metaphyton	$\delta^{15}N$	2.61	1.77	3	0.55
	$\delta^{13}C$	-23.15	-26.33	3	0.13
Sediment	$\delta^{15}N$	3.62	4.84	4	0.22
	$\delta^{13}C$	-22.38	-22.62	4	0.62

*df, degrees of freedom; *for the macrophyte genera selected, we repeated the isotope analysis for each collection site and both seasons. All data were analyzed using Student's t-test.*

Tab. 4. Isotopic signatures δ¹³C and δ¹⁵N of macrophyte-associated fauna in Henrique Pond, Brasilia National Park, Federal District, Brazil.

Taxon	TFG	N	Mean δ^{13}C (‰)	SD	Mean δ^{15}N (‰)	SD
Coenagrionidae	Pr	5	-24.97	1.61	5.17	1.00
Dicteriadidae	Pr	1	-25.68		6.95	
Lestidae	Pr	1	-24.14		7.22	
Libellulidae	Pr	5	-23.19	2.08	3.71	1.08
Tanypodinae	Pr	8	-24.17	1.88	4.09	1.01
Ceratopogonidae	Pr	1	-24.54		3.69	
Chironominae	CG	2	-24.34		3.75	
Culicidae	CG	2	-25.22		3.74	
Hydroptilidae	Sc	1	-26.15		1.69	
Cladocera	Sc	1	-25.24		3.28	
Anura	Sc	3	-24.24	1.36	4.27	0.80

TFG, trophic functional group, according to Ramírez and Gurriérez-Fonseca (2014); N, number of samples; CG, collector-gatherers; Pr, predators; Sc, scrapers. Data are expressed as mean±SD.
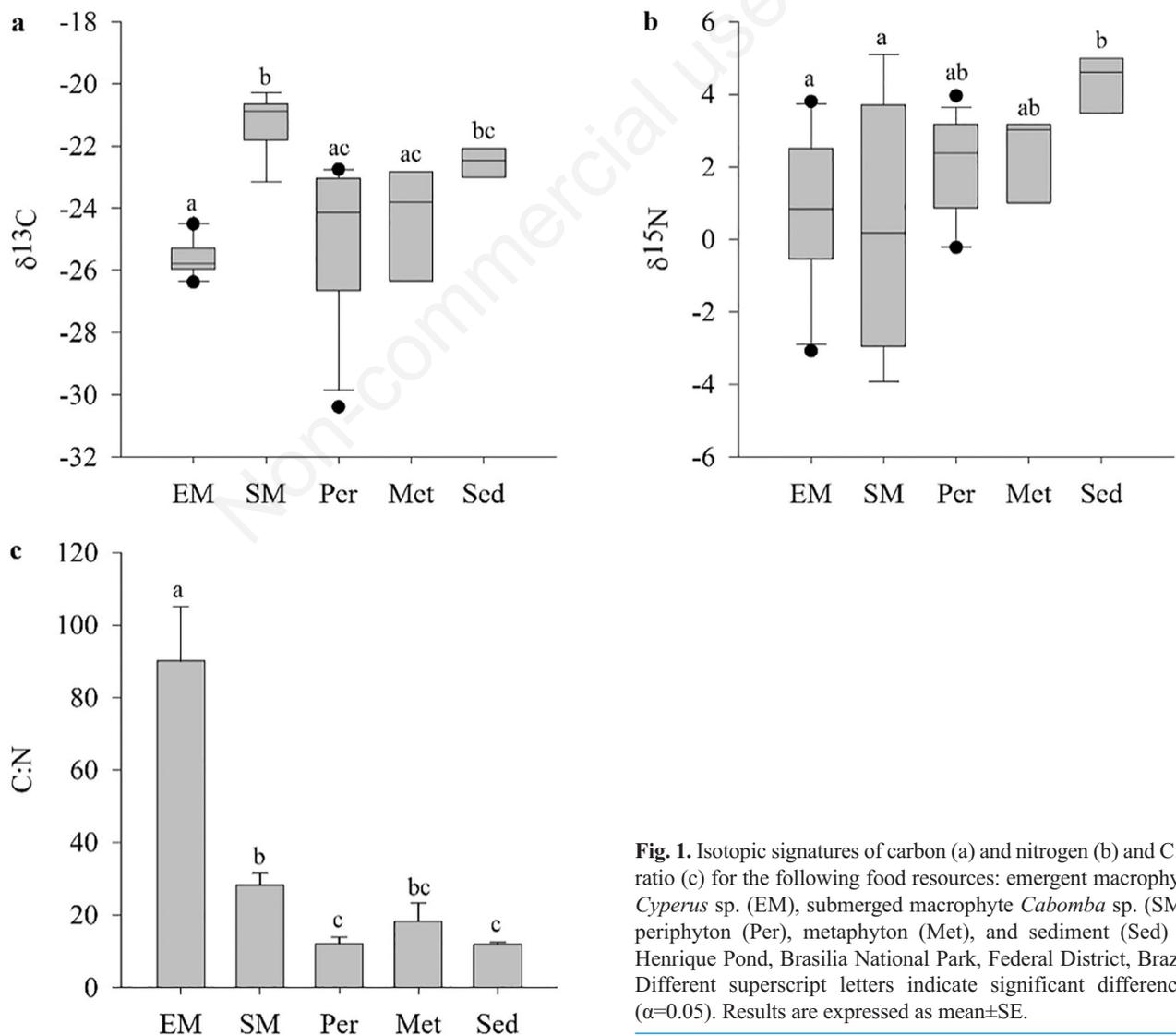
Fig. 1. Isotopic signatures of carbon (a) and nitrogen (b) and C:N ratio (c) for the following food resources: emergent macrophyte *Cyperus* sp. (EM), submerged macrophyte *Cabomba* sp. (SM), periphyton (Per), metaphyton (Met), and sediment (Sed) in Henrique Pond, Brasilia National Park, Federal District, Brazil. Different superscript letters indicate significant differences (α=0.05). Results are expressed as mean±SE.

