

# Evaluation of the feeding habits of *Macrobrachium brasiliense* (Heller, 1862) in the Curral de Arame stream (Dourados/Mato Grosso Do Sul, Brazil)

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**ABSTRACT** - Macrobrachium brasiliense is a cosmopolitan shrimp species found in the channel of large rivers, streams, lakes, igapós and upland igarapés and can present diversity in the use of food resources. This study aimed to evaluate the feeding habits according to sex, molt stage, seasonal and spatial variation, and size class. Organisms were collected every three months from May 2011 to March 2012 at three sampling sites in the Curral de Arame Stream, including the upstream, middle, and downstream sections. Animals were sampled with 50x50cm sieves (1 mm mesh size) on the margin, and with "D" dip nets 40 cm wide (500 µm mesh size) at the bottom. Of each animal we examined the sex, molt stage, carapace length, and stomach content. Data were analyzed through the frequency of occurrence and compared by a chi-square test. Four-hundred-thirty shrimps were collected, of which 26.05% male and 73.95% female; of the total males, 69.64% showed content in the stomach, and females, 72.96%. Among food items stood out: Non-identified Organic Matter (90%), Insects (89.68%), Sand (87.10%), Algae (49.03%), Oligochaeta (29.68%), Fungi (26.77%) and Plant fragment (14.52%). No significant difference was detected in the diet between males and females, but there was a seasonal difference related to the molt stage, and differences along the space and per size classes, and it was concluded that the species has omnivorous habits.

Key words: Crustacea, food habits, frequency of occurrence, Palaemonidae, stomach content

#### Introduction

The genus *Macrobrachium* Spence Bate, 1868 has more than 243 species registered in tropical and subtropical regions worldwide (De Grave and Frasen, 2011). *Macrobrachium brasiliense* (Heller, 1862) is a shrimp with wide geographical distribution, occurring in coastal basins of northern South America, from Venezuela to Brazil, and basins of the rivers

Orinoco, Amazonas, São Francisco, Paraguay and Paraná (Coelho and Ramos-Porto, 1985; Melo, 2003). Recently, the occurrence of this species was expand in several areas of its known distribution, and reported for the first time from the states of Amapá, Tocantins, and Mato Grosso do Sul (Pileggi *et al.*, 2013). It can be found in the main channel of large rivers, streams, lakes, igapós and upland igarapés (García-Dávila *et al.*, 2000).

Studies on *M. brasiliense* include faunistic, taxonomic, population, reproductive and larval morphology surveys (Coelho and Ramos-Porto, 1985; García-Dávila *et al.*, 2000; Vásquez *et al.*, 2000; Mantelatto and Barbosa, 2005; Pereira and Chacur, 2009; Pantaleão *et al.*, 2011), with little information on its feeding dynamics.

The maintenance of a population among other things depends on the food availability, the encounter between predator and prey and an appropriate energy transfer between trophic levels. In this way, the stomach content analysis is common for assessing the importance of the role of food in biological communities (Hyslop, 1980). Information from these studies is applied to several fields of research, such as trophic ecology, and probable uses in optimizing the farming of crustaceans.

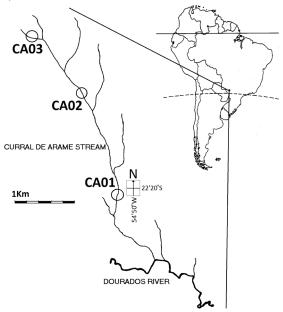
The stomach content analysis could be providing important information on the food habit and its trophic position in a community. Besides that indicate the ecological relationships between organisms and interpretation of general dynamics of the environment (Aguirre-León and Yáñes-Arancibia, 1984), and also indicate their patterns of distribution, migration and ecdysis (Mclaughlin and Hebard, 1961).

Furthermore, the feeding habits may be directly related to the life-cycle stage and the increase in size, and hardness of the prey ingested can also be associated to the increment of the body size of the predator (Collins, 1999; Oh *et al.*, 2001; Roux *et al.*, 2009).

