Activity pattern of the marine shrimp *Litopenaeus vannamei* (Boone 1931) in laboratory as a function of different feeding frequencies

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Abstract

Management practices on most Brazilian shrimp farms have resulted in high expenses for producers and damage to the environment. Applied ethology could provide information on the pattern of shrimp activity, enabling more efficient feeding frequencies. Behavioural activities of the shrimp *Litopenaeus vannamei* were recorded during different feeding frequencies. The shrimp were kept in aquariums on a 12:12 h light/dark cycle. They were fed commercial ration three (at 6:00, 12:00 and 18:00 hours), four (at 6:00, 10:00, 14:00 and 18:00 hours) or seven times per day (at 6:00, 8:00, 10:00, 12:00, 14:00, 16:00 and 18:00 hours). We observed animals at 15 min^{-1} aquarium^{-1} time periods, recording feeding, substrate exploration, swimming and inactivity. Feeding and exploration were the highest for shrimp fed three times per day; swimming was greatest when animals received four feedings, whereas inactivity was higher for shrimp fed seven times per day. There was greater food ingestion between 12:00 and 14:00 hours for animals fed three and four times per day, whereas swimming was high mainly at 18:00 hours for shrimp fed three and seven times per day. The results indicate higher forage-related activities (exploration/ingestion) when feed was offered three or four times, suggesting optimization in the pattern of shrimp activities based on these feeding frequencies.

Keywords: applied ethology, shrimp, feeding frequency, *Litopenaeus vannamei*

Introduction

Shrimp culture has been gaining prominence throughout the world. In Brazil, commercial activity has increased markedly after the introduction of the species *Litopenaeus vannamei* (Boone 1931) in the 1980s (Bueno 1989). Its excellent adaptation to various culture conditions, especially to temperature and water salinity, has made it the main cultivated shrimp species in the country (Wainberg & Câmara 1998). Despite the growth in activity, management (including feeding management) on most farms is still carried out empirically. The feeding frequency used, feeding times and the number of trays per hectare are established by the producer based on traditional practices that can result in unnecessary expenditure because it is likely that not all the ration offered will be consumed. As a result, an accumulation of feed could occur, causing water and soil deterioration in the pond (Nunes, Goddard & Gesteira 1996). This could also lead to eutrophization problems in the pond itself, as well as in adjacent areas where the effluents will be discharged.

Given that feeding is part of the behavioural repertoire of any animal species and considering the importance of activity patterns over a 24-h period, understanding these aspects is critical for developing suitable management policies on shrimp farms. This can be achieved through greater knowledge of shrimp behaviour, because ethological studies are now widely being used to improve the cultures of different animal species (Wechsler, Fröhlich, Oester, Oswald, Troxler, Weber & Schmid 1997; Sambras 1998; Millman, Duncan, Stauffacher & Stooker 2004; Ashley 2007). Furthermore there is a need for more studies related to the feeding frequency to be adopted in shrimp culture that could enable the optimization of shrimp activity. An additional advantage could be lower feed loss to the environment and...
consequently greater benefit to the producers in terms of sustainable culture. Greater knowledge of activity patterns may lead to reduced artificial feed costs (ration), which has accounted for the highest production costs of shrimp farms (Sedgwick 1979; Velasco, Lawrence & Castille 1999; Molina, Cadena & Orellana 2000; Smith, Burford, Tabrett, Irvin & Ward 2002).

Part of the knowledge of shrimp activities has been acquired from studies quantifying activity versus inactivity of the animal through the use of photocells, without discriminating between the different activities performed by these animals (Rodriguez & Naylor 1972; Hindley 1975; Reynolds & Casterlin 1979; Vance 1992; Guerao & Ribera 1996). Ethological studies applied to shrimp farming are rare (Primavera & Lebata 1992; Guerao & Ribera 1996). Ethological studies applied to shrimp farming are rare (Primavera & Lebata 1992; Guerao & Ribera 1996). Ethological studies applied to shrimp farming are rare (Primavera & Lebata 1992; Guerao & Ribera 1996). Ethological studies applied to shrimp farming are rare (Primavera & Lebata 1992; Guerao & Ribera 1996). Ethological studies applied to shrimp farming are rare (Primavera & Lebata 1992; Guerao & Ribera 1996). Ethological studies applied to shrimp farming are rare (Primavera & Lebata 1992; Guerao & Ribera 1996). Ethological studies applied to shrimp farming are rare (Primavera & Lebata 1992; Guerao & Ribera 1996). Ethological studies applied to shrimp farming are rare (Primavera & Lebata 1992; Guerao & Ribera 1996).