the dry and rainy period (t=0.35, P=0.725), samples were composed. Our results showed that $\delta^{13}C$ values varied between -26.2 and -24.1‰ for associated fauna but did not differ significantly between primary consumers (collector-gatherers and scraper) and secondary consumers (predators) (t=-0.92, P=0.383). However, $\delta^{15}N$ values were significantly higher in secondary consumers (mean value of 5.3‰) compared with primary consumers (t=-2.83, P=0.020).

Use of food sources by macrophyte-associated fauna

For collector-gatherers, periphyton was the principal food source, with a proportional contribution of 41.6%, followed by emergent macrophyte *Cyperus* sp. (38.6%) and submerged macrophyte *Cabomba* sp. (19.7%; Fig. 3). For scrapers, the emergent macrophyte *Cyperus* sp. was the principal food source, with a proportional contribution of 42.5%, followed by periphyton (40.0%) and the submerged macrophyte *Cabomba* sp. (17.5%; Fig. 3). For predators, scrapers were the principal food source, with a contribution of 62.0%. Scrapers were represented by three *taxa*: Cladocera, tadpoles and Hydroptilidae (Trichoptera), representing 27% of the total richness and 13% of total abundance of the macrophyte-associated fauna.

Fig. 4 shows the carbon and nitrogen isotopic composition of macrophyte-associated fauna and their food sources. The $\delta^{13}C$ isotopic signature of all fauna groups indicated higher consumption of the emergent macrophyte *Cyperus* sp., and periphyton. Secondary consumers (predators) showed more enriched $\delta^{15}N$ values than the primary consumers (for collector-gatherers and scrapers).
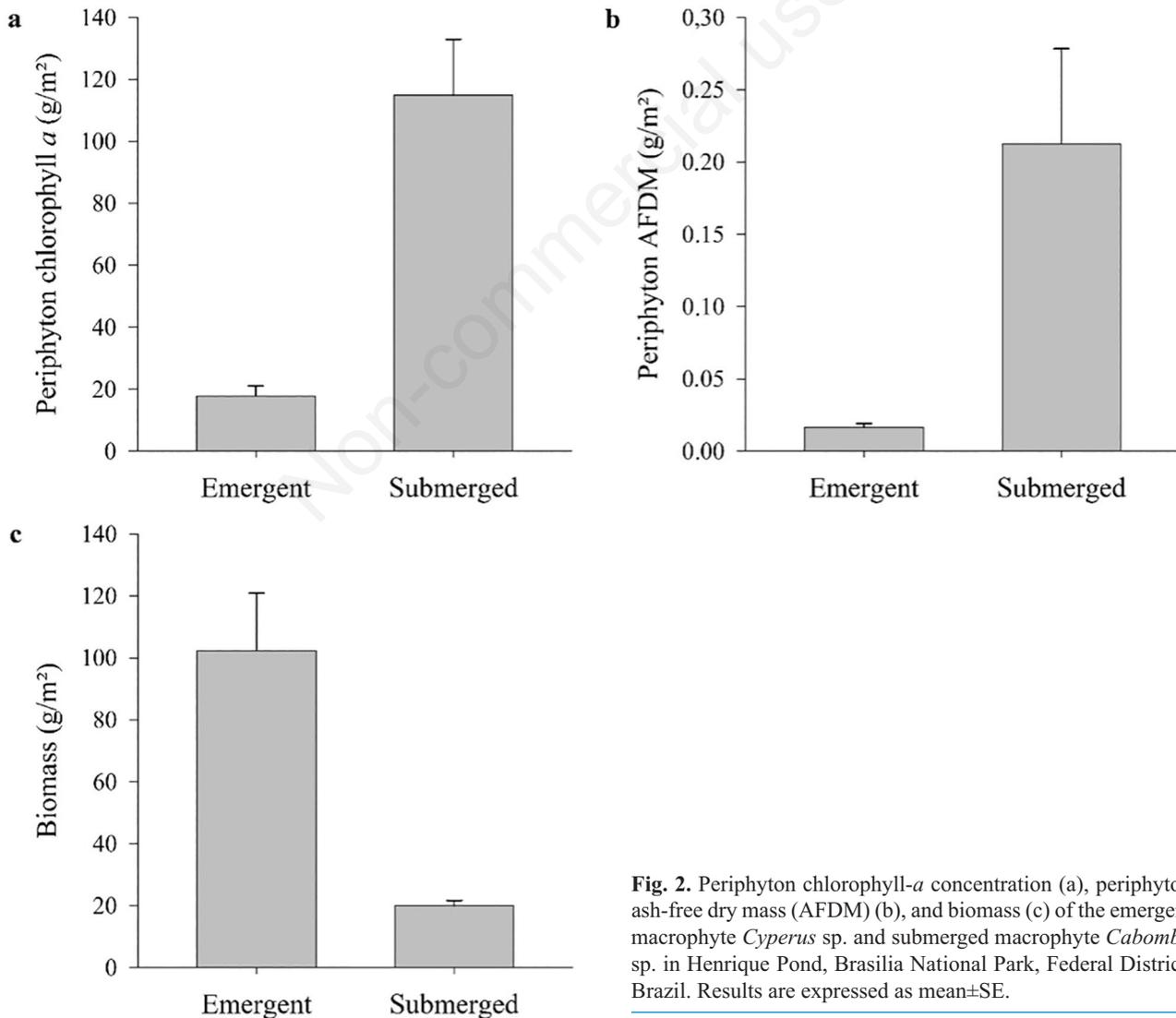
Fig. 2. Periphyton chlorophyll-*a* concentration (a), periphyton ash-free dry mass (AFDM) (b), and biomass (c) of the emergent macrophyte *Cyperus* sp. and submerged macrophyte *Cabomba* sp. in Henrique Pond, Brasilia National Park, Federal District, Brazil. Results are expressed as mean±SE.

DISCUSSION

Primary producers form the base of the trophic web in ecosystems, synthesizing organic compounds and providing nutrients for the other trophic levels. For Henrique Pond, the basal energy sources evaluated were macrophytes (emergent and submerged), macrophyte-

associated periphyton, metaphyton, and sediment. In general, the primary carbon sources of this pond are isotopically similar to those of other tropical environments (Tab. 5).

The $\delta^{13}C$ values for all sources were close to the mean value for C_3 plants, except for *Cabomba* sp. that showed enrichment of $\delta^{13}C$, confirming the first hypothesis (*i.e.*,

Tab. 5. Comparison of isotopic signatures $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of food sources between studies of tropical environments and the present study.

