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ORIGINAL ARTICLE

Behavioral repertoire of the giant freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879) in laboratory

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Abstract An ethogram was prepared to characterize and describe the behavior of the prawn Macrobrachium rosenbergii in the laboratory so that its behavior would foreground in-depth analysis of the species´s culture. Sixty prawns were observed during 30 days through the ad libitum method, featuring one-hour continuous reports, at 07:00-08:00; 08:30-09:30; 10:00-11:00; 11:30-12:30; 13:00-14:00; 14:30-15:30; 16:00-17:00; 17:30-18:30. M. rosenbergii developed several behavioral activities with 28 categories and grouped in activity classes, such as, maintenance, locomotion, feeding and agonism. Characterization, description and understanding of the behavioral repertoire of M. rosenbergii through methodologies and observation tools typical of behavioral studies are an important step towards the improvement of technical management and welfare of the animal in captivity.

Keywords: ethogram, freshwater shrimp farming, prawn

Introduction

Studies on animal behavior have greatly contributed towards improvements in animal breeding (Ashley 2007), actually a great concern for scientific communities (Millman et al 2004). Ethological studies applied to commercial cultivation range from adequate maintenance of the animals in captivity to their welfare (Lawrence 2008; Huntingford et al 2012).

Applied ethology is actually an important tool for animal production systems since it broadens knowledge on behavioral responses of the species in the wake of different pressures and environmental stimuli and provides clues for better well-being and health with subsequent improvements in growth and survival (Lawrence 2008). Research in applied ethology mainly aims at understanding and enhancing welfare, a concern in current society, confirmed by the establishment of the International Society for Applied Ethology (Millman et al 2004). On the other hand, few studies have been undertaken on the theme with water animals, particularly invertebrate ones.

The fresh water prawn Macrobrachium rosenbergii (De Man, 1879) is one of the species marked for shrimp farming due to its easy reproduction, high fertility rates, fast growth and disease-resistance (Karplus 2005; New et al 2010). These factors enhance management and an economically feasible undertaking (Mitra et al 2005).

According to Huntingford et al (2012), high demands exist to maximize profit in all areas in which the organisms are commercially exploited. Research on behavioral activities may be of great help for the application of the species´s best management, behavioral knowledge, physiology and welfare.

In spite of the positive facts on commercial culture, prawns of the genus Macrobrachium are territorialists, aggressive and cannibalistic (Balasundaram et al 2004), while Short (2004) characterizes them as epibentic and nocturnal animals. Similar to most specimens related to the genus Palaemonidae, they have a typically well-developed agonistic behavior preferring a solitary stance, hiding under fallen tree trunks, shaded places and havens beneath stones.
Studies on animal behavior constitute a well-foregrounded kind of research both in Europe and the USA as from the 1930s, by Konrad Lorenz and Nikolaas Tinbergen (Alcock 2011; Yamamoto and Volpato 2011). The first steps in these studies have been the construction of ethograms, characterized by an inventory or list of behavioral units of a determined species, coupled to their description. The ethogram may provide a complete list of behaviors or several specific behavioral classes (Immelmann 1980; Martin and Bateson 1993; Lehner 1996).

The ethogram´s precise descriptions and definitions are a great asset in quantitative behavioral analysis and in the avoidance of contradictions in the behavioral classification during analysis (Immelmann 1980; Grier 1984; Martin and Bateson 1993; Lehner 1996; Freitas and Nishida 2006). Alcock (2011) insists that the ethogram comprises a behavioral repertory or a detailed scheme of all the activities

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developed by the species, with enormous advantages for the understanding and characterization of its history and ecological adaptations.

Similar to those for other species, surveys on behavioral categories in prawns of the genus Macrobrachium provide patterns and standards for quantitative and comparative studies. The agonistic ethogram by Barki et al (1991) described 18 behavioral activities for M. rosenbergii, whilst Kunz et al (2006) established an ethogram for the prawn species Palaemonetes pugio in an artificial environment and with a predator. The authors described 12 categories for the species which included two defense categories hitherto unreported. Palomar et al (2001) describe an ethogram for the feeding habits of the burrowing shrimp Alpheus macellarius, with a series of seven categories. Correa et al (2000) analyzed four events in the mating behavior of Rhynchocinetes typus within a free competition milieu among males. Oullette et al (2003) reported the behavioral patterns of the shrimp Crangon septemspinosa in experimental conditions under several types of substrate, whilst Pontes et al (2006) defined behavioral categories for Litopenaeus vannamei and Silva et al (2012) and Silva and Arruda (2014) registered behavior types of the species Farfantepenaeus subtilis and M. rosenbergii in laboratory conditions.

