BEHAVIORAL RESPONSES OF FRESHWATER PRAWN ACCORDING TO FEEDING MANAGEMENT IN MIXED AND MONOSEX POPULATIONS

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ABSTRACT

The effects of two feeding management methods (substrate spreading and feeding tray) were tested for *Macrobrachium rosenbergii*, evaluating their influence on behavior in mixed (Mi), monosex all-male (Mm) and monosex all-female (Mf) populations. Twelve experimental conditions, totaling 192 animals, with four repetitions each, were performed. Experimental units comprised eight 250-L aquariums (50 x 50 x 100 cm) with soft sand substrate. Behavioral data were collected by focal instantaneous sampling method. Substrate spreading triggered the most extensive behavioral level in all populations under analysis, featuring exploring, autogrooming, crawling, inactivity, burrowing, agonism, swimming and feed ingestion, and revealed that the animals expressed better their behavior when feed was spread. Exceptionally, burrowing in Mm and agonism in Mf had similar level rates for the two feeding managements. Mf had the highest agonistic level when compared to that in Mm and Mi. Data in current analysis indicate that the spread feeding strategy favors animal welfare in all types of population compositions, except in Mf culture, with high agonistic levels in both forms of feeding management.

Key words: ethology; behavior; feeding trays; feed; Malaysia giant prawn.

RESPOSTAS COMPORTAMENTAIS DO CAMARÃO DE ÁGUA DOCE DE ACORDO COM O MANEJO ALIMENTAR EM POPULAÇÕES MISTAS E MONOSSEXO

RESUMO

Foram avaliados os efeitos de dois métodos de alimentação (a lanço e em comedouros) e sua influência no comportamento de populações mistas (Mi), monossexo fêmea (Mf) e monossexo macho (Mm) de *Macrobrachium rosenbergii*, nas fases clara e escura do dia. Foram analisadas 12 condições experimentais, com quarto repetições cada, totalizando 192 animais observados. As unidades experimentais consistiram em 8 aquarios (250 L; 50 x 50 x 100 cm) com substrato de areia fina. Dados comportamentais foram coletados usando método de amostragem focal instantâneo. A oferta a lanço, em todas as composições populacionais, induziu a maiores níveis dos comportamentos de exploração, limpeza, enterramento, inatividade, entocamento, agonismo, natação e ingestão do alimento, indicando que os animais encontraram mais oportunidades para expressão de seus comportamentos com o espalhamento da ração. Como exceção, o comportamento de entocamento em Mm e agonismo em Mf foram semelhantes para ambos tipos de manejo alimentar. O cultivo Mf apresentou maiores níveis de agonismo que Mm e Mi. Nesse estudo, os resultados indicam que, em todos os tipos de composição populacional, a alimentação a lanço favoreceu o bem-estar estar animal, exceto para o cultivo Mf, o qual apresentou altos níveis de agonismo em ambas as formas da estratégia alimentar.

Palavras-chave: etologia; comportamento; comedouros; ração; camarão gigante da Malásia.

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INTRODUCTION

Freshwater shrimp agribusiness is one of the fastest growing aquaculture sector worldwide. It is a sustainable way for the production of crustaceans since the animals are resistant to disease and provide social development with low environmental impact (NEW and NAIR, 2012). High feeding costs, which account for about 60% of production costs, are the main problem that producers of aquatic organisms have to face (MOLINA-POVEDA *et al*., 2013), since feeding management is a critical factor for the farm´s viability.

In the case of marine shrimps, with gregarious habits and group living (DALL *et al*., 1990), feed consumption is usually monitored with trays, considered to be a factor of environmental and economic control on farms, reducing feed wastes, increasing the sustainability of production systems and improving pond water quality (CASILLAS-HERNÁNDEZ *et al*., 2006). On the other hand, the freshwater prawn *Macrobrachium rosenbergii* is characterized as a territorial and aggressive species (NEW and NAIR, 2012) and studies on feeding management strategies are required to evaluate its behavior with regard to access to the feeder.

Natural populations of *M. rosenbergii* are characterized by the development and distribution of big-size animals in a well-defined social structure and morphotype variation. Prawn size variation reflects a complex population structure composed of three major male morphotypes – small males, orange-claw males and blue-claw males, which differ in morphology, physiology and behavior. Since social interactions among juveniles and among sexually mature males affect their growth (KARPLUS, 2005), the monosex culture has been used in some countries to reduce the problem of heterogeneous growth (SIDDIQUI *et al*., 1997; NAIR and SALIN, 2012; MALECHA, 2012).