Source	Range $\delta^{13}C$ (‰)	Range $\delta^{15}N$(‰)	Reference
Emergent macrophytes	-28.6 to -27.4	-0.9 to 5.7	Current study
Emergent macrophyte *Cyperus* sp.	-26.4 to -25.5	-3.1 to 3.8	Current study
Submerged macrophytes	-22.9 to -20.7	0.9 to 5.5	Current study
Submerged macrophyte *Cabomba* sp.	-23.1 to -20.3	-3.9 to 5.1	Current study
C_3 macrophytes	-31 to -27	4 to 9.5	Wantzen *et al.*, 2002
Emergent macrophyte (*Eleocharis interstincta*)	-30.2 to -28.9	-1.3 to 5.1	Reis *et al.*, 2016
Floating macrophyte (*Nymphaea caerulea* and *Nymphoides indica*)	-27.2 to -24.5	0.5 to 3.9	Reis *et al.*, 2016
Aquatic macrophytes	-33 to -24	1 to 3	Iglesias *et al.*, 2017
Periphyton	-30.4 to -22.8	-0.2 to 4.0	Current study
Periphyton	-33 to -26	1 to 3	Wantzen *et al.*, 2002
Periphyton	-26.9 to -18.4	4.1 to 5.6	Reis *et al.*, 2016
Sediment	-23.2 to -21.8	2.1 to 5.2	Current study
Sediment	-38 to -27	1 to 5.7	Wantzen *et al.*, 2002
Sediment	-33.1 to -31.2	1.8 to 3.5	Reis *et al.*, 2016
Metaphyton	-28.2 to -22.5	0.2 to 3.3	Current study
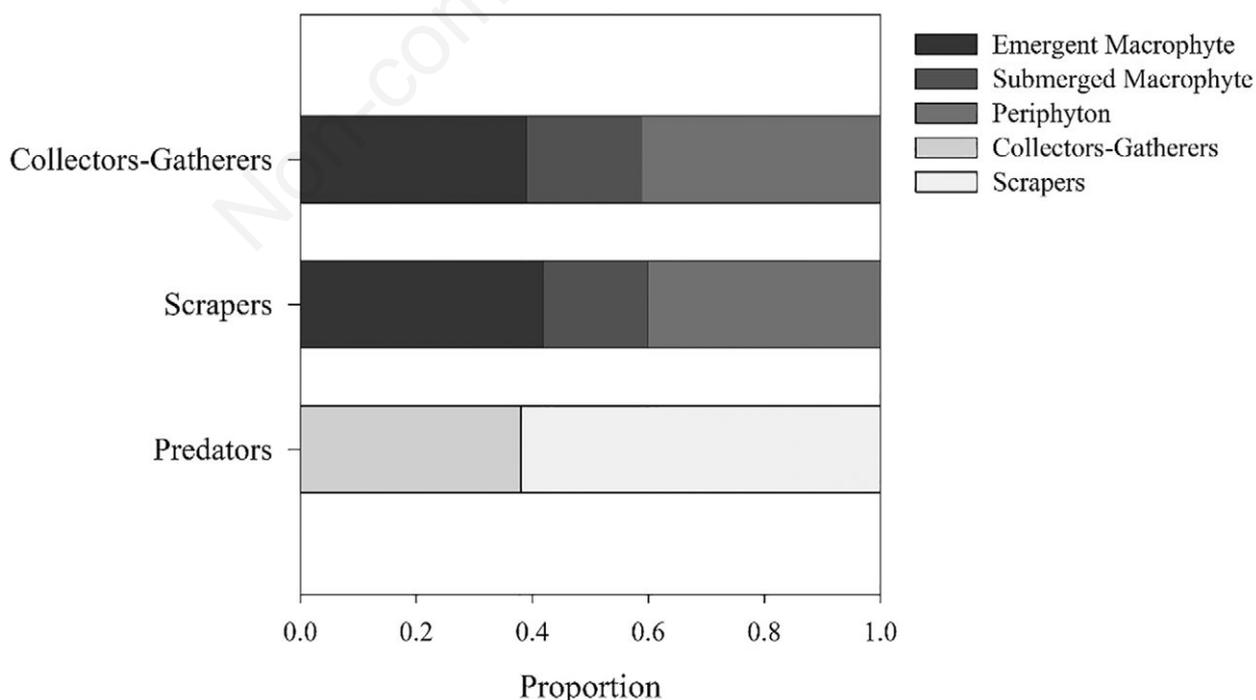
Fig. 3. Stable isotope analysis in R (SIAR) results showing proportions of food resources used by each trophic group (Collectors-Gatherers, Scrapers and Predators) of aquatic invertebrates in Henrique Pond, Brasilia National Park, Federal District, Brazil.