Studies involving the feeding of freshwater shrimps in their natural environment are uncommon due to the difficulty in determining and quantifying the stomach content, but some authors have significantly contributed to the knowledge of the subject (Chong and Sasekumar, 1981; Barros and Valenti, 1997; Albertoni *et al.*, 2003; Jimoh *et al.*, 2011). There is no information available on the natural diet of *Macrobrachium brasiliense* in Brazilian streams. The present study evaluated the feeding habits of *M. brasiliense* in the Curral de Arame Stream according to size, sex, molt stage, the seasonal and spatial variation.

### MATERIAL AND METHODS

For the analysis of the diet of *M. brasiliense* samplings were carried out in May, September, and December 2011, and in March 2012 at three sampling sites in the Curral de Arame Stream, one near the headwaters (CA03 - 22°13'12.6"S / 54°55'05.9"W) with relatively preserved riparian forest, one in the middle stretch of the stream (CA02 - 22°18'02.3"S / 54°51'17"W) an open area with degraded riparian forest and sugar cane plantation in the surroundings, and one downstream (CA01 - 22°20'14.7"S / 54°50'45.7"W) an area with relatively degraded riparian forest and soybean and corn plantation in the surroundings (Fig. 1).



**Figure 1.** Curral de Arame stream showing de sampling sites of *M. brasiliense*.

Animals were collected with 50x50cm sieves (1 mm mesh size) on the margins, and "D"dipnets 40 cm wide (500  $\mu m$  mesh size) for samplings at the bottom with litter, sand or rocks. The capture effort was 3 people for 30 minutes at each sampling site. Animals collected were immediately anesthetized in ice coolers and fixed with 70% alcohol to interrupt the digestion.

In laboratory, each animal was measured: carapace length (CL), distance between the postorbital groove to the dorsal posterior margin of the cephalothorax (0.1 mm) individuals were grouped into six size

classes and sexed according to the presence of internal appendix in the endopod of the second pair of pleopods. It was also observed the condition of molt, by distinguishing the individuals into intermolt with completely rigid exoskeleton (C), newly molted with very soft exoskeleton (A), and in postmolt with the phase of calcification of the exoskeleton (B) not observed D and E stage, according Passano (1960) description for caridean.

Stomachs were removed by dorsal dissection of the cephalothorax and the content was examined by the numerical analysis method and frequency of occurrence, according to Hyslop (1980) and Zavala-Camin (1996). It has been assigned the fullness of the stomach according to Chong and Sasekumar (1981) and Josileen (2011) for all the stomachs, based on the amount of content in each one: 0 indicates an empty stomach, 1 a stomach that is 0.1-25% full, 2 one that is 25.1-50% full, 3 one that is 50.1-75% full and 4 one that is 75.1-100% full. The content was spread on a microscopy slide and analyzed. It was determined the percentage of stomachs of a given food item in relation to the total number of stomachs with any amount of food, ignoring totally empty stomachs, following the formula FO% = ni/N.100, where, (ni) number of stomachs with the item i and (N) total number of stomachs. Items found were identified to the lowest identifiable taxonomic

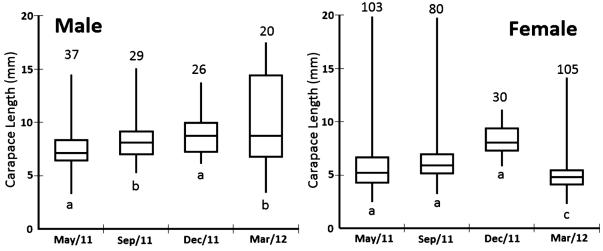
level (Merrit and Cummins, 1996; Bicudo and Menezes, 2006; Mugnai *et al.*, 2010). Items that owing their high level of digestion could not be identified, were considered Non-identified Organic Matter (NIOM) (Branco *et al.*, 2002).

