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Behavioral profile of *Macrobrachium rosenbergii* in mixed and monosex culture submitted to shelters of different colors

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ABSTRACT. Current research analyzed the behavioral activities of *Macrobrachium rosenbergii* and its preference for colored shelters in male monosex, female monosex and mixed culture. Ten shrimps m^{-2} were maintained in eight 250-L aquaria. Three artificial shelters, colored red, black and orange, were placed in each aquarium. Four aquaria were maintained in light/dark photoperiod respectively between 6h00 am and 6h00 pm and between 6h00 pm and 6h00 am, whereas the other four aquaria were submitted to an inverted photoperiod. The animals were observed for 30 days by Focal Animal Method for 15 minutes, with instantaneous recording every 60 seconds, at six different instances within the light and dark phases. Preference for black shelters occurred in male monosex and mixed cultures, whereas red and orange shelters were the preference of female monosex. *M. rosenbergii* kept in the shelter mostly during the light phase in male monosex and mixed populations. Results suggest that black, red and orange shelters may improve the animals' well-being in the culture since aggressive encounters would decrease, especially during the light phase.

Keywords: Malaysian prawn, applied ethology, Caridea, shelter preference.

Perfil comportamental de *Macrobrachium rosenbergii* submetido a abrigos de diferentes colorações em cultivos mistos e monosexo

RESUMO. Esta pesquisa analisou as atividades comportamentais de *Macrobrachium rosenbergii* e a preferência pela coloração de abrigos em cultivos monosexo macho, monosexo femea e mistos. Dez camarões m^{-2} foram mantidos em oito aquários de 250 L. Três abrigos artificiais (vermelho, preto e laranja) foram disponibilizados em cada aquário. Quatro aquários foram submetidos à fase clara de 6h00 – 18h00 e à fase escura das 18h00 – 6h00, e quatro submetidos a fotoperíodo invertido. Os animais foram observados usando Método Animal Focal por 15 minutos, com registro instantâneo a cada 60 segundos, em seis diferentes momentos na fase clara e na fase escura, por 30 dias de observação. Nos cultivos monosexo macho e misto ocorreu uma forte preferência por abrigos de cor preta. No cultivo monosexo fêmea, os camarões preferiram abrigos vermelhos e laranja. *M. rosenbergii* entoca-se mais frequentemente na fase clara, em populacões monosexo macho e mistas. Estes resultados sugerem que a colocação de abrigos nas cores preto, vermelho e laranja, poderão contribuir com a melhoria do bem-estar dos animais em cultivo, uma vez que podem reduzir a ocorrência de encontros agressivos, principalmente na fase clara do dia.

Palavras-chave: gigante da Malásia, etologia aplicada, Caridae, preferência de abrigo.

Introduction

Aquatic animal farming has become an increasingly important source of food due to exponential population growth. Species of the genus *Macrobrachium* are among the most cultivated freshwater prawns worldwide (NEW et al., 2010). In fact, *Macrobrachium rosenbergii* has been the most cultivated species on a commercial scale with a production representing an excellent alternative to agribusiness, due to its biological characteristics such as growth, omnivory, high fertility and resistance to disease. Further, it is widely accepted on the market in all countries because of its soft textured meat and low environmental impact (GUPTA et al., 2007; NEW et al., 2010).

Nevertheless, the *Macrobrachium* species show agonistic and cannibalistic types of behavior, which are enhanced in ponds (COHEN et al., 1981), with consequent low survival rate and productivity (BARKI et al., 1991; KARPLUS et al., 1992), and heterogeneity in the size of the sexually mature population, especially males (SHORT, 2004). Its territorial behavior (KARPLUS et al., 1992) increases aggressive behavior

among members of the same species, with the loss of the queliped, its main attack and defense organs (MARIAPPAN; BALASUNDARAM, 1999).

In natural environment, the crustaceans live under fissures, burrows, stones or gastropod shells that protect specimens during agonistic encounters in intra and interspecific interactions (GARVEY et al., 1994). According to Balasundaram et al. (2004), since cannibalistic and aggressiveness levels may vary according to size and species, the burrowing behavior must be understood to characterize the dominance pattern in the acquisition and defense of the shelter.

