



HABITAT CHOICE BEHAVIOR IN *Macrobrachium brasiliense* (HELLER, 1862) (DECAPODA, PALAEMONIDAE) UNDER LABORATORY CONDITIONS

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Abstract: The main objective of this study was to analyze the choice behavior of adult individuals of *Macrobrachium brasiliense* (Decapoda, Palaemonidae) with regard to different substrates and microhabitats in two distinct experiments. The first experiment offered the prawns three samples of substrates with different granulometries: fine sand, coarse sand, and gravel. The second experiment offered three types of microhabitats: aquatic macrophytes, wood fragments, and rocks. In general, the prawns chose the fine substrate, to a significant extent (ANOVA, $p < 0.01$). In the microhabitat experiment, the prawns selected the wood fragments (ANOVA, $p < 0.01$). In general, these results show that substrates with fine sand and microhabitats composed of fragments of wood might provide direct benefits for *M. brasiliense*, such as protection against predators and food availability through biofilm formation.

Keywords: fine sediment; microhabitat; prawn behavior.

INTRODUCTION

Decapod crustaceans are part of diverse benthic fauna with a wide geographic distribution across marine, freshwater and semi-terrestrial environments. They are highly relevant for their contributions to the complexity, size, and functioning of the ecosystems in which they appear (Hendrickx 1995, Pereira & Chacur 2010). The Palaemonidae family is comprised of small to medium-sized prawns that inhabit marine, estuarine, and freshwater waters in tropical,

subtropical, and temperate regions (Holthius 1952, Silva & Vieira 2012). The genus *Macrobrachium* contains approximately 340 described species which are distributed around the world (De Grave & Fransen 2011).

In Brazil, there are 19 species (Mantelatto *et al.* 2016), including *Macrobrachium brasiliense* (Heller, 1862) (Decapoda, Palaemonidae), which has continental distribution throughout the country (Holthius 1952, Coelho & Ramos-Porto 1984, Melo 2003). Recently, new ecological surveys have reported for the first time the occurrence of

M. brasiliense in the states of Amapá, Tocantins, and Mato Grosso do Sul (Pileggi *et al.* 2013). Given this wide geographic distribution, it is important to highlight that most of the studies which have been conducted to date involve species distribution and taxonomic reviews (Kensley & Walker 1982, Coelho & Ramos-Porto 1984, García-Dávila & Magalhães 2003, Pileggi & Mantelatto 2012).

Studies indicate that substrate choice may be directly related to the ease of finding refuge, since excavation is easier in substrate comprised of smaller grain sizes and vice versa (Dall *et al.* 1990). To understand the general behavior of a species, it is necessary to investigate the behavior of males and females in order to analyze the different choices these animals make, which in this case includes verifying whether higher rates of burial in different types of substrate are seen for each sex (Santos *et al.* 2013). This requires behavioral experiments involving substrates and microhabitats that these animals can find in their natural environments, such as gravel, coarse and fine sand, rocks, wood, and aquatic macrophytes, which usually comprise the beds of rivers and streams (Kikuchi & Uieda 2005).

In the present study, we investigated the behavior of habitat choice by adult females and males of *M. brasiliense* using different substrates (fine sand, coarse sand, and gravel) and microhabitats (rocks, wood fragments, and aquatic macrophytes) under laboratory conditions. We hypothesized that adult females and males of *M. brasiliense* would choose the same type of substrate and microhabitats.

MATERIAL AND METHODS

Sampling and maintenance of animals

Thirty specimens were collected on the banks of the Água Limpa River (19°08'23.5"S, 48°22'44.7"W), near the city of Uberlândia, state of Minas Gerais, Brazil, using sieves (3.0 mm mesh), which were dragged along the submerged substrate. Afterwards, the prawns were individually packed in plastic bags with local water and transported within a maximum of thirty minutes to the Laboratory of Aquatic Ecosystems Ecology (LEEA)

at the Institute of Biology of the Federal University of Uberlândia to perform the experiments.