However, several types of information are required that focus on the behavioral motor patterns of the fresh water shrimp. Current paper aims at complementing the literature and elaborates an ethogram to characterize and describe the behavioral activities of M. rosenbergii within a laboratory milieu so that the acknowledgement of such behavior would provide better experimental conditions for the breeding and cultivation of the species.

Materials and Methods

Post-larvae of M. rosenbergii (PL10) were provided by a commercial larva-breeding enterprise (Larvi Aquicultura Ltda.). They had been acclimatized to culture salinity (0 g/L) and conditioned in a 20,000 L hatching tank in which they were kept for 30 days and fed on 12% of their biomass/day with frozen biomass of adult Artemia alternated by commercial diet (40% CP) every two hours during the daytime. The shrimps were then transferred to breeding farms and kept for 30 days. Research was performed in the laboratory of the Aquiculture Sector (SEAq) of the Center for Animal Sciences at the Universidade Federal Rural do Semi-Árido (UFERSA).

After this phase, 60 shrimps (5.02±0.1 g) were transferred to four 250-L glass aquaria (50 cm x 50 cm x 100 cm) within a closed water recirculation system, with artificial illumination, constant aeration, continuous filtration by biochemical and biological filters (Canister filter). Registers started after 10 days of adaptation of the shrimps to the aquarium´s physical conditions (Sick et al 1973; Pontes et al 2006) and after the establishment of their social hierarchy (Fero et al 2007).

The animals were observed during 30 consecutive days to register the species´s behavior. Shrimps were fed on 10% of their biomass/day, twice a day (08:00 and 16:00 hours) with pelleted diet, containing 35% crude protein, placed in troughs.

Abiotic parameters were assessed daily, featuring water salinity at 0 g/L (portable refractometer), pH at 8.2±0.2 (pH-meter), temperature at 27.7ºC±1 (electrode thermometer), ammonia 0.13±0.06 mg/L and dissolved oxygen above 5 mg/L (oxymeter). Water quality was constant throughout the observation period, or rather, the best for the culture of the species (New et al 2010).

Registers were undertaken daily by the ad libitum method (Martin and Bateson 2007; Yamamoto & Volpato 2011) during 30 days, with 1-hour continuous registers, and distributed into 8 periods throughout the day (07:00-08:00 h; 08:30-09:30 h; 10:00-11:00 h; 11:30-12:30 h; 13:00-14:00 h; 14:30-15:30 h; 16:00-17:00 h; 17:30-18:30 h), with 30-min intervals, totaling 240 hours of observation. The behavioral repertoire was described and reported as from the ethograms of shrimp species (Barki et al 1991; Pontes et al 2006; Silva et al 2012; Silva and Arruda 2014).

Results and Discussion

Since M. rosenbergii demonstrates several behavioral activities, the preparation of an ethogram is an important step anteceding quantification endeavors of behavior types for the species. Ethograms prepared for other shrimp species have focused only on reproduction, agonism (struggle) or feeding (Karplus et al 1991; Pontes and Arruda 2005a; Pontes and Arruda 2005b; Pontes et al 2006; Pontes et al 2008; Silva et al 2012; Silva and Arruda 2014). Current analysis comprises 28 behavioral categories groups under the following classes: maintenance, locomotion, feeding and agonism.

The twenty-eight categories of behavioral expressions described for the giant freshwater prawn *Macrobrachium rosenbergii* in current assay differ from the six activities given by Schmalbach and Qualckenbush (1994), without any behavioral description. The ethogram completes the research by Barki et al (1991) with eighteen behavior activities for *M. rosenbergii*, divided into four categories: locomotion activities involving movement frontward; locomotion activities that involve a non-frontward position or increase of distance; behavior activities based on position and movement of the chelae without touching another animal; behavioral activities based on position, movement and contact with another animal by means of the chelae.