The future prospective of the commercial breeding of *M. rosenbergii* seems to rely on monosex population farming. Several studies in the field of Molecular Biology have introduced new manipulation techniques to obtain monosex populations of the species (VENTURA *et al*., 2009; VENTURA *et al*., 2011; VENTURA and SAGI, 2012; AFLALO *et al*., 2014). LEVY *et al*. (2016) developed a technique by which they were able to induce all-female population of this species with an injection of Hypertrophied Androgenic Gland Cells. Therefore, another important aspect to be addressed is the influence of the population´s gender composition (mixed culture, monosex all-male or all-female) on the animal's behavior and growth.

Information on the species´s behavior in different social and feeding management is needed to improve the technical handling of *M. rosenbergii* culture. According to ROBERTSON *et al.* (1993), feeding offer in the pond must coincide with the species´s active period. Since the daily activity pattern of *M. rosenbergii* has not yet been extensively studied, current assay analyzes the types of behavior that may be triggered through ration supply to the species and tries to identify the best phase of the day (light or dark) to feed them. Further, conditions to improve animal welfare have to be taken into account in management strategies. Current study evaluates the species´s behavior according to the feeding management applied (feeding tray or spreading of feed on substrate) in mixed and monosex (all-male and all-female) populations, in light and dark phases, within a 24-hour period.

METHODS

M. rosenbergii prawns, obtained from a commercial hatchery at the post-larval stage, were kept in 52m^2 earthenware ponds for eighty-four days at the Agricultural Station of the Federal University of Rio Grande do Norte (UFRN), Brazil. During this stage, prawns were fed by spreading feed on the substrate at 10% biomass/day, divided at a frequency of twice a day (8 am and 4 pm) until they reached adult age, weighing approximately 15.84 ± 5.97 g. One hundred and ninety-two *M. rosenbergii* adult prawns were transferred to the Shrimp Behavior Laboratory of the UFRN where the experiments were conducted.

Experimental units consisted of eight 250-L aquariums (50 x 50 x 100 cm) with soft sand substrate (6 cm high), constant aeration and water recycling through biological filters (Canister). Water quality (pH, temperature, dissolved oxygen, salinity) was monitored daily, whilst three PVC pipes (3/4') were added to each aquarium to serve as shelter.

Aquariums were submitted to an artificial 12-h light/dark cycle, using a timer. In one laboratory, the light cycle was equivalent to the natural photoperiod, whilst in another laboratory, by inverted photoperiod, four experimental units in the light phase and the other four in the dark phase were simultaneously tracked. In the case of the inverted photoperiod, white 32-watt fluorescent

lamps and red 15-watt incandescent lamps were respectively used in the light and dark phases. Two different kind of lamps were employed due to the prawn's non-reactivity to luminosity (PONTES and ARRUDA, 2005).

Based on stocking density commonly used by producers for mixed populations (5 to 10 prawns.m^{-2}), each aquarium received four specimens (8 prawns.m^{-2}). The appendage in the second pleopod pair in males was taken into account to sort the animals by gender.

Prawns were weighed immediately after arriving at the laboratory. For ten days prior to behavior recording, the animals were acclimated to artificial light cycle, aquarium physical conditions (PONTES *et al*., 2005), establishment of a social hierarchy (FERO *et al*., 2007) and to the characteristic of each feeding management. Data were collected on a daily basis within six 10-day observation stages. New animals were collected in the ponds for each 10-day stage.

The prawns were fed with pelletized ration containing 35% crude protein (Camaronina 35, Purina Agribrands do Brasil S.A., São Paulo, Brazil), or rather, 10% of their biomass, twice a day - two and ten hours from the start of the behavior recording phase.

Three types of populations were employed in the experimental design, according to gender composition: mixed (Mi), monosex all-male (Mm) and monosex all-female (Mf), subjected to two feeding managements: pellets spread on the substrate and pellets on transparent acrylic feeding tray (2x3x2 cm). Four replicates were used for each type of feed (spread or feeding tray) in each type of culture (Mi, Mm and Mf). The same replicates were observed in both periods (light and dark phase). Thus, 12 experimental conditions were applied, totaling 192 prawns.