In the laboratory, each specimen was individually placed in a 600 mL vessel containing 300 mL of water from the collection site and 300 mL of filtered water for prior acclimatization for twelve hours (from 19:30 h to 07:30 h.) with controlled luminosity, oxygenation and temperature. This method was used to avoid thermal shock and physical/chemical stress from sudden exchange of water. The three aquariums used in both experiments were 44 cm long, 20 cm wide and 27 cm high, with a total capacity of 23 liters. The substrate selection experiment (A) was performed first, followed by the microhabitat selection experiment (B). It is important to note that all the aquariums and materials used were sanitized and sterilized between experiments and between replications; the aquariums, microhabitats, and substrates were cleaned in treated tap water to remove microorganisms that may have developed during previous experiments. In addition, the substrates were sterilized in a 60°C kiln for 48 hours.

Experimental design and procedures

In rivers and streams, a range of substrates and microhabitats can be inhabited by many benthic macroinvertebrates (Cotta *et al.* 2006). Higher-granulometry substrates are generally found in places where there are currents (*i.e.*, lotic environments), while lower-granulometry substrates can be found in lentic environments where waters are still. There are several types of microhabitats, such as rocks, wood fragments, submerged roots, macrophytes, and other vegetation (Sanseverino & Nessimian 2001, Cotta *et al.* 2006). Consequently, an organism's choice of specific substrates and microhabitats can be directly related to its life history, including settlement between different stages, such as protection and feeding, for example.

In each experiment, three options for substrates and microhabitats were offered to 15 prawns in order to evaluate the choices made by adult male and female *M. brasiliense*. The substrates were classified using different granulometry scales as proposed by Wentworth (Wentworth 1922). In experiment A (substrate choice), the three options were: 1) gravel:

sediment measuring > 2.0 mm; 2) coarse sand: 1.0 – 0.5 mm; 3) fine sand: 0.25 – 0.125 mm. These three options were arranged separately in plastic trays measuring 16.5 cm long, 13.0 cm wide, and 4.0 cm high, and placed into each of the three aquariums used in both experiment A and B (Figure 1). In experiment B (microhabitat choice) the options provided were: 1) rock; 2) wood fragment; 3) aquatic macrophyte *Elodea* sp. (Alismatales, Hydrocharitaceae); these microhabitats were arranged on trays containing a fine sand substrate, which the animals chose in experiment A (Figure 1).

In each experiment, three specimens of *M. brasiliense* were placed individually in each of the aquariums, and five replicas were performed for each experiment; in this way, two different groups of 15 individual prawns completed experiments A and B.

After the acclimation period, the collected specimens were introduced individually into each aquarium using an immersion net. In each experiment, the animal remained in the aquarium habitat for three consecutive days, during which its behavior was observed through the focal animal method (adapted from Martin & Bateson 1993). Using this method, the animal's presence in each substrate and microhabitat was recorded every 2 minutes over a period of 20 minutes, totaling 11 records for each interval. These observations were made during four periods throughout the day, using methodology adapted from Freire *et al.* (2011): early morning (08:30 h – 08:50 h, 09:30 h – 09:50 h), late morning (10:30 h – 10:50 h, 11:30 h – 23:50 h), early afternoon (14:30 h – 14:50 h, 15:30 h – 15:50 h), and late afternoon (16:30 h – 16:50 h, 17:30 h – 17:50 h). When the last

observation period ended at 17:50 h, the prawns were individually transferred to 1000 mL vessels, where they were fed to avoid any influence on the experiments; they remained in the vessels until the beginning of the following observation (08:30 h the following morning).

Data analyses

At the end of each experiment, the prawns were sacrificed (using thermal shock), identified (according to Melo 2003), measured (carapace length [CL] in mm), and sexed. The aquariums where the prawns remained during the experiments were numbered, and after the end of each replica, the individuals were sexed using a stereomicroscope, by the presence of secondary sexual characteristics in the second pair of male pleopods (Mantelatto & Barbosa 2005). We analyzed the animals' choice of each substrate or microhabitat as well as excavation behavior using Analyses of Variance (ANOVA) followed by the Tukey test ($p > 0.05$). The data analyses were performed by the software Statistica 7.1 (StatSoft 2005).