General behavioral categories for the sea prawns *Litopenaeus vannamei* and *Farfantepenaeus subtilis* have already been described and include exploring, feeding, grooming, immobility, crawling, burying (Pontes & Arruda 2005a; Pontes & Arruda 2005b; Pontes et al 2006; Silva et al 2012; Santos et al 2013). In the wake of such behavior, adequate feeding management towards the culture of the species may be suggested. However, behavior descriptions failed to take into consideration the richness of detail of the motor patterns of the species.

Table 1 Maintenance behavioral motor patterns of the giant freshwater prawn *Macrobrachium rosenbergii* in the laboratory.

Behavior category	Description of behavior
Burrowing	The animal removes (burrows) the substrate with its pereiopods and by fast digging of the pleopods, causing the movement of sand particles and forming a hole in the substrate in which the animal may remain motionless.
Hiding	The animal enters the shelter and remains alone most of the day. The animal may remain totally hidden or it may expose its second pair of pereiopods or antennae outside the shelter.
Body grooming	The animal frictions its body with the 1st, 2nd, 3rd, 4th, or 5th pair of pereiopods.
Grooming the dorsal region of the body	The animal uses 1st, 2nd, 3rd, 4th, or 5th pair of pereiopods and frictions then to groom the dorsal region (cephalo-thorax and abdomen) by frictioning them on its back.
Grooming the ventral region of the body	The animal uses 1st, 2nd, 3rd, 4th, or 5th pair of pereiopods and frictions then to groom the ventral region (cephalo-thorax and abdomen) by frictioning them on the ventral region.
Grooming of appendixes, antennae and antennules	The animal uses the 1st, 2nd and 3rd pereiopod pairs by frictioning them to clean pereiopods, pleopods, maxilipedes, antennae and antennules.
Total Inactivity	The animal remains immobile, without any movement of locomotion appendixes, antennae, antennules or cephalo-thorax.
Partially immobility	The animal remains immobile, with only slight movements of the locomotion appendixes (pleopods and pereiopods) or antennae or antennules or discrete movement of the cephalo-thorax, to the right and to the left, upwards or downwards.

The assay showed that pereiopods are highly relevant for the daily activities of *M. rosenbergii*, due to their several behavioral expressions (exploring, hiding, feeding, grooming, and displacement). They are primary structures during the agonistic behavior between animals of the same species. According to Mariappanand & Balasundaram (1999), the second pereiopod pair is the main defense and attack structures of *Macrobrachium*. Balasundaram, Jeyachitra & Balamurugan (2004) also reported the importance of the second pereiopod pair in acquiring and defending territory by species *Macrobrachium malcolmsonii, Macrobrachium nobilii* and *Macrobrachium lamarrei*.

Current analysis revealed mutilated animals (cannibalistic mutilation). The most affected parts of the body in (complete or incomplete) struggles and fights were the pereiopods, pleopods, antennae and antennules, uropods and face. Volpato & Hoshino (1987), Souza & Sin-Singer-Brugiolo (2001) also reported mutilations in *Macrobrachium iheringer* and in females of *M. rosenbergii*, respectively, due to cannibalistic attacks. Brugiologo et al (2007) registered in the laboratory that the amputation of the chelae inhibited cannibalistic behavior and enhanced the survival of *M. rosenbergii* specimens.

The animals explored the environment in a homogeneous manner and at all times. Pontes et al (2006) and Santos et al (2013) perceived a similar behavior in shrimps of the species *L. vannamei* and by Santos et al (2015) in *M. rosenbergii*.

Entering the refuge and hiding are usually reported for the genus *Macrobrachium* in a natural environment or in captivity, since both activities are a protection against predators, besides enhancing access to food and partners (Stein 1977; Garvey et al 1994; Soderback 1994; Rebach & Dunham 1983; Cobb & Phillips 1980; Kurihara et al 1989). Santos et al (2015) also registered that prawns are prone to choose shelter according to color.

Table 2 Behavioral locomotion patterns of the giant freshwater prawn *Macrobrachium rosenbergii* in the laboratory.