Shelters are relevant resources to increase access to partners, minimize aggressive encounters and predation, and protect submissive animals when faced with dominant ones (ENGLUND; KRUPA, 2000; ALCOCK, 2011). In monocultures, the use of shelters in nurseries has become an important tool that reduces growth variation inside a freshwater prawn population (TIDWELL et al., 1998), increases survival rate and full production and decreases cannibalism (BALASUNDARAM et al., 2004).

Several species of crustaceans have the capacity of color vision (MARSHALL; OBERWINKLER, 1996), which provides a clear evolutionary advantage in multi-colored habitats. Thus, the perception of colors is a useful resource (shelter, food, female, etc.) for the animals to recognize the details of the environment (LUCHIARI et al., 2012). Some studies have demonstrated the influence of color in the biological processes of crustaceans and other aquatic animals (MARIAPPAN; BALASUNDARAM, 1999; VOLPATO et al., 2004; LUCHIARI; PIRHONEN, 2008; LUCHIARI et al., 2012).

According to Penn (1984), the prawns' diurnal activity varies among the species, ranging between those that completely cease their activities (such as the burrowing prawn) and those that fail to reduce their activities. Several studies have been conducted on the efficiency of shelters for the prawns *M. rosenbergii* (MURTHY et al., 2012), *Macrobrachium nobilli* (MARIAPPAN; BALASUNDARAM, 1999) and *Macrobrachium australiense* (LAMMERS et al., 2009).

Current paper evaluates the occupation frequency of shelters for *M. rosenbergii* in male monosex, female monosex and mixed cultures, and analyzes the behavioral activities of the prawn in environments that offer different color shelters during light and dark phases.

Material and methods

The study was developed in the Laboratory of Shrimp Behavioral Studies of the Physiology Department, Biosciences Center, where *M. rosenbergii* adult prawns (25.12 ± 7.49 g) were maintained and marked with blue and pink silicone rings on the optical peduncles (PONTES; ARRUDA, 2005).

Eight 250-L aquaria (50 x 50 x 100 cm) were used in the experiment within a closed system of uninterrupted filtration and water reuse (Canister filter), with artificial lighting, constant aeration, and fine sand as substrate. Four animals were introduced into each aquarium, with a stocking density of 10 prawns m^{-2}, following producers' usage pattern (five to ten prawns m^{-2}). Three artificial cylindrical polyethylene red, black and orange colored shelters, 8 cm wide and diameter 5 cm, were added to the aquaria. The colors of the shelters were selected according to Balasundaram et al. (2004) and Yasharian et al. (2005). The quality of the water (pH, temperature, dissolved oxygen, ammonia and salinity) was daily monitored.

The experimental units were distributed in two rooms with artificial lighting system, where the photoperiod was controlled by a timer, with a 12:12h light/dark cycle. In one of the rooms, the light phase comprised the 6h00 am - 6h00 pm period and the dark phase comprised the 6h00 pm - 6h00 am period (natural photoperiod); in the other room, the photoperiod was inverted, allowing the monitoring of four units in the light phase and four units in the dark phase, simultaneously. For direct lighting of the aquaria, 32W white fluorescent lamps during the light phase and 15W red incandescent lamps for the dark phase were used, due to the lack of reaction of the shrimps to this kind of luminosity (PONTES; ARRUDA, 2005).

Experimental delineation was in randomized blocks, with four repetitions, to record behavioral activities, occupation and preference of the shelter according to color. Selection of different colored shelters by the animals was recorded in three treatments (male monosex, female monosex and mixed cultures), in both light and dark phases of the 24-hour photoperiod, by analyzing the shelter's color preference according to occupation frequency. In the case of monosex treatments, the presence of egg-bearing females was the parameter used to separate the animals according to the sex. In each experimental condition, the animals were observed during 10 uninterrupted days and observations started after 10 days of the prawn's adaptation to the physical conditions of the aquaria (PONTES et al., 2006) and after the establishment of the social hierarchy (FERO et al., 2007). Prawns were fed by feeders (transparent acrylic bowls) with 10% of their biomass day^{-1}, with shrimp ration containing 35% of crude protein, offered twice a day at random to avoid the interference of feed offer on behavioral activities.