RESULTS

Experiment A - Substrate choice

Of the 15 prawns were used in this experiment, 9 were males and 6 were females. The mean carapace length (CL) for the males was 14.3 ± 2.6 mm, and for the females was 13.6 ± 2.0 mm. The lowest and the highest CL values of males were 9.9 and 15 mm, respectively, while for the females these CL values ranged from 8.9 mm to 13.6mm.

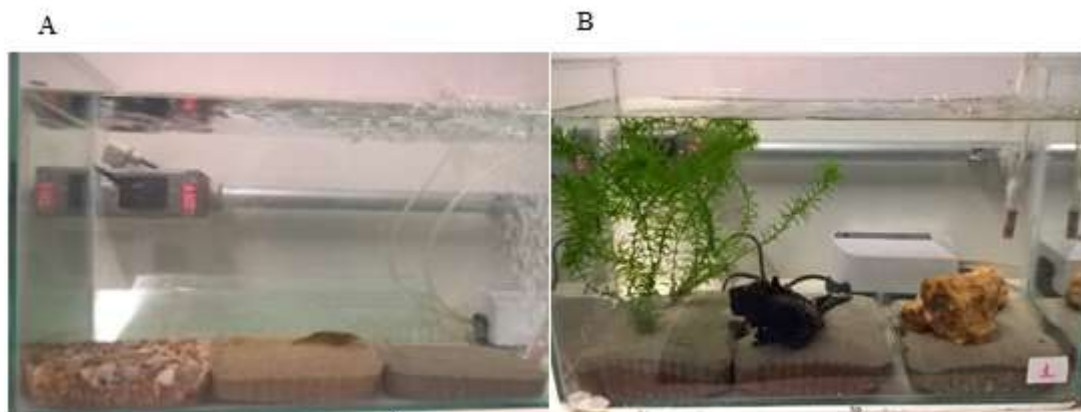


Figure 1. Aquarium overview with substrates (A) and microhabitat (B), showing experimental setup.

In general, the individuals of *M. brasiliense* were seen to choose the substrate composed of fine sand in this study ($p < 0.01$) (Figure 2). However, when this choice was analyzed according to sex, the results were significant for the males ($p < 0.01$) but not significant for the females ($p > 0.05$). The females were more frequently observed in the coarse sand substrate (40.6%), but also occurred in the substrates composed of gravel (29.4%) and fine sand (29.9%) (Figure 3). In the substrate choice (A), both males and females excavated, to a significant extent ($p < 0.01$).

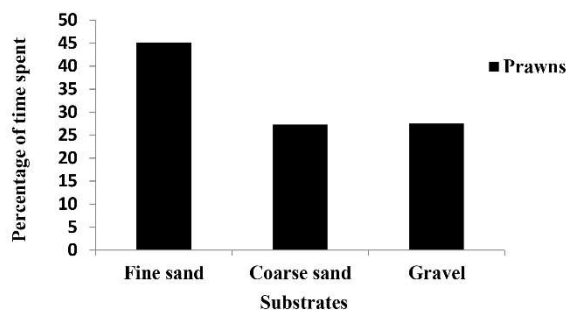


Figure 2. Substrate choice among adults of both sexes of *Macrobrachium brasiliense* (Decapoda, Palaemonidae).

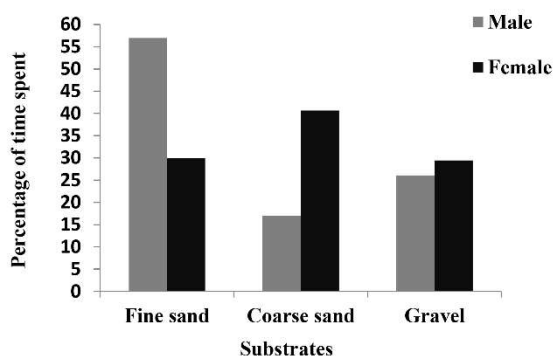


Figure 3. Substrate choice by sex of adult specimens of *Macrobrachium brasiliense* (Decapoda, Palaemonidae).