Behavioral category	Description of behavior
Exploring the environment	The animal uses the first pereiopod pair (chelae) to explore the substrate (introduction and distancing). The chelae pinch the substrate or other material on the ground, sometimes moving along. The animal may hold the item (substrate or food particles) with the chelae and may release them. During exploration, the cephalo-thorax and the head slightly incline downwards, and the animal may move along the substrate. During exploration, the animal may be displacing itself or it may be immobile.
Moving on the substrate	The animal displaces itself by its 1st, 2nd, 3rd, 4th, or 5th pereiopods. The pleopods may participate: they are important for fast displacement.
Swimming	The animal displaces itself along the water column (vertically or horizontally), moving by continuous fast beatings of the pleopods. The abdomen and the pleopods have wavy movements; abdominal contraction occurs for fast displacement.

Although the burying behavior by *M. rosenbergii*, has not been reported in current assay, the species exhibited a type of substrate burrowing behavior which is different from that by penaeid shrimps with their burrowing and burying habits (Dall et al 1990; Pontes et al 2006; Silva et al 2012).

Prawns are reported to have grooming behavior on their own body. According to Bauer (2002) and Barr et al (2008), several decapod crustacean species spend much time and effort on this activity. Karplus et al (1992) and Karplus (2005) analyzed the social interactions of *M. rosenbergii* males and suggested that high grooming rates were extant due to the aquarium used for experiment (restricted place). Closeness to dominating prawns may possibly affect such behavior.

According to Lawrence & Rushden (1993), grooming may be considered a displacing behavior undertaken out of context and compulsively to ease tension. Current assay suggests that high occurrence of apparently displaced grooming (tension) may be related to the limited size of the aquarium, closeness to another prawn, microorganisms within the carapace and communication mechanisms. Moreover, prawns reveal grooming behavior after agonistic activities. It seems that such behavior may affect energy reserves: instead of directing energy towards growth, it is used to exhibit such behavior. Long-term studies should be performed to explain the survival or reproduction rates of such behavior.

Table 3 Behavioral feeding patterns of the giant freshwater prawn *Macrobrachium rosenbergii* in the laboratory.

Behavior category	Description of behavior
Ingestion	The animal holds the food (diet pellet, dead animal or *exuvia* from molting) with the first pereiopod pair and takes it to the maxilípedes. The prawn pinches the food, turns and puts it into its mouth, removing some pieces. The 2nd, 3rd and 4th pereiopod pairs may also participate in food intake.
Cannibalism	The animal holds the dead animal, uses the first pereiopod pair and takes parts of the body or exuvia of another animal up to the maxilípedes. The prawn pinches the food, turns it and then takes it to its mouth, removing some pieces. The 2nd, 3rd and 4th pereiopod pairs may participate in food taking.

Hierarchy among prawn have been reported in agonistic behavior, affected by the size and color of the chelae of the male´s 2nd pereiopod pair. Dominating males had big size blue chelae and revealed territorial behavior; they were followed by mid-sized orange-colored chelae; followed by small males with transparent chelae. Karplus (2005) noted that the effect of animal size and morphotype (color of chelae) in *M. rosenbergii* occurs specifically with competition for resources and that alpha position was always occupied by blue chelae males.

A major obstacle in the cultivation of *M. rosenbergii* is the typical heterogeneity of the size of specimens (mainly males) in the population affected by social dominance in the morphology of the blue chelae male. The above characteristic, underscored by increase in population density, is the effect of a complex social structure composed of three main morphotypes that differ in morphology, physiology and

behavior (Short 2004). Although specialized literature reports on mere morphotype differences and the color of *M. rosenbergii* males, current analysis revealed females with different colors on their chelae (blue and orange-colored chelae) and with agonistic behavior and dominance patterns. Further studies should investigate individual behavior and difference in females of the species according to morphotype.

Although *M. rosenbergii* is one of the best prawn types for cultivation, its aggressive behavior may worsen in agglomerated cultivation conditions. Several researchers, such as Fujimura & Okamoto (1970) Seagal & Roe (1975), Cohen et al (1981), Aiken & Waddy (1988), Karplus et al (1989) reported that aggressive behavior decreases survival and production rates in prawn cultivation. According to Del-Claro et al (2002), studies of behavioral repertoires or ethogram are basic tools for a better understanding of the biology, ecology and behavior of an animal, especially in captivity.

