TROPHIC STRUCTURE OF AQUATIC INVERTEBRATES IN AMAZON FOREST STREAMS USING STABLE ISOTOPES

ESTRUTURA TRÓFICA DE INVERTEBRADOS AQUÁTICOS EM RIACHOS FLORESTAIS DA AMAZÔNIA USANDO ISÓTOPOS ESTÁVEIS

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ABSTRACT

The approach of stable isotopes has allowed to investigate the trophic structure in food webs and to determine the food source of the nutrient cycling pathways in terrestrial and aquatic ecosystems. Thus, the main objective of the present study was to investigate the trophic structure of macroinvertebrates in Amazonian streams. The research was carried out in 15 streams of the Alter do Chão Environmental Protection Area and surroundings, in Santarém-Pa city. A total of 3,150 individuals were collected, being 1,459 in the less rainy season and 1,691 in the rainy season, distributed in total in 65 taxa. The isotopic analysis was performed with 37 most representative taxa (57%), totaling 2,895 (92.57%) of the total number of individuals collected. We compared the isotopic analysis between the seasons (dry and rainy). In the less rainy season there were four trophic functional groups (herbivore, detritivore, omnivore and carnivore), while for the rainy season there were three groups (herbivore, detritivore, omnivore), with emphasis on the occurrence of herbivores in the rainy season. Most of the taxa collected showed a different isotopic signature between the two seasonal periods, changing the functional group, indicating food plasticity. The trophic classification currently used, based on studies in temperate areas, showed divergence largely with the results in the present work, based on stable isotopes. Trophic groups were related to abiotic variables such as temperature, dissolved oxygen, these variables being important for structuring the macroinvertebrate community, perhaps more important than the food source.

Key words: ecosystems aquatic, isotopic analysis, trophic groups.

RESUMO

A abordagem dos isótopos estáveis tem permitido investigar a estrutura trófica em teias alimentares e determinar a fonte alimentar das vias de ciclagem de nutrientes em ecossistemas terrestres e aquáticos. Assim, o principal objetivo do presente estudo foi investigar a estrutura trófica de macroinvertebrados em riachos amazônicos. A pesquisa realizou-se em 15 riachos da Área de Proteção Ambiental

Alter do Chão e entorno, no município de Santarém-PA. Um total de 3. 150 indivíduos foram coletados, sendo 1.459 no período seco e 1.691 no período chuvoso. A análise isotópica foi realizada com 37 táxons (57%) mais representativos somando 2.895, (92.57%) do total de indivíduos coletados. Comparamos a análise isotópica entre os períodos sazonais (seco e chuvoso). No período seco ocorreram quatro grupos funcionais tróficos (herbívoro, detritívoro, onívoro e carnívoro), enquanto para o período chuvoso ocorreram três grupos (herbívoro, detritívoro, onívoro), com destaque para a ocorrência de herbívoros no período chuvoso. A maioria dos táxons coletados apresentaram assinatura isotópica diferente entre os dois períodos sazonais, mudando de grupo funcional indicando plasticidade alimentar. A classificação trófica utilizada atualmente baseada em estudos em áreas temperadas apresentou divergência em grande parte com os resultados no presente trabalho, baseados em isótopos estáveis. Os grupos tróficos foram relacionados às variáveis abióticas como temperatura, oxigênio dissolvido, sendo essas variáveis importantes para estruturação da comunidade de macroinvertebrados, mais importante talvez do que a fonte alimentar.
Palavras-chave: ecossistemas aquáticos, análise isotópica, grupos tróficos.

INTRODUCTION

In ecological studies of aquatic environments, two themes are frequently addressed: the sources of organic matter that support the food webs and the trophic position of organisms[1]. This would be directly linked to the availability of food and energy along the river, and intimately linked to the influence of riparian vegetation on watercourses[2]. Many of these studies have used functional food groups (GFA) due to the broadness of information they represent[3,4,5]. Classification into functional groups is based not only on the type of resource consumed but also on the morphological and behavioral mechanisms for acquiring these resources[6,7]. Being classified in the functional groups as fragmenters, scrapers, collectors-filters, collectors-catchers, and predators[5,8].

Such characteristics make it possible to monitor the trophic dynamics of any aquatic ecosystem, given the proportions between the trophic functional groups (e.g., level of environmental trophy)[8,9]. Thus, these organisms are essential for nutrient cycling, for understanding the predator-prey dynamics and functioning of aquatic systems[2].

Despite the practicality of using this tool, some studies suggest disadvantages in the use of functional food groups because, according to these studies, the species' diet can vary according to the availability of food[10], throughout the life of these organisms[11], or it can vary depending on the seasonal period[12], even if the morphology of the oral parts remains constant. Considerations have been made especially for tropical environments[13].