Experiment B – Microhabitat choice

The second group of 15 prawns used in experiment B was comprised of 8 males and 7 females. The mean CL in the males was 13.1 ± 2.4 mm and in the females was 10.6 ± 0.73 mm. Among the males in this group, the lowest and the highest CL values were 10 and 16.7 mm,

respectively, and in the females this number ranged from 9.9 mm to 11.9 mm.

In general, most of the animals (both male and female; 65%) chose the microhabitat composed of wood fragments, to a significant extent ($p < 0.01$) (Figure 4). When the sexes were analyzed separately, the wood fragment habitat was chosen to a significant extent for both sexes ($p < 0.01$). However, a significant choice was also seen for the rock microhabitat (39.7%) (ANOVA, $p < 0.01$) only in the males of *M. brasiliense* (39.7%) (Figure 5). In the choice of microhabitats (B), both males and females chose to excavate in the microhabitat with wood, to a significant extent ($p < 0.01$).

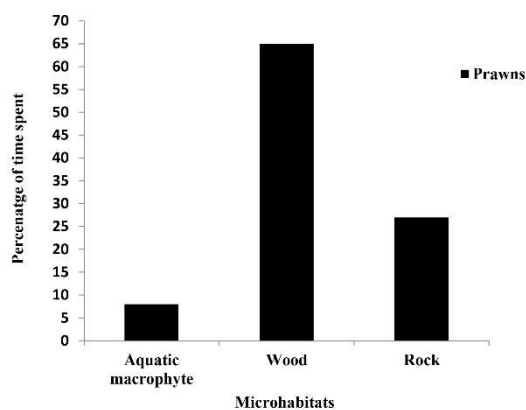


Figure 4. Microhabitat choice for adults of both sexes of *Macrobrachium brasiliense* (Decapoda, Palaemonidae).

DISCUSSION

The laboratory experiments showed that *M. brasiliense* chose fine substrate. Our hypothesis is that this choice is directly related to protection and energy economy; in other words, the species is better able to protect itself from potential predators in finer substrate because excavation is easier and requires less energy expenditure (Dall *et al.* 1990). Santos *et al.* (2013) observed the same substrate choice in the marine shrimp *Litopenaeus vannamei* (Decapoda, Penaeidae), which was seen to prefer fine and very fine sand. Even though these animals live in a different environment and consequently differ physiologically from the species studied in this present study, both results confirm the statement by Dall *et al.* (1990) that substrates with particles between 1.0 mm and 62.0 μm provide food availability and facilitate escape

from predators. In a study on the caridean prawn *Crangon septemspinosa* (Decapoda, Crangonidae), Oullette *et al.* (2003) also found that this species preferred finer substrates, and connected this choice with survival, since *C. septemspinosa* lives much of its life cycle on this type of substrate. This finding agrees with our observation that *M. brasiliense* individuals spent most of their time buried in this type of substrate. This behavior may be considered a protection and escape strategy which enables *M. brasiliense* to survive predation in its natural habitat.

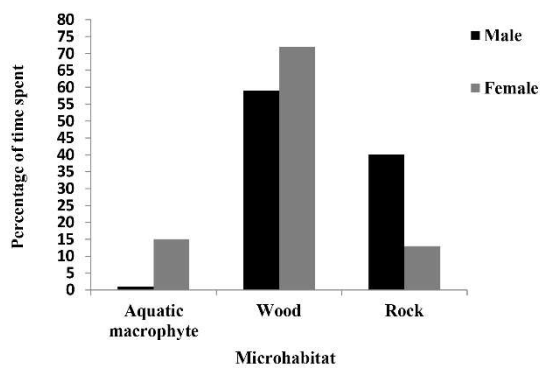


Figure 5. Microhabitat choice by sex of adult specimens of *Macrobrachium brasiliense* (Decapoda, Palaemonidae).