Table 4. Behavioral agonistic patterns of the giant freshwater prawn *Macrobrachium rosenbergii* in the laboratory.

Behavior category	Description of behavior
Front approach	The prawn displaces itself for more than 5 cm towards another prawn. The chelae of the 2nd pereiopod pair may be open and may be directed or not towards another animal. The prawn may or may not attack (threaten).
Non-front approach	The prawn displaces itself sideways for more than 5 cm towards another animal of the same species which moves in a position that its front is directed far from the other animal. Chelae of the 2nd pereiopod pair may be open or not towards the other animal. The prawn may or may not attack (threatening).
Approach with flectioned abdomen	The prawn displaces itself with flectioned abdomen to thrust the other prawn behind it, increasing the distance between the two. During this activity, the prawn´s body becomes arched in such a way that its tail takes a front position; it loses contact with the substrate and its claws are moved behind its body.
Threatening	The prawn shows the 2nd pereiopod pair with open chelae (dactyl) when another co-species is present; it may raise and/or shake the 2 pereiopod pair or merely show and open its chelae.
Attacking	The prawn pinches (attacks) another prawn with the chelae of its pereiopods. The attack generally occurs with the 2nd pereiopod pair although it may be undertaken by the 1st pair. The attack may occur at the frontal or caudal regions, even in the dorsal, ventral or lateral region of the other animal. It may merely pinch the other animal, pushing it away.
Front hug	The dominating animal, generally with a blue or orange chela, approaches at a mean distance of 5 cm from the other prawn, opens the 2nd pereiopod pair at an angle of 90º from its body, contracts the abdomen and moves the pleopods fast; all of a sudden it approaches the other animal and quickly closes the 2nd pereiopod pair, sometimes pinching the other animal (aggression).
Meral extension	The animal raises its body with the two pereiopods above the substrate and parallel to the other animal. The ischium and mere remain perpendicular to the body axes. A fast and simultaneous opening of the pereiopods may occur towards the other prawn or demonstrated between the two animals.
Persecuting	The prawn displaces itself fast on the substrate or in the water column towards the other animal, normally showing the 2nd pereiopod pair with open chelae.
Fighting (total fight)	The animal raises the body´s front part and the pereiopods towards the other prawn. It stays in an erect position where the carapace forms a 45°- 90° angle horizontally. The abdomen extends; the uropods open and close their caudal fan whilst the pleopods shake vigorously. The pincers are raised at an angle of 45° - 90° from the horizontal plane. At this time, the animal opens and closes the dactyls and an attack occurs by one of the prawns.
Fighting (incomplete fight)	In current behavior, the animal initially acts as above, with no attack by one of the prawns at the end.
Retreating	The animal moves its cephalo-thorax downwards in the presence of another prawn and displaces itself approximately 5 cm backward or sideward. Displacement may occur towards the side regions and also on the prawn´s

	own axes. Behavior is normally followed by lowering of the body and the 2nd pereiopod pair close to the substrate.
Giving place	When the immobile and subordinated animal, either in the shelter or elsewhere, perceives the presence of the other dominating prawn (approach or attack), it lowers the cephalo-thorax and displaces itself on the substrate by means of its pereiopods towards another place, giving up the place previously occupied. Displacement may occur in a fast way by beating the pleopods.
Divert	The animal, with its front part facing the other, turns on its own axes sideward or backward, without facing the other prawn.
Flight	When the animal perceives the other, or when it is attacked or persecuted, it contracts its abdomen and swims fast using its pleopods towards places which are distant from the other animal.
Defense	When the animal is attacked by another prawn, it raises the 2nd pereiopod pair in front, above the cephalo-thorax. During its defense, the animal may run away, give up the place or retreat.

Conclusions

The prawn species *M. rosenbergii* has several behavioral activities, or rather, twenty-eight categories that make up its behavioral repertoire, grouped in general classes of motor patterns for maintenance, locomotion, feeding and agonism. The characterization and description of the behavioral repertoire of *M. rosenbergii* complements data of specialized literature and may be employed in studies that aim at using the species for cultivation. It also provides improvement for management and welfare of the animal in captivity.

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