Studies have been conducted not only considering the oral parts but also the stomach contents[10,14] and its isotopic signature[15,16]. The isotope approach has allowed researchers to investigate the trophic structure and its connections in food webs[17]. The principle of these studies is that the isotopic composition of the food is reflected in the consumer[18], while the analysis of stomach content determines only the food that was ingested and not its assimilation effectively and, thus, difficult the determination of which and how much of the food sources available in the habitat are important in feeding the species[19].

The difference between the isotopic signal of animal tissue and the tissue of its diet is called isotopic fractionation. It results from the processes occurring during digestion, absorption, assimilation, and excretion of nutrients[20]. The isotopic composition of stable nitrogen ($\delta^{15}N$) can be used to estimate the trophic position of organisms in a community. This is possible because the preferential excretion of $\delta^{14}N$ atoms by the organisms[21] causes an isotopic fractionation along the food chains, increasing the $\delta^{15}N$ values as the trophic level rises.

Knowledge of trophic ecology, based on stable isotopes, became very important to conservation studies in general, as it determines the strength of the relationship of different species, their eating habits and seeks to understand how the flow of energy can be influenced by the presence of riparian forest in the headwaters of the streams. Considering its potential, the present study seeks to address the trophic dynamics of communities in these environments based on stable isotopes.

METHODOLOGY

The research was carried out in 15 streams of the APA Alter do Chão and surroundings (Table 1, Figure 1), in the municipality of Santarém-PA. The APA Alter do Chão was created in July/2002 (Law No. 17,771), comprising an area of 16,180 ha, with the southern limit coinciding with the border between the municipalities of Santarém and Belterra and the northern limit coinciding with the right margin of the Tapajós River.

The dominant climate in the region, as characteristic of tropical regions, is hot and humid classified as Am- monsoon following Köppen's classification[22]. The maximum temperatures regulate between 26 ° C and 31 ° C and the minimum vary between 21 ° C and 23 ° C. The average rainfall in the region varies around 2,000 mm, with an irregular distribution during the months. Rainy season takes place between December and June, the rainy season rainfall accounts for 80% of the annual rainfall. The dry period occurs between July and November[23].

The predominant vegetation in the region is of the dense ombrophilous forest type, characterized by its large trees with a height between 25 and 35 m. Other formations can also be found, such as open ombrophilous forest, seasonal forest, but in smaller proportions, as well as the presence of savanna patches that are characterized by an essentially herbaceous lower stratum of variable height and density, a shrub stratum of 60-80 cm in height and a tree stratum that can reach up to 10 meters in height[24].

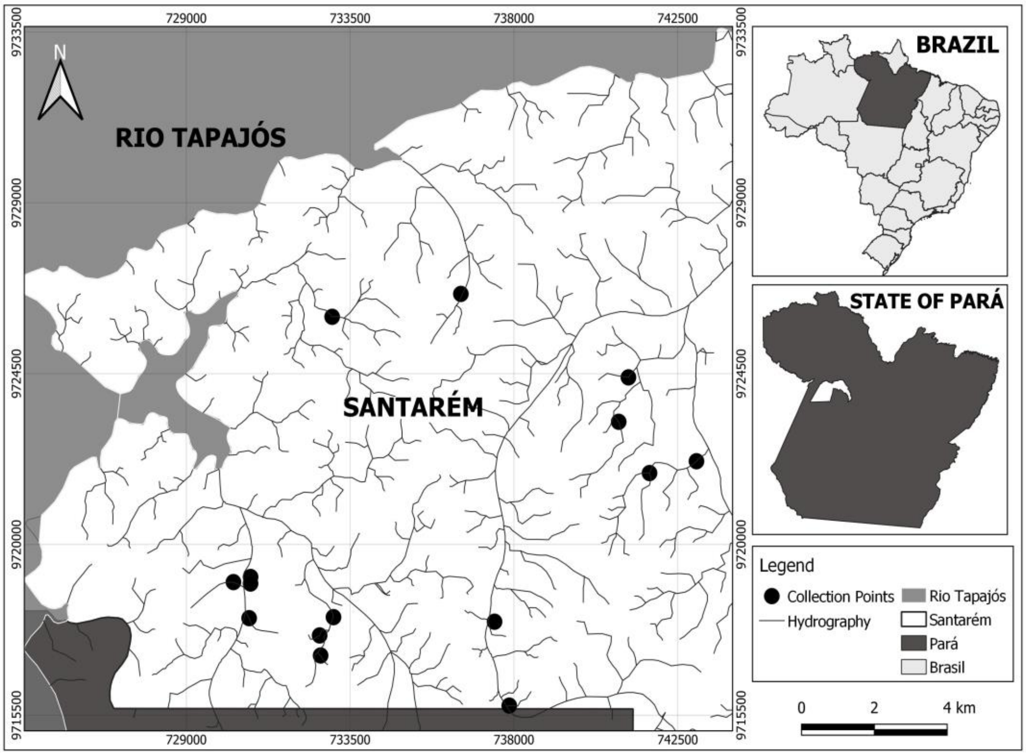
SAMPLING

Macroinvertebrate collections and measurements of abiotic variables were carried out in two seasonal periods: dry (October to November 2014) and rainy (March to June 2015). The sampling occurred in a 50 m transect of each stream, at three points equidistant ~ 17 m.