Observations were performed by the Focal Animal Method (MARTIN; BATESON, 2007) for each treatment, during 30 days. The animals in each aquarium were observed for 15 minutes, with instantaneous recording at every 60 seconds, at six different times, during light and dark phases, totalizing one hour of register each time. Observation times in both phases were distributed as follows: 1st observation time (immediately after the change of phase 0L and 0D); 2nd observation time (2 hours after change of phase – 2C and 2D); 3rd observation time (4 hours later – 4C and 4D); 4th observation time (6 hours later – 6C and 6D); 5th observation (8 hours later – 8C and 8D); 6th observation time (10 hours later – 10C and 10D).

Since observations of the aquarium lasted 15 minutes, the registers of the behaviors occurred simultaneously in both light and dark phases - an observer would observe the animals in one aquarium during the light phase while another would record the behavior in another aquarium during the dark phase and so forth, until the 8 aquaria were observed (four during the light phase and four during the dark phase). Ninety-six prawns were observed (32 animals in mixed culture, 32 in male monosex and 32 in female monosex culture) totalizing 160 hours of observation in 30 days and 2,880 registers of behavioral activities.

Abiotic parameters were verified at 11h00 am, on a daily basis. Water salinity was kept at 0 ppt (portable refractometer Instrutherm RTS-101ATC); pH at 7.6 ± 0.6 (pH-meter Instrutherm PH-710); ammonia 0.17 ± 0.09 mg L^{-1}; temperature at 26.9ºC ± 1 and dissolved oxygen over 5 mg L^{-1} (oximeter Instrutherm MO-900). Water quality was kept constant throughout the assay, at optimal levels for farming of the species (NEW et al., 2010). Survival of the animals in different treatments was also reported (BAUTISTA-TERUEL et al., 2003).

Statistical programs SIGMASTAT 3.1 (2004) (Systat, Erkrath, Germany) and SIGMAPLOT 10.0 (2006) were used for analysis. Results were analyzed with the parametric aspect of data (Normality – Kolmorov-Smirnov; Homoscedasticity – Shapiro-wilks) (ZAR, 1999), ANOVA or Kruskal-Wallis, followed by the post-hoc Dunn's test, to analyze the preference for shelter color and behavioral activities; Mann-Withney U Test was employed to analyze the occupation of the shelter during a 24-hour period and according to the photoperiod. The significance level was $p < 0.05$.

Results

Periods during the light phase (0C, 2C, 4C, 6C, 8C and 10C) were more suitable for burrowing behavior, regardless of the culture system (mixed or monosex) (Kruskal-Wallis, H = 1672.930, gl = 11, $p < 0.001$) (Figure 1).

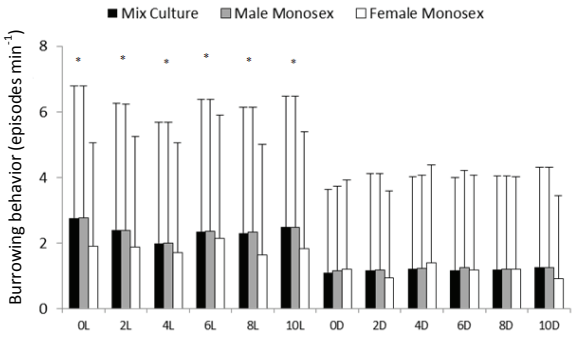

Figure 1. Frequency of occupation of the shelter (mean ± standard deviation) of *Macrobrachium rosenbergii* in mixed crops, male monosex and female monosex during phases of light (L) and dark (D), in 24 hours: 0L/0D (immediately after phase change); 2L/2D (2 hours after); 4L/4D (4 hours after); 6L/6D (6 hours after); 8L/8D (8 hours after); 10L/10D (10 hours after). ⋆ indicate a significant difference ($p < 0.05$).