$\delta^{13}C$ values are enriched for submerged macrophyte than for the other food sources). A study evaluating aquatic macrophytes in 19 shallow ponds in South America (Mendonça *et al.*, 2013) suggested that the $\delta^{13}C$ signal of submerged macrophytes depends on the intrinsic characteristics of the species and the availability of dissolved CO_2. Because the resources required by submerged macrophytes are found almost exclusively in water and sediment, these organisms have developed morphological and physiological adaptations to overcome carbon limitation in the aquatic environment such as C_4 metabolism, crassulacean acid metabolism (CAM), use of HCO_3, and use of CO_2 from interstitial sediment water (Pierini and Thomaz, 2004). Chappuis *et al.* (2017) analyzed aquatic plants from a wide range of stagnant water bodies in the Mediterranean region and also found that functional group (an intrinsic factor) is the most important factor determining $\delta^{13}C$ variability. The submerged macrophyte *Cabomba* sp. likely uses dissolved carbon from the water column and often uses carbon from the sediment. According to Chappuis *et al.* (2017), these carbon sources exhibit considerable $\delta^{13}C$ variability and fractioning during inorganic carbon uptake. Consequently, submerged macrophyte species exhibit less negative $\delta^{13}C$ mean values since the aquatic environment generally decreases fractionation. Our analysis of stable isotope ratios for all the macrophytes of the pond did not show differences in photosynthetic metabolism (C_3 *vs* C_4; Tab. 2), suggesting that differences in carbon source (carbon dioxide as gas or dissolved in water as bicarbonate) accounted for the difference in
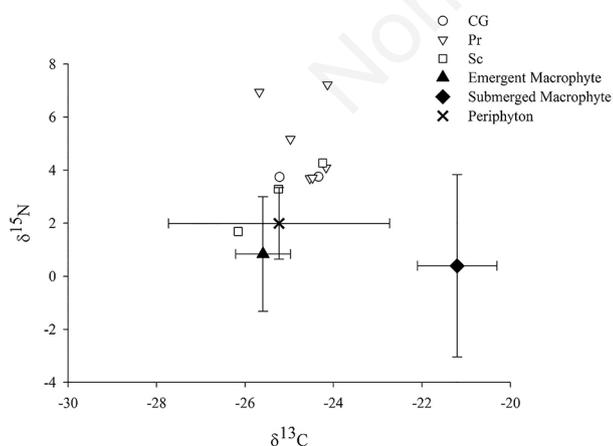
Fig. 4. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic signatures for associated fauna and mean ($\pm$ SD) isotopic values for the emergent macrophyte (*Cyperus* sp.), submerged macrophyte (*Cabomba* sp.), and periphyton in Henrique Pond, Brasilia National Park, Federal District, Brazil. CG, collectors-gatherers; Pr, predators; Sc, scrapers.

carbon stable isotope ratios between emergent and submerged macrophytes.

In spite of five different food sources considered in this study, their nitrogen stable isotope ratios were similar, leading to less ambiguity in the interpretation of trophic transfer through the food web and, supporting our second hypothesis (*i.e.*, food resources present similar values of $\delta^{15}N$). Both of the macrophyte species studied are rooted, and they can obtain nitrogen from both the water column and sediment, whereas periphyton can obtain nitrogen from the water column and the substrate, in this case, the macrophyte. Furthermore, the studied pond is limited by nitrogen and phosphorus and, comparing macrophytes, periphyton and sediment, periphyton shows the highest concentrations of nitrogen (Alvim, 2017). There is evidence that nitrogen availability in the Cerrado is low and the nitrogen cycle is conservative (Bustamante *et al.*, 2004). Isotope discrimination does not occur in nitrogen-limited ecosystems, and macrophyte $\delta^{15}N$ values tend to increase if the limitation is greater (King *et al.*, 2009).