Table 1. Collection points and geographic coordinates.

Streams	Latitude (S) and Longitude (W)
Point 1	2°32'21.5"S 54°55'56.3"W
Point 2	2°32'27.1"S 54°55'28.6"W
Point 3	2°33'25.9"S 54°55'43.8"W
Point 4	2°33'11.5"S 54°54'27.1"W
Point 5	2°32'55.8"S 54°54'14.8"W
Point 6	2°33'28.7"S 54°54'26.2"W
Point 7	2°32'56.8"S 54°55'29.8"W
Point 8	2°28'18.5"S 54°52'22.0"W
Point 9	2°28'38.3"S 54°54'16.4"W
Point 10	2°30'51.8"S 54°49'34.0"W
Point 11	2°30'41.6"S 54°48'52.3"W
Point 12	2°30'07.9"S 54°50'01.5"W
Point 13	2°34'11.4"S 54°51'38.4"W
Point 14	2°32'59.5"S 54°51'51.6"W
Point 15	2°29'29.8"S 54°49'52.9"W

Figure 1. Location of the study streams in which the macroinvertebrates were sampled in western Pará, Brazil.



MACROINVERTEBRATES

The macroinvertebrates were collected using an entomological net (340 cm² and 1 mm² mesh). Each sample consisted of three subsamples that were screened in the field under plastic trays with the aid of tweezers. A minimum of 100 specimens was collected per stream. After screening, the individuos were conditioned in vials with absorbent paper, placed in thermal boxes for transport to the laboratory, where they were identified under a stereomicroscope, using regional taxonomic keys[25] and, stored (stream/taxon lots) in microtube for centrifugation, in a refrigerator at -35 ° C until isotopic analysis.

ABIOTICAL VARIABLES

Instant measurements of abiotic variables such as water temperature, hydrogen potential (pH), dissolved oxygen, electrical conductivity, flow, river width, and depth, were performed at three points equidistant ~ 17 m along the sampling transect. For this purpose, portable devices such as oximeter (Oakton, model: DO 300 Series), potentiometer / conductivimeter (Oakton, model: PC 10), flow meter (General Oceanics, model: 2030 series), and metric ruler were used.

STABLE ISOTOPE ANALYSIS

For isotopic analysis, the specimens, separated in glass flasks by taxa and by sampling period (sample), were dried in an oven at 60 °C. Then, these samples were transferred to polycarbonate flasks with two stainless steel balls (model 440) each, the vials were properly closed and immersed in liquid nitrogen for three minutes for freezing the samples and, later grinding in a cryogenic mill (Spex Sample Prep, model Geno / Grinder 2010) at -196ºC. After grinding, the samples were weighed (50-70µg) and packed in tin capsules for isotopic analysis.

The isotopic compositions were determined by the in-line combustion of the sample by a CF-IRMS in an elementary analyzer (Carlo Erba, model CHN-1110) coupled to a mass spectrometer (Delta Plus). This process consists of the release and purification of the gas to be investigated from other combustion by-products and separated by gas chromatography before being injected into the mass spectrometer.

Many ecological studies express the isotopic composition in terms of δ, which represents parts per thousand (‰) because the values of these ratios are numerically small and the results are expressed in δ notation[26].

With analysis error of the order of 0.2‰ and calculated by the formula of the sample isotope difference in relation to the standard. δ X=[(Sample/Rstandard)– 1]x 10, where: δ = relative enrichment of the sample in relation to the standard. R = isotopic ratio of sample and standard. All these analyzes were performed at the Cento de Isótopos Estáveis – CIE (Center for Stable Isotopes) at Universidade Estadual Paulista (UNESP). In order to estimate the trophic position, the equation was used: PTcons=(δ^1⁵Ncons - δ^{15} Nbasal)/2.3+2[27]. Where δ^{15}Ncons is the isotopic signal of the taxon from which the trophic position was estimated, δ^{15}Nbasal is the value of $\delta^{15}N$ of the trophic web base and 2.3‰ is the fractionation value between each trophic level. For this work we adopted the δ^{15}Nbasal, the isotopic value of the consumer taxon with the lowest isotopic value of $\delta^{15}N$, the Oligochaeta taxon. The specimen closest to the base of the food web should be chosen and, assuming it to be a primary consumer[28], the value 2 is added to the trophic position calculated for each taxon[27,29]. For the fractionation value of $\delta^{15}N$, the value of 2.3‰ was adopted, which is presented for the fractionation of aquatic specimens[29].