The specimen´s behavior in each treatment was registered after daily feed offer (two hours and ten hours after the onset of the behavior recording phase) - lights on for natural photoperiod and lights off for inverted photoperiod. Experiments occurred during six stages with 10 days each, equivalent to 60 days of behavioral recordings, 4 hours/day (2 hours/observer), totaling 240 hours for each population type.

The focal instantaneous sampling method (MARTIN and BATESON, 2007) was employed in 15-minute windows for each aquarium, with instantaneous recording at every 60 seconds. Two observers collected data on check sheets, simultaneously, during the light and the dark phases until the 8 aquariums of each population type were observed (four during the light phase and four during the dark phase). Lots were drawn at the beginning of the day to determine the aquariums that would be recorded by each observer at each observation window. Reliability tests performed by the observers produced 93% accuracy in all parameters.

Behavioral categories comprised exploring substrate, autogrooming (cleaning), crawling, inactivity, burrowing, agonism, swimming and feed ingestion (SANTOS and PONTES, 2016).

Measurements for latency to access feed were taken for each specimen, following MARTIN and BATESON (2007). The time elapsed between stimulus presentation (pellet) and the time the animal reached it on the feeding tray or spread on substrate was recorded (PONTES and ARRUDA, 2005).

Abiotic parameters were verified daily at 11 am. Water salinity was kept at 0 gL^{-1} (measured by portable refractometer); pH at 8.2 +- 0.2 (pHmeter); temperature at 27.7 ± 1ºC (electrode thermometer); dissolved oxygen above 5 mgL^{-1} (oximeter), appropriate for cultivating *M. rosenbergii* prawns (NEW *et al*., 2011).

Data analysis was performed by Univariate Analysis of Variance test which evaluated the main effect of variables: type of culture (mixed and all-male and all-female monosex) and feeding management applied (feeding tray and spreading feed on substrate) on each behavioral category recorded. Response variables used were frequency of occurrence (episodes.min^{-1}) of each behavioral category, and latency of access to feed. Tukey's post-hoc test was applied after verifying the effect of culture type on the variables. Significance level at all tests was 5% bi-caudal (ZAR, 1999), with PASW Statistics 18.

RESULTS

The behavior of exploring the substrate was influenced by feeding management ($F = 365.080$; $p<0.05$) and by the type of culture ($F = 12.178$; $p<0.05$). The two variables interacted ($F = 9.168$; $p<0.05$) and affected behavior. Exploratory behavior was more frequent within the monosex all-male population and with feed spread on the substrate (Figure 1a).

Autogrooming (Figure 1b) was influenced by culture type ($F = 12.408$, $p <0.05$), with a higher

frequency in the monosex all-male population. The type of feed management also affected autogrooming (F = 367.878, p <0.05), inducing higher frequency of the behavior in the pellet spread management, albeit greater in mixed cultivation. The effect of interaction between these two independent variables was observed only in exploration (F = 9.168, p <0.05) and autogrooming (F = 9.103, p <0.05). Since a greater frequency of behaviors in supply for all culture types was registered, particularly in mixed populations, there was a greater difference between spread and feeding trays treatments and suggesting the combined effect of the two independent variables for these behaviors. Crawling was influenced by sex composition (F = 12.533, p <0.05) and the type of feed supply (F = 44.189, p <0.05), with higher frequencies when feed was spread on the substrate. Monosex all-male culture induced the highest frequency of this behavior, while mixed population was responsible for lowest one (Figure 1c).