The lack of seasonal pattern in carbon and nitrogen stable isotope ratios of the basal energy sources in this study can related to subtle seasonal changes in limnological variables as only water temperature, depth, dissolved oxygen and turbidity differed between seasons (Tab. 1), This contradicts our third hypothesis (*i.e.*, seasonality affects the carbon and nitrogen stable isotope ratios of food resources). In a remnant of the Atlantic rainforest tropical lake, Reis *et al.* (2016) investigated basal carbon sources and found that carbon isotope ratios were generally more negative during the dry season; however, this difference was significant only for seston (-32.6‰) and emergent macrophytes (31.3‰; e.g., *Eleocharis interstincta*). The authors did not observe differences in $\delta^{15}N$ values between seasons. Nutrient concentrations and pH could predict the variability in $\delta^{15}N$ of macrophytes in the Pantanal floodplain and that water pH could predict differences of $\delta^{13}C$ between periphyton and C4 plants (Alves *et al.*, 2017). In a shallow eutrophic lake in China, the isotopic signatures for food sources varied widely (-31.4‰ to -14.2‰ for $\delta^{13}C$, and 6.4‰ to 12.6‰ for $\delta^{15}N$), with $\delta^{13}C$ and $\delta^{15}N$ values generally enriched in summer because of increased productivity (due to high temperature and irradiance levels) and compared with winter (Mao *et al.*, 2014).

The study of Alves *et al.* (2017) was carried out in environments with a pH range of 6.0 to 6.9. Studies with a similar approach to ours are not common in the aquatic environments of the Cerrado, making it difficult to compare them with environments that present a pH range close to our study area. Although the relatively low pH, normally found in the aquatic environments of Cerrado, favors the presence of C in the form of CO_2, the relatively high temperatures, on the other hand, potentiate its

diffusion into the atmosphere. Thus, the environment is conducive to carbon limitation in water, since acidic pH does not favor the maintenance of inorganic carbon reserves in the form of HCO_3^- or CO_3^{2-} (Maberly *et al.*, 2009). The underlying mechanisms involving pH changes and C isotopic signature need further investigation in Cerrado aquatics environments.

In the Cerrado pond analyzed in this study, periphyton and emergent macrophyte were the main food sources for collector-gatherers and scrapers, respectively, partially confirming the fourth hypothesis. This result may be due, in part, to the low C:N ratio of periphyton, indicating its high palatability. Our finding is consistent with that of Brett *et al.* (2009), who reported that autochthonous sources (phytoplankton) have high nutritional quality and enhance the growth, reproduction, and lipid composition of zooplankton. Similarly, De Kluijver *et al.* (2015) showed that macrophytes and associated periphyton subsidize zooplankton, supporting its growth in a subtropical shallow eutrophic lake in China. Although the C:N ratio of the submerged macrophyte *Cabomba* sp. was lower than that of the emergent macrophyte *Cyperus* sp., its proportional contribution as food source was the lowest of the resources studied (18%). This finding may be due to the greater biomass of *Cyperus* sp. in this environment (Fig. 2c).

The emergent macrophyte *Cyperus* sp. also contributed to the consumers analyzed, despite its higher C:N ratio. Cladocera species are capable of foraging in the periphyton and have a $\delta^{13}C$ signature similar to this source. In the present study, the trophic niche overlap was low, which suggests more selective feeding habits of the associated fauna and, consequently, less competition for resources. Parreira de Castro *et al.* (2016) found that land use changes can alter the feeding behavior of macroinvertebrates, making them more generalist and increasing trophic niche overlap.

The investigation of biogeochemical compartments (*i.e.*, macrophytes, periphyton and sediment) of five different oligotrophic Cerrado ponds in four sampling seasons found that phosphorous concentrations are higher in periphyton than in macrophytes in these ponds (Alvim, 2017). This finding, along with the low C:N ratio observed in the present study, indicates that the periphyton of Henrique Pond is a nutrient-rich food source capable of maintaining the trophic web in this system. Based on the biogeochemical functioning of these ponds, we may also assume similar trophic interactions, indicating that the results reported here are relevant for similar environments. The elemental stoichiometry appears to be strongly related to the functional characteristics of the different primary producers in this environment.