As determined the position of the trophic level by the isotopic signatures, we divided the macroinvertebrates into categories. The trophic level classification was adapted from Winemiller (2006)[30]: herbivores (T = 1.0 to 1.3), detritivores (T = 1.3 to 1.5), omnivores (T = 1.5 to 2.0) and primary carnivores (2.0 to 2.5). Considering the functional food groups described in Merrit et al. (2008)[6] and other related studies, shredders and scrapers were classified as herbivores, collector-gatherers and collector-filter were classified as detritivores, predators were classified as carnivores, and those taxa that feed on more than one food level were classified as omnivores.

DATA ANALYSIS

The physical and chemical parameters measured in the streams were tested using the t-test ($\alpha < 0.05$) to verify whether or not there are significant differences between the seasonal periods.

Functional groups were established by isotopic analysis and compared with the classification of Merritt and Cummins (1996)[8] and Merritt et al. (2008)[6].

To verify if there was a significant difference between the isotopic signatures reflected in the macroinvertebrates between the two seasonal periods, t-test for $\delta^{15}N$ was performed.

Differences in isotopic values for taxa collected between trophic levels were tested using Analysis of Variance (ANOVA), followed by Tukey's test. Anovas were also performed to compare the abundance of trophic groups in the streams sampled.

To verify the influence of abiotic variables on the distribution of trophic groups in the studied streams, a Canonical Correspondence Analysis (CCA) was performed.

Anovas and t-tests were performed using the Statistica program (STATSOFT v. 10.0) and the CCA was performed using the Paleontological Statistics software (Past v. 3.15)[31].

RESULTS

CHARACTERIZATION OF STREAMS

The abiotic variables measured in the field characterized the streams with an average temperature of 25.85°C (± 0.78°C), moderate concentration of dissolved oxygen (4.96 mg/L ± 1.11 mg/L), acidic waters (pH 4.1 ± 0.45), low electrical conductivity (16.26 µS/cm ± 3.72 µS/cm), small magnitude, width 119 cm (± 56.95 cm), depth 29.73 cm (± 18.28 cm), flow rate 0.019 cm^3/s (± 0.014 cm^3/s). Typical low-order and preserved Amazon streams. Between seasonal periods, only significant differences were observed in the values of temperature ($p=0.03$, $t= 2.36$

and gl=14) and pH (p<0.01, t= 3.19 and gl=14), being higher in the less rainy period; and in depth (p<0.01, t= -3.90 and gl=14) and width (p=0.04, t= -2.18 and gl=14), these being greater in the rainy season (Table 2).

Table 2. Comparison of the abiotic variables recorded between the dry and rainy seasons.

Abiotic variables	Season		Statistic		
	Dry	Rainy	t-test	p-value	df
pH	4.10 ± 0.45	3.78 ± 0.19	3.19	<0.01*	14
Water temperature (ºC)	25.85 ± 0.78	25.59 ± 0.76	2.36	0.03*	14
Electrical conductivity ($\mu S.cm^{-1}$)	16.26 ± 3.72	16.08 ± 3.65	0.41	0.68	14
Dissolved Oxygen ($mg.L^{-1}$)	4.96 ± 1.11	4.46 ± 1.19	1.58	0.13	14
Depth (m)	0.30 ± 0.18	0.32 ± 0.20	-3.90	<0.01*	14
Width (m)	1.19 ± 0.57	1.38 ± 0.70	-2.18	0.04*	14
Discharge ($m3.s^{-1}$)	0.019 ± 0.014	0.016 ± 0.011	0.89	0.38	14

The asterisks (*) indicate significant differences (p < 0.05) for analyses of paired t-test. Degrees of freedom (df).

TROPHIC STRUCTURE OF MACROINVERTEBRATES

A total of 3,150 individuals were collected in the 15 streams sampled, 1,459 of which were collected in the dry season and 1,691 in the rainy season, distributed in total in 65 taxa. The most representative taxa for the dry period were Rhagovelia (22.20%), Campylocia (7.26%), Baetodes (7.19%) and Macronema (5.34%). And for the rainy season were Rhagovelia (13.06%), Caenis (12.89%), Leptonema (11.88%), Brachymetra (6.80%), Campylocia (6.19%) and Baetodes (4, 19%).

The isotopic analysis was performed with 37 taxa (57%) most representative, totaling 2,880 (92.42%) of the total of individuals collected with 1,332 individuals from the less rainy season and 1,548 from the rainy season. These numbers guarantee the representativeness of the sampled fauna (dry period R2 = 0.91, P < 0.0001, rainy period R2 = 0.89, P < 0.0001).