In all treatments, prawns of the species *M. rosenbergii* were mostly outside the shelters (Mann-Withney, $p < 0.001$). In the mixed (Kruskal-Wallis, H = 11.542, gl = 2, $p = 0.003$) and male monosex (Kruskal-Wallis, H = 15.830, gl = 2, $p < 0.001$) cultures, there was a stronger preference for the black shelter (0.71 ± 2.38; 0.76 ± 2.28 respectively) due to higher occupation frequency. The animals preferred the orange and red shelters in the female monosex culture (0.55 ± 2.01; 0.56 ± 2.06 respectively) (Kruskal-Wallis, H = 7.960, gl = 2, $p < 0.019$).

There was no statistical difference with regard to the preference for the color of the shelter according to the photoperiod between the culture system in the dark (Table 1) and light phase (Table 2), although animals tended to occupy (episodes $min.^{-1}$) the black shelter in mixed and in male monosex cultures and the orange one in the female monosex culture, in both phases.

Differences in behavior activities were demonstrated by *M. rosenbergii* in the light and dark phases. The light phase evidenced the relevant occurrences (episodes $min.^{-1}$) in cleaning (2.86 ± 2.81), followed by burrowing, exploration, inactivity, crawling and agonism (Kruskal-Wallis, H = 2126.594, gl = 9, $p < 0.001$, post-hoc Dunn's test, $p < 0.05$) (Figure 2.1). Differences in types of culture for burrowing, inactivity and agonism were also reported during the light phase ($p < 0.001$). In the female monosex culture, the animals burrowed less often and revealed higher inactivity and agonistic behavior than animals in mixed and male monosex cultures (Figure 2.1).

Table 1. Preferential occupation of shelter (mean ± standard deviation) in episodes min.$^{-1}$ of *M. rosenbergii* in mixed crops, male monosex and female monosex, during the dark phase (6h00 pm - 6h00 am), according to the shelter color.

Population	Occupation (6h00 pm - 6h00 am)		Color of occupied shelter (6h00 pm - 6h00 am)		
	Outside	Inside	Black	Red	Orange
Mix culture	6.15 ± 4.57^{A}	2.40 ± 3.93^{B}	0.93 ± 2.71	0.86 ± 2.65	0.61 ± 2.19
Male monosex	6.12 ± 4.48^{A}	2.42 ± 3.94^{B}	0.99 ± 2.45	0.89 ± 2.69	0.45 ± 1.69
Female monosex	8.16 ± 3.42^{A}	1.85 ± 3.42^{B}	0.43 ± 1.78	0.65 ± 2.17	0.76 ± 2.39
	Mann-Withney $p < 0.05$		Kruskal-Wallis $p > 0.05$		

Different letters in the same column represent statistical differences.

Table 2. Preferential occupancy of shelter (mean ± standard deviation) in episodes min.$^{-1}$ of *M. rosenbergii* in mixed crops, male monosex and female monosex, during the light phase (6h00 am - 6h00 pm), according to the shelter color.

Population	Occupation (6h00 am - 6h00 pm)		Color of occupied shelter (6h00 am - 6h00 pm)		
	Outside	Inside	Black	Red	Orange
Mix culture	8.12 ± 1.17^{A}	1.17 ± 2.85^{B}	0.50 ± 1.98	0.41 ± 1.76	0.27 ± 1.38
Male monosex	8.14 ± 1.08^{A}	1.22 ± 2.78^{B}	0.57 ± 1.88	0.46 ± 2.25	0.31 ± 1.76
Female monosex	8.20 ± 3.21^{A}	1.25 ± 2.55^{B}	0.34 ± 1.56	0.44 ± 1.83	0.36 ± 1.65
	Mann-Withney $p < 0.05$		Kruskal-Wallis $p > 0.05$		

Different letters in the same column represent statistical differences.