Material and methods

We used juvenile shrimp of L. vannamei species after 2 months of culture and a mean weight of 8.04 ± 1.5 g obtained from a commercial shrimp farm in the state of Rio Grande do Norte (NE of Brazil). The temperature was maintained at 28 ± 1°C and salinity at 34 g L⁻¹ throughout the experiment.

The experimental units consisted of glass aquaria (0.3 × 0.5 × 0.4 m) containing 30 L of seawater; a closed water recirculation system, constant aeration and continuous filtration through a 3 cm high substrate of gravel and crushed oyster shells.

Five animals were placed in each aquarium (41 shrimps m⁻²) although during the observations only four of these animals were observed, owing to the observation capacity of the researchers. In order to recognize each animal individually during the observations, we placed different-coloured silicone rings on the ocular peduncle. The observations began 7 days after the animals were placed in the aquaria to enable them to acclimatize to the experimental conditions.

The shrimp were subjected to a 12:12 hour light–dark cycle, with behavioural observations performed from 6:00 to 18:00 hours. Illumination in the light phase was provided by 12 timer-controlled white fluorescent light bulbs (32 W) distributed across the laboratory ceiling. The last observation (18:00 hours) was performed under individual aquarium illumination, consisting of one red incandescent light (15 W) (Rodriguez & Naylor 1972; Hindley 1975). The white fluorescent lights generated a mean luminosity of 180 lx for each aquarium while the red incandescent light provided a mean luminosity of 1 lx.

During the entire experiment, the shrimp were fed an amount equivalent to 10% of the biomass of each aquarium using pelleted shrimp ration with 35% crude protein (Camaronina 35, Purina®. Agribrands do Brasil S.A., São Paulo, Brazil), according to the treatment adopted: three, four or seven daily portions. The same amount of ration was offered independent of the feed frequency adopted; only the number of daily feedings varied. The ration was offered on transparent acrylic trays (4 × 3 × 2 cm), left for 1 h and removed at the end of this period.

The animals were subjected to the following feeding frequencies: three feedings per day every 6 h
(6:00, 12:00 and 18:00 hours); four feedings per day every 4 h (6:00, 10:00, 14:00 and 18:00 hours); or seven feedings per day every 2 h (6:00, 8:00, 10:00, 12:00, 14:00, 16:00 and 18:00 hours). Independent of the treatment adopted, the first feeding was performed immediately after the onset of the light phase (6:00 hours) and the last after the onset of the dark phase (18:00 hours).

The observations of each aquarium followed the feeding regime adopted, starting immediately after introduction of the ration and lasting 15 min, considering the following behavioural activities: (a) feeding – introduction of artificial feed (ration) in the oral cavity with subsequent ingestion; (b) exploration of the substrate – continuous insertion and removal of chelated pereiopods with the cephalothorax inclined slightly downward; (c) swimming – continuous vertical or horizontal displacement or suspension in the water column through movement of the pleopods; and (d) inactivity – stationary with or without locomotor appendage movement. The behaviours were recorded using the instantaneous focal method every 60 s (Martin & Bateson 2007).

A total of seven replicate tanks were applied for each treatment; the duration of each repetition was 33 consecutive days, with four weekly observations. The experiment included 84 animals and 490 observation hours. Given the non-adherence of the data to normal distribution and the heterogeneity of the factor variances shown by the Shapiro–Wilks and Levene tests, respectively, we used non-parametric statistical analysis. To analyse behavioural activities as a function of feeding frequency, we used the Kruskal–Wallis test while analysis of activities at different feeding times was performed using the Friedman test. For significant differences, we applied the post hoc Mann–Whitney U-test (activity versus feeding frequency) or the Wilcoxon test (activities versus feeding times). The significance level was set at 5%, and the results in graph form were based on median values and inter-quartile range (75–25%). Different letters indicate a statistically significant difference.

Results

Behavioural activities as a function of feeding frequency: feeding, substrate exploration, swimming and inactivity

We observed significant differences in feeding as a function of the three different feeding frequencies [Kruskal–Wallis, \(H(2; 8570) = 312.043; P < 0.05\)]. The shrimp that were fed three times a day were observed ingesting artificial feed more frequently than those that received four or seven feedings (Fig. 1a).