The macroinvertebrate taxa collected and classified into functional food groups by Merritt et al. (2008)[6] were compared with trophic groups based on the isotopic signature of $\delta^{15}N$. There was confirmation of the classification by Merritt et al. (2008)[6] for only one taxon, Neotrichia (Trichoptera), as a herbivore. Eight taxa partially confirmed the classification and 28 did not confirm the classifications based on mouthparts and behavior, also showing an effect of the seasonal sampling period on the diet of these taxa (Table 3). A reservation is made for Chironomidae, which was identified in a family resolution, harboring a range of food resource use that determined its omnivorous and detritivorous feeding habits, partially confirming the literature. 27 taxa showed omnivorous feeding habits in at least one of the seasonal periods (Table 3). A significant difference was observed between the $\delta^{15}N$ isotopic signatures of macroinvertebrates between the two seasonal periods ($P < 0.0001$, $t = 10.73$ and gl= 35) (Figure 2). In the dry period, four groups of $\delta^{15}N$ isotopic signatures ($P < 0.0001$, $F = 58.74$ and gl=35) were observed, being herbivores, detritivores, omnivores and primary carnivores. While in the rainy season, three signatures were observed ($P < 0.0001$, $F = 94.67$ and gl= 35), being herbivores, detritivores and omnivores (Figure 3).

Figure 2. Representation of $\delta^{15}N$ isotopic signatures of macroinvertebrates in the dry and rainy seasons in the streams sampled

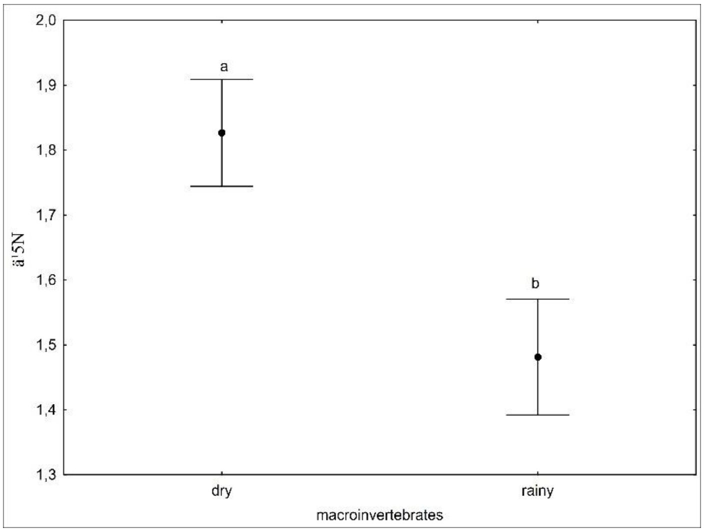
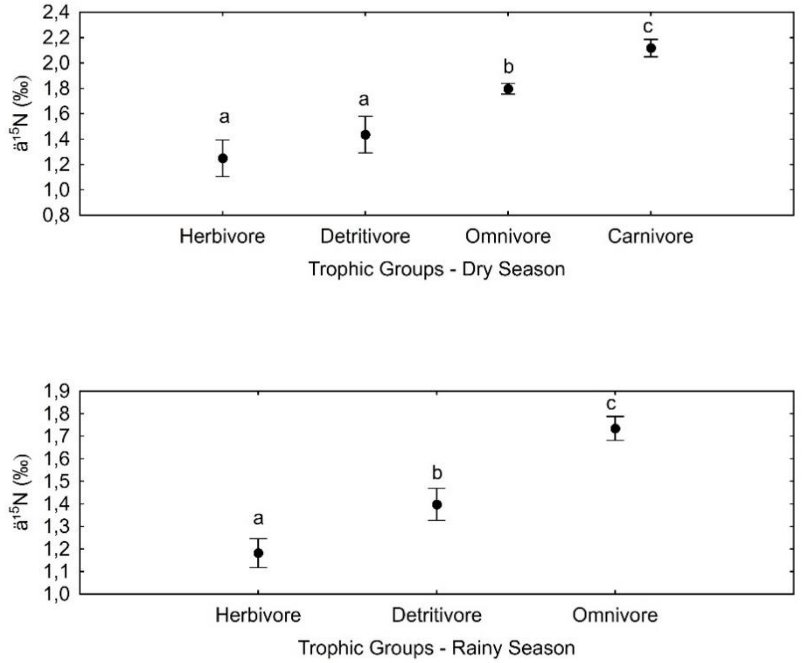


Figure 3. Representation of the categories of trophic groups (herbivores (H), detritivores (D), omnivores (O) and carnivores (C) based on the isotopic value of $\delta^{15}N$ in the dry and rainy seasons in the streams sampled.



In the dry period, two taxa were classified as herbivores, two taxa as detritivores, nine taxa as carnivores and 23 taxa as omnivores. For the rainy season, 11 taxa were classified as herbivores, nine taxa as detritivores and 16 taxa as omnivores (Table 3).