To compare differences in carapace length between sex, sites and months were used Kruskal-Wallis test complemented with the Student-Newman-Keuls (p < 0.05) and to compare stomach contents between size, sex, molt stage, spatial distribution and seasonal changes were used the chi-square test (p < 0.05).

## RESULTS AND DISCUSSION

We collected 430 shrimps of the species *M. brasiliense* in the Curral de Arame Stream, of these 112 (26.05%) were males and 318 (73.95%) were females, with carapace length ranging from 3.25 to 17.45 mm and 2.3 to 19.85 mm, respectively.

The size comparison between the sampling sites and sex demonstrated differences H = 90.3056 (p <0.001) between sexes but not between the sampling sites, otherwise when comparing between sex and month of sampling a significant differences was observed H = 144.6169 (p < 0.001) between the sexes in the months of September and March and in the sampling sites for both males and females (Fig. 2).



**Figure 2.** Box-plot (median, quartile and range) of the carapace length between male and female of *M. brasiliense* by sample month. The same letter below box-plot indicate that no has statistic difference between the box-plots, and the number above box representing total specimens caught in month.

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Size class (mm)	Total (n)	%	Females (n)	Males (n)	Empty Stomachs (n)
2.30 + 5.23	154	35.81	148	6	38
5.23 ⊦ 8.15	175	40.70	120	55	48
8.15 + 11.08	65	15.12	30	35	25
11.08 + 14.00	22	5.12	14	8	4
14.00 + 16.93	11	2.56	4	7	4
16.93 + 19.85	3	0.70	2	1	1
Total	430		318	112	120

**Table 1.** Frequency distribution by size classes. n = number of individuals; % = percentage of the class in the total sample.

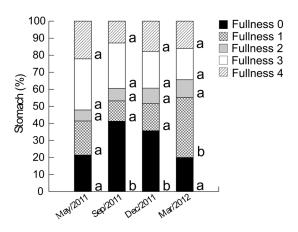
The size class that had the highest percentage was the class 5.23 + 8.15 mm length, representing 40.70% of the total, followed by the class 2.30 + 5.23 mm representing 35.81% indicating that the population consists mainly of juveniles individuals (Table 1).

Among males, 34 (30.36%) have presented completely empty stomachs and 78 (69.64%) presented stomach with content, even in small amount. For females, 86 (27.04%) have presented completely empty stomachs and 232 (72.96%) had content in the stomach.

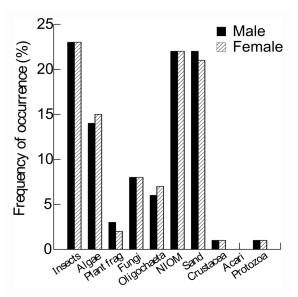
Of the 430 individuals analyzed, 28% had an empty stomach (stomach repletion 0), 22% with stomach repletion 1, 8% with stomach repletion 2, 25% with stomach repletion 3, and 17% completely filled with stomach repletion 4.

No significant difference (p > 0.05;  $X^2$  = 9.2836; df 4) was found when analyzed the degree of stomach repletion by sex or spatial variation, as well as for the stomach repletion 2, 3 and 4 in the months of sampling, but a significant difference (p < 0.05) was detected for the fullness of the stomach at degrees 0 and 1 (Fig. 3).

The more representative food items registered (Fig. 4) were not significantly different between males and females (p > 0.05;  $X^2 = 5.6853$ ; df 9) evidencing a very similar pattern of food items, that can be because both sexes occupy the same habitat during most life cycle. Collins (1999) and Carnevali *et al.* (2012) observed similar results for other palaemonid shrimps, without significant



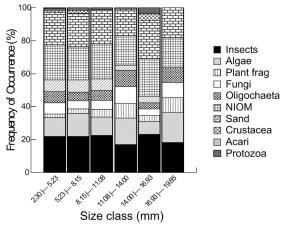
**Figure 3**. Fullness of the stomach of *M. brasiliense* by month of sampling. The letter beside column indicates the comparison within each degree of repletion between the months, the same horizontal letter indicate that no has statistic difference between the fullness of the stomach and the sazonal variation.