As was the case with feeding, substrate exploration behaviour showed significant differences between all feeding frequencies used [Kruskal–Wallis, \(H(2; 8570) = 380.974; P < 0.05\)], with greater exploratory activity observed in the animals fed three times per day (Fig. 1b).

Swimming showed differences as a function of the three feeding frequencies adopted [Kruskal–Wallis, \(H(2; 8570) = 82.314; P < 0.05\)], the highest levels being found in shrimp that received four daily feedings (Fig. 1c).

There were also differences in inactivity as a function of the three feeding frequencies [Kruskal–Wallis, \(H(2; 8570) = 394.235; P < 0.05\)], with higher inactivity recorded in the animals fed seven times a day than those fed three or four times a day (Fig. 1d).

Behavioural activities as a function of feeding time and different feed frequencies: feeding, substrate exploration, swimming and inactivity

There were differences in feeding according to feeding times for the animals fed three (Friedman, \(\chi^2(2600) = 32.077; P < 0.05\)), four (\(\chi^2(3600) = 90.774; P < 0.05\)) or seven times a day (\(\chi^2(6600) = 74.912; P < 0.05\)). The shrimp that received three daily feedings consumed the most at 12:00 hours: those that received four feedings ingested feed mainly at 14:00 hours. For the shrimp receiving seven daily feedings, artificial feed was ingested more frequently at 8:00 up to 14:00 hours (Fig. 2).

Exploratory activity as a function of feeding times showed no difference for the animals fed three times a day [Friedman, \(\chi^2(2600) = 1.247; P > 0.05\)]. However, there were differences for the animals that received four (\(\chi^2(3600) = 59.740; P < 0.05\)) or seven feedings (\(\chi^2(6600) = 86.983; P < 0.05\)). Exploration activity of the shrimp fed four times a day was high at 6:00, 10:00 and 14:00 hours, decreasing only during the last hour. The shrimp that were fed seven times a day displayed greater exploratory activity of the 8:00 up to 14:00 hours (Fig. 3).

The shrimp showed differences in swimming when they were fed three (Friedman, \(\chi^2(2600) = 20.742; P < 0.05\)) or seven times a day (\(\chi^2(6600) = 54.206; P < 0.05\)). When receiving three feedings, the shrimp swam more at 12:00 and 18:00 hours. The animals...
that received seven daily feedings also showed more swimming activity at 18:00 hours. There were no significant differences in the animals fed four times a day ($\chi^2 (3;600) = 5.198; P > 0.05$) (Fig. 4).

There were differences in the inactivity of the animals when they received three (Friedman, $\chi^2 (2;600) = 34.585; P < 0.05$), four ($\chi^2 (3;600) = 59.352; P < 0.05$) or seven feedings ($\chi^2 (6;600) = 75.338; P < 0.05$). When fed three times a day, the shrimp were more inactive at 6:00 and 18:00 hours; the animals fed four times were more inactive at 18:00 hours. The shrimp that received seven feedings were more inactive at 6:00 and 16:00 hours (Fig. 5).

### Discussion

The behaviours of feeding, substrate exploration, swimming and inactivity varied as a function of the different feeding frequencies.
The ingestion of artificial feed was the highest in the shrimp fed three times a day, indicating greater food consumption by the animals when feeding is more evenly spaced throughout the day. This response could be more advantageous to the shrimp producer in cost–benefit terms.

Corroborating our results, Smith et al. (2002) assessed the effect of feeding frequency on the growth of the black tiger shrimp _Penaeus monodon_ (Fabricius 1798) when they were fed at different frequencies (three, four, five or six times per day), and found no significant differences in growth, food...
conversion and animal survival. Based on these results, the authors recommend that once a nutritionally suitable feed is used, frequencies of more than three times a day provide no additional benefit to the animal.

Carvalho & Nunes (2006) pointed out that the number of feedings adopted on shrimp farms varied as a function of the cultivation method and management system used. These authors subjected *L. vannamei* to different feeding frequencies (two, three, four, five or six times per day) in a grow-out pond and found that when feed rations were adjusted weekly only, using estimated shrimp biomass as the criterion, more than two daily feedings were not advantageous. Only in cases where feeding occurs exclusively in trays, or when low nutritional and physical quality feeds are used, should multiple feedings be considered as a strategy for reducing the food conversion rate.