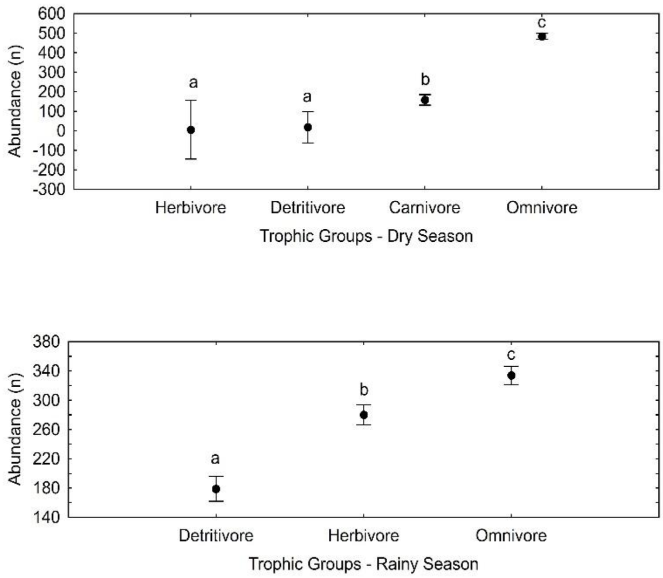
Table 3. Macroinvertebrate categorized into functional food groups according to Merritt *et al.*[6] (S=Scraper, F=Fragmenter, CC= Collector-Catcher, CF= Collector-Filter and P=Predator) and the isotopic categorization (C. isotopic) for the dry and rainy periods in the streams sampled.

Family	Genus	GFA[6]	Dry isotopic C.	Rainy isotopic C.	Comparison with Merritt *et al.*[6]
Veliidae	*Rhagovelia*	P	Omnivore	Omnivore	Does not confirm
Veliidae	*Stridulivelia*	P	Carnivore	Detritivore	Partially
Gerridae	*Brachymetra*	P	Omnivore	Detritivore	Does not confirm
Gerridae	*Cylindrostethus*	P	Herbivore	Herbivore	Does not confirm
Gerridae	*Limnogonus*	P	Detritivore	Herbivore	Does not confirm
Notonectidae	*Buenoa*	P	Omnivore	Detritivore	Does not confirm
Naucoridae	*Limnocoris*	P	Omnivore	Omnivore	Does not confirm
Naucoridae	*Ambrysus*	P	Carnivore	Omnivore	Partially
Perlidae	*Anacroneuria*	P	Carnivore	Omnivore	Partially
Perlidae	*Macrogynoplax*	P	Carnivore	Omnivore	Partially
Euthyplocidae	*Campylocia*	CC	Omnivore	Omnivore	Does not confirm
Baetidae	*Baetodes*	S	Omnivore	Detritivore	Does not confirm
Caenidae	*Caenis*	CC	Omnivore	Herbivore	Does not confirm
Hydroptilidae	*Neotrichia*	S	Herbivore	Herbivore	Confirm
Hydropsychidae	*Smicridea*	CF	Omnivore	Herbivore	Does not confirm
Hydropsychidae	*Macronema*	CF	Omnivore	Herbivore	Does not confirm
Hydropsychidae	*Leptonema*	CF	Omnivore	Herbivore	Does not confirm
Calamoceratidae	*Phylloicus*	F	Detritivore	Herbivore	Partially
Odontoceridae	*Marília*	P	Omnivore	Herbivore	Does not confirm
Gyrinidae	*Gyrinus*	P	Carnivore	Detritivore	Partially
Hydrophilidae	*Tropisternus*	CC	Omnivore	Herbivore	Does not confirm
Noteridae	*Suphis*	P	Omnivore	Herbivore	Does not confirm
Gomphidae	*Phyllogomphoides*	P	Omnivore	Omnivore	Does not confirm
Gomphidae	*Zonophora*	P	Carnivore	Omnivore	Partially e
Gomphidae	*Cyanogomphus*	P	Omnivore	Detritivore	Does not confirm
Libellulidae	*Peristhemis*	P	Omnivore	Omnivore	Does not confirm
Megapodagrionidae	*Heteragrion*	P	Carnivore	Omnivore	Partially
Cordulidae	*Aeschnosoma*	P	Omnivore	Omnivore	Does not confirm
Gomphidae	*Praeviagomphus*	P	Omnivore	Omnivore	Does not confirm
Libellulidae	*Tauriphila*	P	Omnivore	Omnivore	Does not confirm
Chironomidae	—	CC	Omnivore	Detritivore	Partially
Tipulidae	*Hexatoma*	P	Omnivore	Omnivore	Does not confirm
Corydalidae	*Corydalus*	P	Omnivore	Detritivore	Does not confirm
Crambidae	*Parapoynx*	F	Omnivore	Detritivore	Does not confirm
Paleomonidae	*Palaemon*	F	Carnivore	Omnivore	Does not confirm
Paleomonidae	*Macrobrachium*	F	Carnivore	Omnivore	Does not confirm