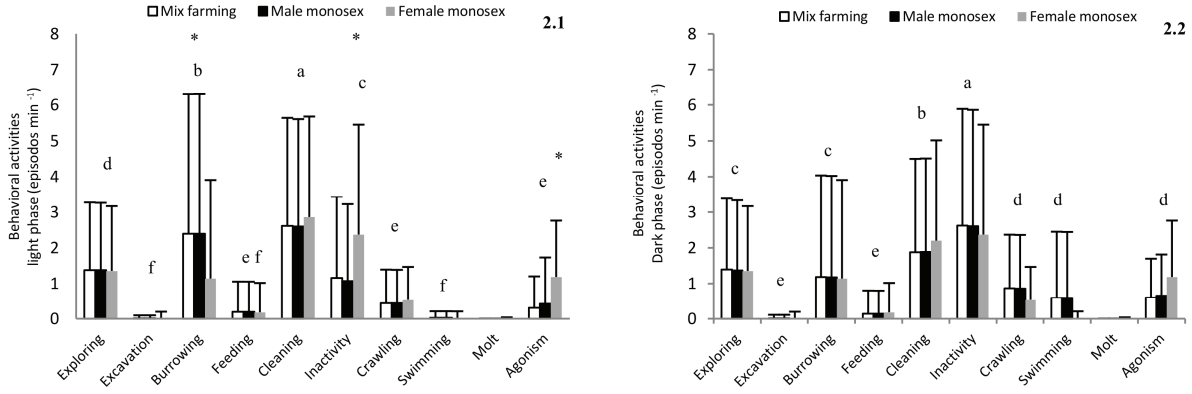

Figure 2. Behavioral activities (mean ± standard deviation) of *M. rosnbergii* in mixed, male monosex and female monosex cultures during light (6h00 am - 6h00 pm) (Figure 2.1) and dark phases (6h00 pm - 6h00 am). Different letters indicates significant difference among behavioral activities in treatments ($p < 0.05$).

Differences in behavioral activities (Kruskal-Wallis, H = 2007.233, gl = 9, $p < 0.001$, post-hoc Test of Dunn's, $p < 0.05$) (Figure 2.2) were also registered during the dark phase. Inactivity was the most frequent behavior (2.61 ± 3.22) in all treatments, followed by cleaning (2.23 ± 2.81), exploration (1.39 ± 2.08) and burrowing (1.20 ± 2.81). Only the agonistic behavior was different among treatments, with higher rates in the female monosex culture.

Discussion

Results show that *M. rosenbergii* seeks shelter more often during the light phase. When submitted to a monosex culture (male, female) the animals tend to choose the color of the shelters. Several reasons have been forwarded to justify this preference linked to the animal's welfare, such as acquiring and defending shelters help the crustacean to reduce risks against predation and optimizes access to resources (feeding, partners).

Current study failed to register sharing of shelter by the prawns in any of the tested conditions; during the few recorded visits to the shelter, an agonistic behavior (attack or threat) was expressed towards other co-species.

In a study by Englund and Krupa (2000), the shelters proved to be important resources against aggressive encounters and predation since they provided protection to submissive specimens when in the presence of a dominant male. Murthy et al. (2012) informed that cylindrical shelters (PVC pipes) play an important role in the survival and growth of *M. rosenbergii* and were significantly associated to bigger prawn growth. Balasundaram et al. (2004) observed that *M. nobilli* and *M. malcolmsonii* shared the shelter, coupled to a burrowing behavior and to a longer permanence in the shelter during the light phase.

The behavioral activities shown by the prawn in the treatments under analysis comprised cleaning as the most frequent during the light phase. Several species of decapod crustaceans spend much time and

energy in this activity (BAUER, 1989). Karplus et al. (1992) report that high frequency in body cleaning occurs because of the experimental tank (restricted space). The proximity with the dominant prawn may influence this behavior.

The animals explored the environment homogeneously, at all times of the phases and in all the culture systems. The same behavior was also observed by Pontes et al. (2006) and Santos et al. (2013). It has been registered that inactivity in *M. rosenbergii* occurred more often during the dark phase.

The movement of the animals (swimming and crawling behavior) was more frequent during dark phase and in the male monosex culture. This behavior may be due to the fact that prawns migrate between several habitats to complete their life cycle (DALL et al., 1990; PONTES et al., 2006; FREIRE et al., 2011, SANTOS et al., 2013). However, few registers of swimming were observed in the female monosex culture which may be related to the fact that the culture was carried out in freshwater (0 ppt). Hughes and Richard (1973) observed that egg-bearing *Macrobrachium acanthurus* females move (swim) towards the estuary during the dark phase and with alterations on salinity. Future studies must deal with the movement pattern of the prawn *M. rosenbergii* in its different stages of life - larvae, juvenile and male and female adults.