Although we did not observe a seasonal effect on isotopic signatures, the community structure of the macrophyte-associated fauna studied (abundance of individuals) differed between seasons. In the dry season, macrophyte-associated fauna increased in abundance in both macrophytes *Cyperus* sp. and *Cabomba* sp. (Tab. S1). This result may be due to a lower water level, which decreased the size of the aquatic system and increased the concentration of organisms. In addition, the concentration of periphyton chlorophyll-*a* in the submerged macrophyte (Fig. 2a), suggests that higher biomass of attached algae favors the greater abundance of associated fauna. Previous studies have described the relationships between macrophyte architecture and the community structures of invertebrates (Vieira *et al.*, 2007; Thomaz and Cunha, 2010; Lucena-Moya and Duggan, 2011) and periphyton (Santos *et al.*, 2013; Souza *et al.*, 2015; Pettit *et al.*, 2016). The submerged macrophyte *Cabomba* sp. appears to indirectly increase basal resources by increasing habitat for periphyton (primary food source) and providing shelter for the associated fauna.

Our results showed that $\delta^{15}N$ values varied from 3.7 to 7.2‰ for secondary consumers (predators) and from 1.7 to 4.3‰ for primary consumers (collector-gatherers and scrapers). This variation may be related to differences in feeding strategies and biochemical/metabolic pathways (Vander Zanden and Rasmussen, 2001; Cremona *et al.*, 2010). Similarly, in a study that evaluated the ability of isotopic signatures to distinguish between eight *taxa* of tropical benthic invertebrates, $\delta^{15}N$ values varied from 6.9 to 13.3‰ for predators (five genera of Odonata and one genus of Hemiptera) and from 3.2 to 4.5‰ for shredders (two genera of Trichoptera) (Carvalho *et al.*, 2015). The results presented here are consistent with the idea that $\delta^{15}N$ values for consumers differ between functional trophic groups, despite the high degree of variability between *taxa* within the same functional trophic group.

CONCLUSIONS

In this small shallow oligotrophic pond in the Cerrado, the stable isotope ratios of food sources did not vary between seasons but did differ between aquatic macrophyte life forms (emergent *vs* submerged). Although seasonality is an important factor in the dynamics of aquatic ecosystems of the Cerrado, it was not a determining factor in the stable isotope ratios of the food sources analyzed. The difference in carbon isotopic signatures between emergent and submerged macrophytes may be related to the different carbon sources in the aquatic environment. The nitrogen stable isotope ratios showed that they are similar, leading to less ambiguity in interpretation of trophic transfer through the food web. Periphyton and emergent macrophyte are the primary resources in this environment, possibly because of the low C:N ratio of periphyton, indicating a higher-quality resource, and the greater biomass of emergent

macrophyte, indicating an increase in functional diversity and a more complex environment. Although the submerged macrophyte *Cabomba* sp. had a lower C:N ratio and higher abundance of associated fauna and algal biomass (chlorophyll-*a*) than the emergent macrophyte *Cyperus* sp., it contributed less to the trophic web. These data indicate different trophic roles for the primary producers in this ecosystem, with the emergent macrophyte representing a primary food resource, and the submerged macrophyte providing physical structure to serve as shelter for periphyton, protection from predation, and substrate for colonization. Despite sampling restricted to one pond, our data provide empirical evidence indicating that the structure and interactions of the trophic webs in shallow oligotrophic ponds are more complex than previously thought. In addition, our results point out an increase in functional diversity and a more complex environment. The results reveal important insights into trophic web structure of a peculiar environment of the Cerrado. This type of aquatic ecosystem has not yet been extensively investigated in terms of the interactions of its very oligotrophic conditions with high richness of different aquatic groups (algae, Protozoa, microcrustaceans, and aquatic insects). Our study provides a baseline for the understanding of patterns and processes in pristine tropical environments, which are being rapidly modified by anthropogenic processes.

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