TROPHIC STRUCTURE OF STREAMS

In the less rainy period, a higher abundance of omnivores (average 72.81% ± 2.26) was recorded, followed by carnivores (average 23.79% ± 1.00) and, in a smaller proportion of detritivores (average 2.63% ± 0.29) and herbivores (mean 0.75% ± 0.16) ($F_{(3,\ 1324)}$ =179.97, p= <0.01) (Figure 4), contrary to the study hypothesis, which herbivores would be the dominant group. In the rainy season, although there was still a higher proportion of omnivores (average 42.18% ± 4.56), herbivores stand out, which in the less rainy season represented less than 1% of the analyzed fauna as the second most abundant group (mean 35.35% ± 3.98). Detritivores were also more representative in this seasonal period (mean 22.45% ± 5.25), ($F_{(2, 1580)}$ = 102.46, p= <0.01) (Figure 4).

Figure 4. Representation of the categorization of taxa into trophic groups based on the isotopic value of $\delta^{15}N$ for the streams sampled in the dry and rainy seasons.



ABIOTIC CHARACTERISTICS RELATED TO TROPHIC GROUPS

In the dry period, the main abiotic variables influencing the trophic structure were dissolved oxygen, water temperature, stream depth and pH. The CCA explained 94.58% of the data variability, with 69.11% explained in axis 1. The greater abundance of omnivores and detritivores was related to dissolved oxygen concentration and depth (positive correlations) and pH (negative correlation), while the abundance of carnivores and herbivores was related to streams with higher temperatures (negative correlation) (Figure 5, Table 4). In the rainy season, the abiotic variables explained 100% of the variability, with 71.07% explained in the first axis. The herbivore group was influenced by temperature, width (negative correlations) and the omnivore group was influenced by dissolved oxygen (negative correlation). And for the trophic group of detritivores, there was no relationship with the measured variables (Figure 5, Table 4).

Figure 5. Canonical ordering diagram between the abiotic variables: Temperature (Temp), Dissolved Oxygen (DO), Electrical Conductivity (Cond), Discharge (Disch) and the trophic groups of aquatic macroinvertebrates Herbivores (Herb), Detritivores (Det), Omnivores (Omn) and Carnivores (Carn) collected in the dry and rainy season in streams (black dots).

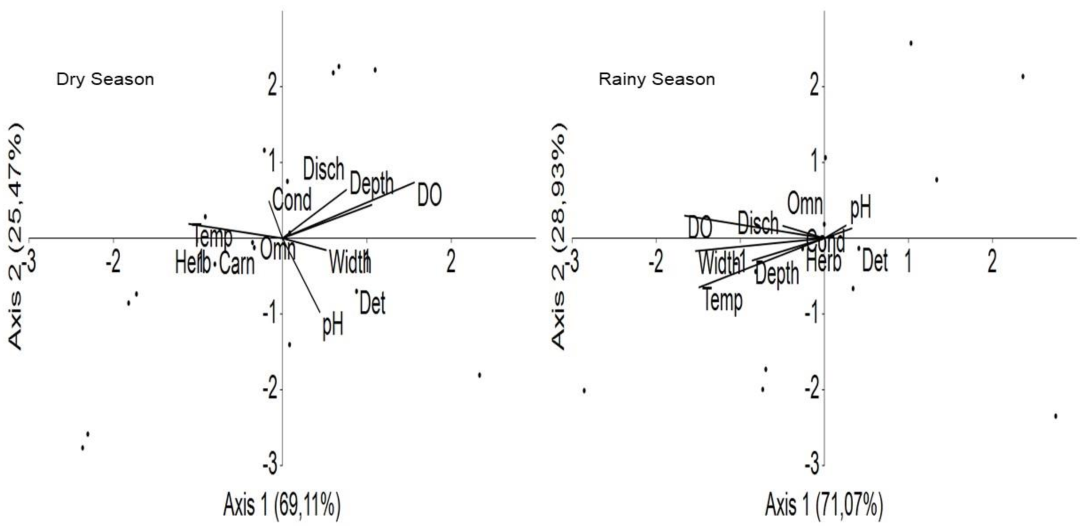


Table 4. Result of Canonical Correspondence Analysis expressing the relationship of environmental variables with trophic groups in the dry and rainy season in the streams sampled.