Hence, the environmental discrimination becomes useful to indicate the perception of the animal and the specific stimulus that makes the environment more attractive than the others when choices offered may be indicative of well-being conditions to the animal, providing possible advantages in feed, migration or reproduction (VOLPATO et al., 2004). According to Luchiari and Pirhonen (2008), knowledge on the effects of the colors on the farmed aquatic animals is still very restricted. Studies on the effect of environmental coloration may contribute to improve the culture environment and increase productivity.

Some of these environmental manipulations that alter the culture environment have provided interesting results for aquaculture. Color changes of tanks, shelters, substrate or light/darkness are parameters that bring positive results to the culture (MEAGER et al., 2005; YASHARIAN et al., 2005; LUCHIARI; PIRHONEN, 2008; LUCHIARI et al., 2009; FREIRE et al., 2011; LUCHIARI et al., 2012).

Preference tests have been used as a methodological option to determine the environmental parameters, such as temperature (KRAUSE et al., 1998), food (GALEF; WHISKIN, 2001) and type of substrate (DAWKINS, 1998). Although preference may be specific to each species, one possibility may be the preference for a specific color as indicative of the environmental factor that promotes the animals' well-being (DAWKINS, 1998). Thus, tests of color preference may be useful to understand how animals recognize the colorful environments and which ones represent the best photic environment for the animal. In the case of prawn cultivation, few color tests have been done in species which are relevant to farming. Lin and Omori (1993) reported that tank color affected the behavior of *M. rosenbergii* larvae in swimming.

Mariappan and Balasundaram (1999) observed that the color and the texture of the shelter influenced burrowing behavior of *M. nobilli* and verified that the animals avoided transparent shelters. With regards to the color of the shelter, the authors spotted a clear preference of young and adults for dark-colored shelter (black). Balasundaram et al. (2004) reported that in a 24-hour period, the prawn *M. malcolmsonii* spent 60.5% of the time inside brownish shelters, while *M. nobilli* spent 71% of the time inside a black one. Further, at certain instances, the prawn allowed the sharing of the shelter by other co-species. In current study, the sharing of the shelter was not reported. Yasharian et al. (2005) evaluated the effect of the color of farming tanks in *M. rosenbergii* post-larvae and observed that the color of the tank did not influence the animals' weight gain; however, survival was greater than in the red tanks. Luchiari et al. (2012) observed a bigger growth and food efficiency in the cultivation of *Litopenaeus vannamei* in yellow and red substrates.

Murthy et al. (2012) reported that the introduction of shelters provided beneficial effects on the culture of *M. rosenbergii*. The authors believe that these effects may be related to the night habit of seeking food and to the agonistic behavior of the animal. Higher peaks of agonism in the dark phase of the day have been registered, especially in the female monosex culture. Current results are different from those by de Barki et al. (1991), Karplus et al. (1992), Short (2004) and Karplus (2005) who observed that the male prawn of this species are more aggressive in mixed culture.

The methodological approach of applied ethology has seldom been explored for crustaceans. However, in different animal species, from mammals to fishes, this method of investigation has brought excellent results. Moreover, welfare has been one of the requirements of the commercial trade of farming animals. The discussion on crustaceans is still in the beginning, but shows a growing and relevant development (SILVA et al., 2012; SILVA; ARRUDA, 2014).

In current study on *M. rosenbergii*, the best conditions for burrowing are black for mixed and male monosex populations and red and orange for female monosex populations. In the case of females, the occupation of orange or red shelters may be related to egg coloring. They are carried outside the pleopods and their color ranges between orange and brown as they develop (HABASHY et al., 2012).

Conclusion

Shelters of different colors for prawns *M. rosenbergii* - black for males and orange or red for females – are recommended for the culture of the species to decrease the incidence of agonistic encounters between the animals during the light phase. It is important to take into account the consequences of different conditions of environment on the welfare of animals in farming, underscoring that which provides greater freedom for animals to express their natural behavioral repertoire. The method will probably increase growth and decrease production costs.

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