**Figure 4.** Percentage of main food items in the diet of males and females of *M. brasiliense*.

difference in diet composition between sexes. Once there was no difference between the diet of males and females, analyses were performed with data of grouped sexes.

There was a significant difference for the items consumed between size classes, in the items Fungi (p < 0.05,  $X^2 = 41.509$ ; df 5); Algae  $(p < 0.05; X^2 = 58.617; df 5); Plant fragment$  $(p < 0.05; X^2 = 70.90; df 5)$  and Oligochaeta  $(p < 0.05; X^2 = 38.699; df 5), of these, Algae,$ Plant and Fungi have increased their frequency of occurrence according to the increase of the carapace length, and the opposite being observed for the item Oligochaeta. Some authors such as Oh et al. (2001) and Spanjersberg et al. (2006) in studies developed on the feeding of different shrimp species have mentioned that the size and hardness of the preys could be directly related to the increase in size of individuals that capture (Fig. 5).



**Figure 5.** Frequency of occurrence (%) of the items found in the stomach contents of *M. brasiliense* for size class (mm).

The qualitative analysis of the stomach content *M. brasiliense* has revealed a great array of items adding up a total of 34 (Table 2). The most important items by the frequency of occurrence (FO%) were Insects with 94.52%, followed by NIOM (Non-identified Organic Matter) with 90.00%, and Sand with 87.10%. This latter item is widely discussed in literature because it is not known if such intake is intentional or accidental. In this way, Branco and Verani (1997) have considered that the sand is accidentally ingested along with prey, but also stressed that it can be intentionally consumed if considered that the sediment is

colonized by several microorganisms, such as Bacteria, Fungi and Protozoa, being the last two found in this study with FO 26.77% and 4.52% respectively. According to Pru *et al.* (1994, *apud* Spanjersberg *et al.*, 2006, p. 103) the sand besides being an item accidentally ingested, it also assists the process of crushing organic particles ingested, and can be used for the formation of a new exoskeleton (Choy, 1986). The item Oligochaeta had frequency of occurrence of 29.68% and Plant fragment had 14.52%.

Among Insects that could be identified due to the presence of specific parts, the family Chironomidae (Diptera) occurred with the highest frequency, occurring in almost nearly half of the stomachs containing food, similarly to Collins (1999), Carnevali et al. (2012) for other species. The orders Ephemeroptera and Trichoptera have had a frequency of occurrence of 18.06% and 9.35% respectively, representing important items in the diet of M. brasiliense. The other orders, such as Coleoptera, Plecoptera, Odonata, Megaloptera and Hymenoptera, have presented a lower frequency of occurrence. Aquatic insects are items constantly found in stomach content analysis in crustacean and fish, and are important in the maintenance of the food chain because they occupy almost all levels (Johnson and Triplehorn, 2004).

Among Crustacea found in the stomach content, stands out the order Cladocera represented by the genus Daphnia sp. (FO% = 3.22). Cladocerans have been registered by several other authors (Collins, 1999; Albertoni et al., 2003; Jimoh et al., 2011; Carnevali et al., 2012). It was also verified locomotor appendages, part of the rostrum and antenna of M. brasiliense (FO% = 1.93). Probably the ingestion of these parts is a mechanism to obtain calcium carbonate to form a new exoskeleton, however Carvalho and Collins (2011) have stated that the cannibalism is common given the competition for food, space and sexual partner. Araujo and Valenti (2005) argued that species of the genus Macrobrachium are competitive, with possible cannibalism and social stress, which was proved by Souza