Abiotic variables	Dry Season		Rainy Season	
	Axis 1	Axis 2	Axis 1	Axis 2
pH	0,14	**-0,32**	0,10	0,04
Water temperature (ºC)	**-0,36**	0,06	**-0,49**	-0,21
Electrical conductivity (µS cm^{-1})	-0,05	0,16	0,08	0,05
Dissolved Oxygen (mg L^{-1})	**0,52**	0,24	**-0,55**	0,09
Depth (m)	**0,35**	0,14	-0,28	-0,09
Width (m)	0,17	-0,05	**-0,51**	-0,05
Discharge (m^3 s^{-1})	0,25	0,21	-0,16	0,05

DISCUSSION

Stable isotopes have been used to establish a general outline of food webs, considering that the composition of consumer tissues reflects their diet. Thus, it allows verifying changes in the animals' eating and foraging habits[32].

Two key issues can be discussed with our results. The first demonstrates that part of the groups categorized into functional food groups, based on the studies by[6] and correlates, is not confirmed by isotopic signatures. This means that even preferentially ingesting a certain food item, as with Odonata, typically classified as predators[6,33], there may be a greater nutritional contribution to their body structure from resources other than prey. As a result, these taxa were mostly classified as omnivores[12]. Of the 37 taxa evaluated, only Neotrichia (Trichoptera) confirmed the findings in the literature and eight taxa partially confirmed it, given the seasonality effect.

Thus, the second question is related to seasonality. Temporal variations modify the characteristics of streams and exert a strong influence on the invertebrate community[13], which may interfere with the availability or ease of these organisms in obtaining food resources[34,35].

The predominance of generalist or omnivorous species in tropical environments reflects spatial and temporal fluctuations in the relative abundance of food items in these environments[36,37]. Perlidae, for example, is classified by the literature as a typical predator[38], a fact corroborated by the analyzes of the dry period of our study. However, Perlidae used more than one resource in the rainy season, being classified as omnivore.

In the rainy season, the volume of water in the streams increases, and this can be a risk for predators that venture out in search of prey[39], decreasing this specific food item[40], with this, insects may prefer to make use of any available resource, rather than having a food preference[36,10,35,13].

These two questions indicate that macroinvertebrates, especially insects, would be more related to habitat selection, as demonstrated in other studies[41], than to the availability of certain (specific) food resources, given that of the 37 taxa analyzed, 27 showed omnivores habits in at least one of the seasonal periods sampled and only 11 taxa showed the same resource use regardless of the seasonal period sampled, 10 of these taxa classified as omnivores.

In the rainy season, there was a greater number of herbivores, which may be related to the availability of leaves that fall into the streams via fall or leaching from the banks in this period[42]. Herbivores in the present study represent shredders and scrapers together, since the resource used in food would be the same (vegetable debris or coarse particulate organic matter), despite differences in the form of food acquisition (chewing or scraping).

The role of macroinvertebrates in the transformation of coarse particulate organic matter into fine particulate organic matter is described by many authors as one of the steps in the decomposition process of detritus[43,44,45]. In general, in this process, the importance of shredders is considered[43], without considering the potential of scrapers.

In tropical environments, in general, there is a low abundance of shredders[46,47], so that the transformation of coarse particulate organic matter into fine particulate organic matter is believed to be carried out mainly by microorganisms[48] or by the action of macroconsumers such as fish and crustaceans[49]. If we also consider the role of scrapers, we may have other conclusions, since the isotopic signatures do not differentiate them.

Our results indicate that trophic categories for aquatic macroinvertebrates are not fixed, since changes in levels may occur according to the seasonal periods sampled and that external morphology does not necessarily represent energy or nutrient dynamics. Thus, a greater number of regional studies must be developed for the correct establishment of trophic relationships for each taxon.

Changes in physical and chemical variables can cause changes in the trophic structure of biological systems, food chains and energy flows[50], pH, dissolved oxygen, turbidity and electrical conductivity of water affect the quality of the environment and, consequently, aquatic organisms[51,52]. Temperature, dissolved oxygen, depth, width were the main variables influencing the trophic structure of macroinvertebrates. Temperature exerts a direct and indirect influence on macroinvertebrate populations, as it interferes with the solubility of gases in water, especially oxygen, which also influence the community[53]. In streams with warmer waters, a greater number of herbivores was observed. The distribution of aquatic insects is the result of the interaction between the physical conditions that characterize the habitat and food availability[8,54], where the current speed, temperature and dissolved oxygen are the most important abiotic variables[53,55].

CONCLUSIONS

Considering the results obtained in the present study, the organization of macroinvertebrates seems to be more related to the characteristics of the environment, especially temperature and dissolved oxygen, than to food availability.

The classic trophic classification based on the morphology and behavior of macroinvertebrate taxa is different from that observed with the isotopic signature in the present study. Taxons routinely classified as shredders and predators consumed more than their basic food item (leaves and prey, respectively), presenting themselves mostly as omnivores taxa. Also, several taxa changed from omnivores to herbivores in the rainy season in the region, demonstrating the effect of seasonality, which is probably related to resource availability. These results indicate that the classification of these organisms should not only be defined by their external morphology, but by what they are actually consuming and assimilating.

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