Macrobrachium prawns are known to have individual aggression (Valenti *et al.* 1993), and when they occur in high densities, larger males occupy the substrates where sheltering is easier, while smaller males occupy the other substrates where they are generally more exposed (Ling 1969, Peeble 1979a,b, Cohen *et al.* 1981, Valenti *et al.* 1993). In our study, we showed that adult individuals of *M. brasiliense*, from the smallest to the largest individuals, choose the same substrate. In our experiment, each prawn's choice was observed individually, which prevented aggressive behavior in this species. In the study conducted by M. S. Melo (unpublished data) on the same species, he evaluated individuals from three different size classes (juvenile and adult individuals) with regard to their choice of different substrates (fine sand, coarse sand, light pebbles, stem fragments, and *Elodea* sp.). This author introduced five animals of different sizes into the same aquarium and observed that the adult prawns preferred *Elodea* sp., while the smaller individuals preferred the substrate composed of light pebbles. Such results

highlight the importance on prior knowledge of species behavior in order to carry out experimental work.

Our results for microhabitat choice show that males and females of *M. brasiliense* choose wood fragments, with males also selecting rock microhabitats. In its natural habitat, *M. brasiliense* occurs in rocks, roots, submerged vegetation, or litter in watercourses (Mantelatto *et al.* 2016). Lima & Oshiro (2002) evaluated two species of *Potimirim* (Decapoda, Atyidae), which selected different microhabitats along the river where they were sampled. Individuals of *P. glabra* were found in rocky environments, while individuals of *P. potimirim* were found in environments without rocks but with abundant decomposing vegetation. The results of Lima & Oshiro (2002) show that prawns can select different microhabitats. Although Mantelatto *et al.* (2016) suggest that *M. brasiliense* may use wood fragments more frequently as a refuge in its natural habitat, the present study is the first to investigate this choice in the laboratory. Our results on microhabitat choice may support future research in experimental studies with *M. brasiliense*, considering that wood fragments and rocks can often be found in its natural habitat.

Many factors can influence microhabitat choice, including indirect factors such as the development of biofilms. These may change some microhabitat characteristics, especially in rocks and wood fragments (O'toole *et al.* 2000). Biofilms generally are composed of bacteria and fungi that can provide essential biomolecules for the development of crustaceans (Thompson *et al.* 2002, Ballester *et al.* 2003). Rocks and wood fragments are easily found at the site where the *M. brasiliense* specimens were sampled, and are considered important retainers of organic matter (Tank & Winterbourn 1995). In the present study we did not evaluate the presence or growth of biofilm. Even though the materials used were sanitized and sterilized between experiments and between replications, the microhabitats remained inside the aquariums for 72 hours, which may have contributed to the formation of biofilms within them, especially on the rocks and wood fragments since these have a larger contact surface. The formation of such biofilms may consequently have influenced microhabitat choice by *M.*

brasiliense, and could represent an alternative food source for this species and an important survival factor. In addition to biofilm and feeding opportunities, the wood fragments also contain small cracks which serve as hiding places.

In summary, we demonstrated experimentally that male and female *M. brasiliense* choose substrate composed of fine sand and a microhabitat composed of wood fragments, corroborating our initial hypothesis. The reasons why these prawns choose this microhabitat and sediment should still be evaluated in future studies. We hypothesize that the choice of the substrate composed of fine sand and the microhabitat composed of wood fragments is directly related to the benefits that these provide with regards to protection and possibly food, through the hiding places they provide and the formation of biofilms, respectively. By demonstrating how *M. brasiliense* adults choose their habitat, our study made an important contribution to understanding the occurrence and distribution of *M. brasiliense* in its natural environment, as well as its interactions with this environment.

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