Vauplius

**Table 2**. Frequency of Occurrence for the food items found in the stomach of *M. brasiliense*.

Food Items	FO % May/2011	FO % Sept./2011	FO % Dec./2011	FO % Mar./2012	FO % Total
Phylum Sarcomastigophora	2.73	4.69	2.78	7.00	4.52
Phylum Heterokontophyta	8.18	3.13	2.78	3.00	4.84
Class Bacillariophyceae (Diatom)	62.73	54.69	25.00	39.00	49.03
Phylum Chrysophyta	0.91	0.00	2.78	0.00	0.64
Phylum Rodhophyta	0.91	0.00	0.00	0.00	0.32
Phylum Chlorophyta	3.64	0.00	5.56	0.00	1.93
Genus Spirogyra	4.55	1.56	5.56	3.00	3.55
Class Chlorophyceae	4.55	0.00	0.00	0.00	1.6
Plant fragment	15.45	3.13	8.33	23.00	14.52
Fungi	57.27	9.38	5.56	12.00	26.77
Phylum Annelida					
Class Oligochaeta - Tubificida	17.27	48.44	25.00	33.33	29.68
Class Hirudina	0.91	0.00	0.00	0.00	0.32
Insects (fragments)	84.45	96.88	100.0	86.00	89.68
Phylum Arthropoda					
Subphylum Cheliceriformes					
Order Acari	0.91	1.56	2.78	3.00	1.93
Subphylum Crustacea					
Order Cladocera					
Genus <i>Daphnia</i>	0.91	6.25	2.78	4.00	3.22
Order Decapoda					
M. brasiliense	1.82	1.56	2.78	2.00	1.93
ClassCopepoda	0.00	0.00	0.00	1.00	0.32
Subphylum Hexapoda					
Class Insecta					
Order Coleoptera	2.73	0.00	0.00	5.00	2.58
Order Ephemeroptera	15.45	17.19	25.00	19.00	18.06
Order Plecoptera	1.82	0.00	8.33	0.00	1.61
Order Trichoptera	12.73	7.81	11.11	6.00	9.35
Order Odonata	3.64	0.00	1.82	0.00	1.93
Order Megaloptera	0.00	0.00	2.78	0.00	0.32
Order Lepdoptera	0.00	0.00	0.00	1.00	0.32
Order Hymenoptera					
Family Formicidae	0.00	3.13	0.00	0.00	0.64
Order Diptera	11.82	3.13	5.56	7.00	7.74
Family Chironomidae	35.45	53.13	33.33	32.00	37.74
Subfamily Tanypodinae	0.91	1.56	0.00	0.00	0.64
Subfamily Orthocladiinae	0.91	0.00	0.00	1.00	0.64
Subfamily Chironominae	1.82	0.00	0.00	0.00	0.64
Genus Polipedium	1.82	0.00	0.00	0.00	0.64
Genus Stenochironomus	5.45	3.13	0.00	6.00	4.52
Insects (fragments)	84.45	96.88	100.0	86.00	89.68
Sand	87.27	95.31	97.22	78.00	87.1

and Singer-Brugiolo (2001) who observed cannibalistic behavior among *M. rosenbergii* shrimps in laboratory.

Algae were also present in the stomach, representing 55.81% of the total sample. It was recorded numerous genera from different phyla. The most frequent were Diatoms (FO% = 49.03), followed by Algae of the family Bacillariophyceae (FO% = 4.52), *Spirogyra* sp. (FO% = 3.55), also genera Spiniferomonas sp., Nitzchia sp., Hydrodictyon sp., Selenastrum sp., Chlorococcum sp., Peniun sp. and Closteriumsp., both with frequency of occurrence of 0.48%. Collins (1999), Albertoni et al. (2003), Spanjersberg et al. (2006), Jimoh et al. (2011), and Carnevaliet al. (2012) also verified Algae in their studies, sometimes with the greatest importance, this item has a seasonal and size class variation significantly, but not having a significant spatial variation (Table 3).