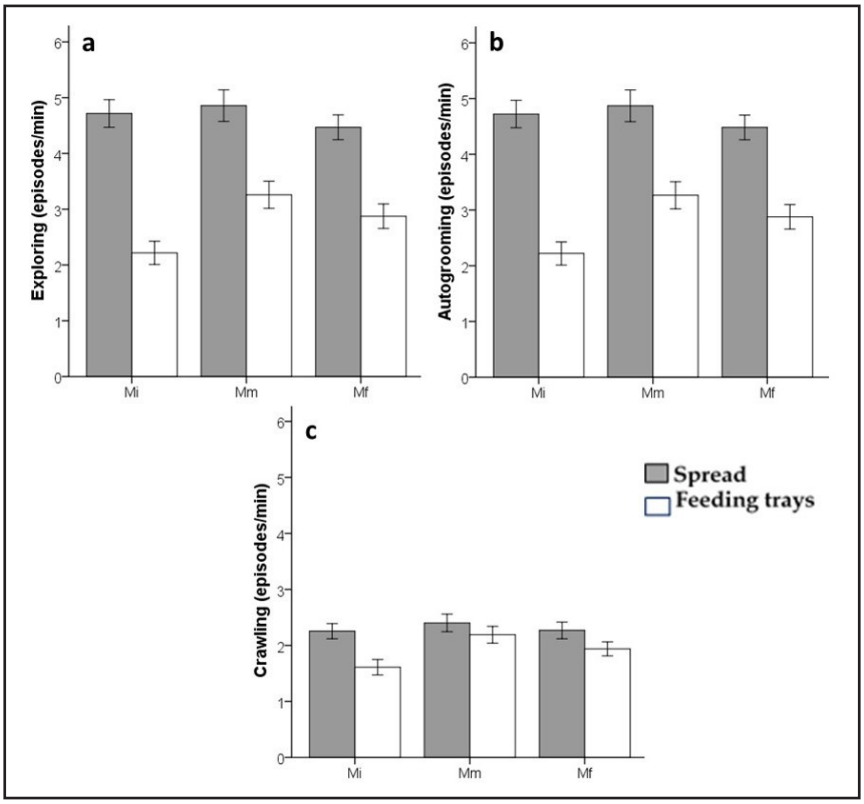

Figure 1 . Frequency (mean ± standard deviation) of *Macrobrachium rosenbergii* prawns' exploratory (a) autogrooming, (b) crawling, (c) behaviors in mixed (Mi), monosex all-male (Mm) and monosex all-female (Mf) cultures with feed spread on the substrate or on feeding trays.

Inactivity showed a significant difference according to feeding management used (F = 23.377, p <0.05), with higher frequencies observed for the feed spread management. This type of culture (F = 21.451, p <0.05) also had an influence on shrimp inactivity, with monosex all-male culture revealing higher frequency rate of this behavior (Figure 2a).

The analysis of the burrowing behavior indicated differences according to the type of culture (F = 4.608, p <0.05) and feeding management (F = 25.973, p <0.05). Burrowing frequency was lower for monosex male culture submitted to feed spread management. Further, frequency of burrowing increased when feed was spread in monosex female culture and in mixed culture (Figure 2b).

Agonistic behavior was also affected by culture type (F = 36.461, p <0.05) and by feed supply management (F = 6.560, p<0.05). Similar to other types of behavior, the occurrence of agonism had a higher rate in feed spread treatment. This behavior showed a higher frequency in the monosex all-female population and a lower one in the mixed culture one (Figure 2c). The type of culture interfered in the swimming behavior (F = 34.336, p <0.05), with

lower frequencies for mixed culture and type of feed supply ($F = 30.240$, $p < 0.05$), with highest frequencies when feed was spread in all culture types tested (Figure 2d).

Feed ingestion was influenced only by the type of culture ($F = 4.829$, $p < 0.05$), with the highest frequency for monosex all-female culture (Figure 3a). The latency to access the feed was influenced only by the feeding management applied ($F = 652.418$, $p < 0.05$), with faster access to feeding for animals in the feed spread management (Figure 3b).

In current research, the feeding management (spread on substrate and feeding trays) and the sexual composition of the specimens (mixed, monosex all-male and monosex all-female) failed to influence the final survival rate of *M. rosenbergii* giant freshwater prawn. At the end of the experiment, the survival rate of the prawns was 100% for all treatments.

Since day phases (light and dark) in which the animals were observed had no effect on the response variables analyzed, the results for the 24-hour observation have been given.