With respect to preferred feeding times at different feeding frequencies, there was greater ingestion between 12:00 and 14:00 hours for the animals that received three or four daily feedings. Animals fed seven times a day consumed more feed at 8:00 up to 14:00 hours. This shows that feeding of shrimp, in addition to undergoing alterations in the number of feedings, is also influenced by the times at which the feed is made available.

Molina et al. (2000) obtained similar results when they tested *L. vannamei* kept in aquariums and offered artificial feed twice a day at different feeding times (8:00 and 16:00, 10:00 and 18:00, 12:00 and 20:00 or 14:00 and 22:00 hours). They found that the shrimp fed at 12:00 or 14:00 hours consumed more feed and had a higher final biomass, gradually ingesting less food until 24:00 hours. They also observed that the peak of amylase, lipase and protease enzymes occurred at 14:00 hours, independent of feeding time. Thus, the authors suggested feeding the shrimp larger amounts at 12:00 hours, the time at which greater food ingestion was observed and followed by higher digestive enzyme activity.

A lower amount of artificial feed was consumed at 18:00 hours independent of the feeding frequency used (three, four or seven times per day). Because 18:00 hours was the only dark phase time recorded in our study, our results agree with those obtained by Pontes & Arruda (2005a). These authors offered juvenile *L. vannamei* ration four times a day (8:00, 16:00, 20:00 and 4:00 hours) and found that feeding occurred more intensely during the light phase than it did in the dark phase.

Exploratory activity as well as artificial feed ingestion showed higher values for the shrimp fed three times a day. This may be explained by the direct relation between these two behaviours because when searching for food in general, exploration is an important component. Exploration occurred throughout the day and the animals fed four or seven times daily showed a predominance of exploratory activity during the light phase. The lowest activity was recorded at 18:00 hours, in contrast to the animals that received three daily feedings, for which no differences were observed in relation to time.

Pontes et al. (2006), observing *L. vannamei* juveniles fed once a day at random times, showed that substrate exploration occurred both in the light and in the dark phase, suggesting that the search for food occurs in both phases. The animals reached higher peaks 7 h after the onset of the light phase, although our data point to increased exploration up to 8 h after the lights are turned on, after which time exploratory activity decreases.

Swimming was more frequent among the shrimp that received four feedings. This behaviour showed high values, independent of the feeding frequency adopted (three, four or seven times) at 18:00 hours, the only observation time in the dark phase. Pontes et al. (2006), studying the daily behavioural pattern of *L. vannamei* over a 24-h period, found that they swam predominantly in the dark phase.

With respect to inactivity, it was observed that the shrimp were more inactive when they were fed seven times a day. The shrimp that received three or seven feeds remained stationary at 6:00 hours, becoming more active over the course of the day until they once again registered high values for this behaviour at the end of the day. Owing to large energy expenditures during swimming, the shrimp seem to alternate between moments of activity and inactivity. This may be due to the relation between activity/inactivity and oxygen consumption.

Dall (1986) related shrimp activity with oxygen consumption in his study performed with *Penaeus esculentus* (Haswell 1879) to measure the metabolic routine of shrimp through oxygen consumption. He found small fluctuations in O2 consumption during the day when the shrimp were more inactive. At night, when they become more active, oxygen consumption triples.

Primavera & Lebata (2000), based on 25 hourly laboratory observations, also found higher activity levels between *Metapenaeus ensis* (De Haan 1844), *Penaeus latisulcatus* (Kishinouye 1896) and *Fenmerope-
naeus merguiensis (De Man 1888) juveniles during the dark phase. M. ensis and P. latisulcatus juveniles displayed a pattern of burrowing themselves into the substrate during the day and emerging at night. Diurnal burrowing was greater in Metapenaeus than in Penaeus while activities above the substrate (cleaning, feeding, sheltering, inactivity, swimming and crawling) were more frequent in Penaeus than in Metapenaeus species. This pattern was less pronounced in E. merguiensis, which is equally active in the light and dark phases.

Thus, it can be affirmed that feeding frequency influenced the pattern of shrimp activities. The highest feeding and exploration levels resulted from three feedings per day, followed by four feedings. When fed seven times a day, the animals explored less and were more inactive. Given that exploration is an activity related to the search for food, the less frequent feedings (three or four times a day in the light phase) seem to optimize activity patterns directed towards both searching for and consuming food, suggesting that the shrimp should receive three daily feedings. Three feedings per day is beneficial both to the animal and to the producer, who can improve management and achieve better business results.

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