Seasonal variations in the diet of M. brasiliense (Table 3) were observed, but not all food items found have presented significant difference (p > 0.05), only the items Fungi (p < 0.0001;  $X^2 = 84.072$ ; df 3); Algae (p = 0.002;  $X^2 = 14.795$ ; df 3); Oligochaeta (p = 0.0006;  $X^2 = 17.222$ ; df 3) and Plant fragment (p = 0.0004,  $X^2 = 17.963$ ; df 3), analyzed by the chi-square test have shown significant difference in the frequency of occurrence during the study, and the items Algae and Fungi have had a higher frequency of occurrence in May 2011, with a reduction in September and December 2011, and a lower value in March 2012.

The items Plant fragment and Oligochaeta presented an inverse pattern, the first was more frequent in May 2011 and March 2012, and Oligochaeta was more frequent in September 2011 and March 2012. Only the items Fungi (p = 0.0396;  $X^2 = 6.459$ ; df 2) and Oligochaeta (p = 0.0028;  $X^2 = 11.734$ ; df 2) have obtained a significant difference in the spatial variation, with the first being the most frequent at the site 2, and less frequent at the site 3 and less frequent at the site 2.

Moreover, the feeding of these shrimps has varied according to their molt stage (Table 3), the food intake was not significantly different between stages, except for the items Fungi (p < 0.0001;  $X^2 = 32.71$ ; df 2) and Plant fragment (p = 0.0178;  $X^2 = 8.056$ ; df 2). For the molt stage A, the items with higher FO were Insects remains (86.67), NIOM (80) and sand (73.33), without record of Fungi, Crustacea, Acari, and Protozoa; the molt stage B had Insects remains/sand (90.79), NIOM (89.47), Algae (51.32) and Oligochaeta (26.67) as the most frequent items, and Crustacean, Acari, Protozoa, Plant fragments and Fungi as the least frequent; and in the molt stage C, the most frequent items were Insects remains (95.89), NIOM (90.87), sand (86.76), Algae (58.45) and Fungi (32.42), and with lower frequencies the same items cited for the stage B.

Studies on *Macrobrachium* species have indicated that, as observed in the present study, this genus has a diet made up by several food items. Albertoni *et al.* (2003) and Jimoh

**Table 3.** Chi-square test ( $X^2$ ) applied to the components of the diet of *M. brasiliense* for grouped sex, seasonality, spatial variation, class size and molting stage variation. (\*): P < 0.05. (-) (Data insufficient to calculate chi-square test).

Food Items	Sex Variation(X <sup>2</sup> )	Seasonal Variation (X <sup>2</sup> )	Spatial Variation(X <sup>2</sup> )	Size Class Variation(X <sup>2</sup> )	Molt Stage Variation (X <sup>2</sup> )
Insecta	0.036	1.253	0.077	1.292	0.468
Algae	0.056	14.795*	2.576	58.617*	3.468
Plantfragment	1.234	17.963*	0.307	70.9*	8.056*
Fungi	1.325	84.072*	6.459*	41.509*	32.71*
Oligochaeta	0.513	17.222*	11.734*	38.699*	1.542
NIOM	0.001	1.153	0.077	1.997	0.806
Sand	0.602	2.578	0.299	1.787	1.999
Crustacea		2.59			
Acari					
Protozoa	0.622			22.396*	

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et al. (2011) found similar results related to the wide composition of the diet of *M. acanthurus*, *M. lamarrei* and *M. vollenhovenii*, respectively. Studies on other genera also evidenced that shrimps are usually omnivorous and feed on a wide variety of food (Chong and Sasekumar, 1981; Collins, 1999; Branco and Junior, 2001; Spanjersberg *et al.*, 2006; Carnevali *et al.*, 2012).

In summary, the results of the qualitative analysis of the stomach content of *M. brasiliense*, using the frequency of occurrence, have shown that the species has omnivorous habit, being registered diverse items, such as Insects remains particularly *Chironomidade* larvae, Diatoms, Oligochaeta, Plant fragment, filamentous Fungi, Sand, or Non-identified Organic Matter. Furthermore, it was observed a spatial and seasonal variation in the feeding of this species, as well as different size classes feed on different prey.

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