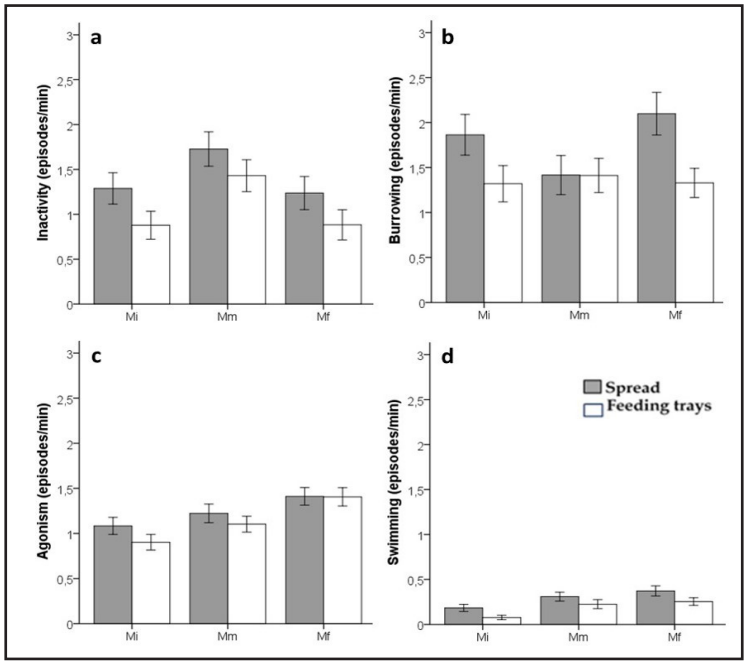

Figure 2 . Frequencies (mean ± standard deviation) of behavior of *Macrobrachium rosenbergii* prawns: inactivity (a), burrowing (b), agonism (c) and swimming (d) behavior in mixed (Mi), monosex all-male (Mm) and monosex all-female (Mf) cultures, with feed spread on substrate and on feeding trays.

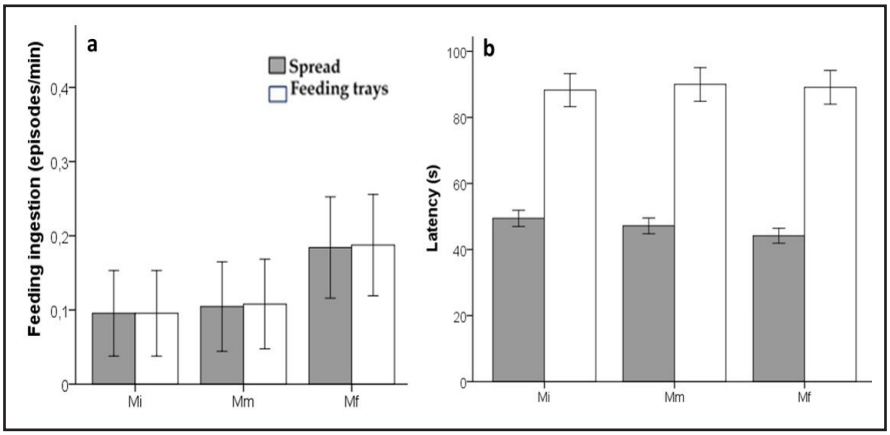

Figure 3 . Frequency (mean ± standard deviation) of *Macrobrachium rosenbergii* prawns' feed ingestion (a) and latency (b) in mixed culture (Mi), monosex all-male (Mm), monosex all-female (Mf) in two feed managements - spread on substrate and feeding trays.

DISCUSSION

As a rule, when feed spread on substrate was compared to feed on trays, the former triggered greater exhibition rates in behavior, regardless of population composition, with the exception of burrowing in monosex all-male culture and of agonism in monosex all-female culture, with similar levels in the two types of feeding management tested. Further, monosex all-female population had the highest agonic levels when compared to those of monosex all-female and mixed populations.

The animals found more space and opportunities to express their behavior when feed was spread on the substrate. Feed spread favors the scramble competition since apparently resources cannot be monopolized by dominating animals and are equally available for all (STRIER, 2000). In current analysis, concentrated resource probably favored behavior inhibition of subordinated specimens.

In their research on the behavior of *M. rosenbergii*, VanMAURIK and WORTHAM (2014) argue that foraging, fighting a predator or a conspecific specimen and mating, are normally deemed primary behavior types and are usually considered high in behavioral hierarchy as they are evolutionarily relevant for reproductive fitness and survival conditions. Secondary behavior types occur when primary behavior ones are not crucial, like body grooming, which is a behavioral adaptation to fouling pressures. In fact, secondary behaviors may be inhibited when more vital types of behavior are beneficial to survival. Furthermore, organisms in resource-limiting environments or social situations (i.e. competition, mating, agonistic interactions) give priority to behaviors with the greatest resource profitability. Therefore, yields depend on the size of the hierarchical groups in the grow-out tanks or ponds. Research, therefore, demonstrated that grooming in prawns is a secondary behavior, which is an important behavior as an antifouling adaptation, particularly in high-density populations such as aquaculture settings.

Agonistic behavior in current assay was more frequent in feeding management conditions for the monosex all-female culture system when compared to monosex male and mixed, characterizing a greater competition for ration by females. Lower agonism levels were registered for mixed cultivation, mainly when feed supply was on trays. This information is possibly related to a better establishment of dominance relations between males and males *vs* females in natural populations.

NUNES *et al*. (1996) observed that feed ingestion always increased in *Farfantepenaeus subtilis* culture, in dark and light phases, after feed was spread in the pond. This fact demonstrated that feed supply is an important factor for environmental exploration, stimulating the animals to ingest natural and feed items. Registering ingestion by *M. rosenbergii* in light and dark phases, HARPAZ (1997) showed that, since the animal fed during the two phases, the strong influence of chemicals added to the ration caused the animal to search and ingest the feed even outside its natural feeding phase.

In general, animals compete among themselves for food, shelter and mate, particularly through aggression (MARTIN and MOORE, 2007). According to current results revealing higher aggression levels by females fed on feed spread on substrate or on feeding trays, it seems that feeding resources are more limited for females than for males. Current data suggest that in the case of *M. rosenbergii* males, obtaining a sexual partner is probably a more critical resource than it is for females.

KARPLUS (2005) and KARPLUS *et al*. (1992) evaluated aggressiveness in *M. rosenbergii* only in confrontations with adult males. SILVA and ARRUDA (2014) observed that although there was no correlation between dominance and general behavioral activities in *M. rosenbergii* juveniles, dominants had greater weight gain and faster access to feed.

Several studies have also shown that the use of trays in marine shrimp culture improves water quality (MARTINEZ-CORDOVA *et al*., 1998). Nevertheless, PRETO *et al*. (2008) warn that when feed is given on trays, it may restrict access to feed to a larger number of animals, even though it reduces dirt formation. In current study, latency rate to access feed was lower than for feed spread, in all forms of culture. Feed ingestion was higher for monosex all-female culture when compared to monosex all-male and mixed populations, although the type of feed management did not interfere with the ingestion behavior, in all forms of culture. PRETO *et al.* (2008) reported that shrimp *Macrobrachium amazonicum* adapted well to feed on tray. However, due to its territorial and aggressive behavior, the authors suggested that producers should use a greater number of trays per area.

The effects of feeding trays have also been

compared among species. NUNES and SANDOVAL (1997) compared F. *subtilis* and *L. vannamei* in pond cultures fed on trays during approximately 100 days and observed that growth and survival rates of F. *subtilis* were higher than those of *L. vannamei*. NUNES and PARSONS (1999) observed F. *subtilis* in an 89-day rearing cycle and recorded no difference in growth, feed consumption or survival rate under the two feeding managements (spread on the substrate and feeding tray), despite the fact that stomach contents were greater when individuals were fed by feed spread.

NAIR *et al*. (2006) conducted a study on population composition to compare the economics of all-male, mixed and all-female culture in fifteen 4000 m ponds each for a period of 5 months after the nursery phase. Average weight, productivity and specific growth rates were highest for all-male culture, or rather, 80.92±2.41 g, 1532 kg ha^{-1} and 1.97±0.02 respectively. All-female culture significantly registered a higher survival rate (89.16±0.77%) and the best apparent feed conversion ratio of 1.26±0.02.

The economic analysis revealed that all-male monosex culture was 63.13% and 60.20% more profitable than mixed and all-female cultures, respectively. Results demonstrate the valorization of behavior as an influence in the profitability of a culture system with regard to the social relationships of animals that integrate each population in culture ponds, especially dominance relationships that interfere in animal growth.

Current results demonstrate that behavioral differences must be related to environmental conditions (feeding management strategies), which seem to influence exploratory behavior levels, or rather, adjusting animals´ interaction with their environment according to feed conditions. The use of feeding trays for shrimps have both positive and negative consequences. In feed spread management, greater care with water and soil quality is recommended for a more favorable environment to the cultivated animals.

It is important to take into account the consequences of different conditions of feed offer on the welfare of farming animals, allowing greater possibilities for the animals to express their natural behavioral repertoire, as emphasized in the five freedoms that must guide animal culture systems (BOX, 2006). Knowledge on animal behavior favors the establishment of animal welfare (GONYOU, 1994) since a more uniform behavior has been detected in animals fed on trays, perhaps indicating better welfare conditions.

CONCLUSION

Differences between types of population should be considered when planning a culture system. In relation to feeding management, data indicate that the feed spread strategy favors welfare of the animals in all types of population composition. Considering the sexual composition of the population and the search for animal welfare, female monosex culture is not recommended, since high agonistic levels were recorded when feed occurred in the two modalities analyzed.

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