Feeding responses of juvenile shrimp *Litopenaeus vannamei* (Boone) fed at different frequencies under laboratory conditions

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Abstract
In shrimp farms, food partitioning during the course of the day is contradictory; ethology studies may help in determining the proper frequency. *Litopenaeus vannamei* juveniles were placed in 30L aquariums (41 m⁻²), exposed to a 12:12 h light–dark cycle. Feeding was provided at 10% of biomass three, four and seven times per day, with observations made in 15 min windows, initiated after the ration was offered. Latency to access the feeding tray and to start eating was recorded using instantaneous focal sampling and digestive tract filling (DTF) by the continuous focal method. Weight gain was recorded at the end of the experiment. We performed seven repetitions, with 28 individuals observed for 33 days for each treatment (490 h of observation). The three-times offering induced less latency for accessing the tray and for ingesting the feed as well as a higher DTF when compared with the other frequencies. Animals fed three and four times had similar weight gains, and were greater than those fed seven times. Our results indicate that a more spaced food offering stimulates the search for and ingestion of feed. As compared with other frequencies, the three-times-per-day option assumes lower labour costs and a more efficient use of the feed.

Keywords: activity, applied ethology, behaviour, shrimp, *Litopenaeus*

Introduction
In Brazil, the marine shrimp *Litopenaeus vannamei* (Boone) is cultivated using the semi-intensive methodology, characterized mainly by the use of ponds dug into the ground, by the offering of ration as a complement to the natural food of the pond and sometimes by the use of paddle-wheel aerators at times of insufficient dissolved oxygen. The shrimp are kept in these ponds for about 3 months (PL₀ for juveniles), then harvested at about 12 g and sent to market.

Shrimp is generally fed in feeding trays. Feed amounts and feeding times are determined by the farmer's operational criteria. Circular feeding trays are made from the inner edge of car tyres with a 1.3-mm-diameter mesh net fixed to the inner wall. These are placed on poles and introduced across the length of the pond at 30 units ha⁻¹ (Nunes & Sandoval 1997). The number of feeding trays introduced in a pond is also dependent on shrimp stocking density, which can vary from 5 to 60 shrimp m⁻². Bador (1998) considers the use of feeding trays as a way of controlling feed costs and decreasing the ecological changes that may result from uneaten feed left at the pond bottom.

In shrimp farming, feed is commonly offered two or three times per day. Ration is determined by feed tables, which mainly consider stocked shrimp biomass. Rations are also determined through daily recording of uneaten feed left in the feeding tray. Because this practice does not consider the shrimp physiological factors involved in the feeding, inefficient use of the feed may result, with consequent economic and environmental losses. Jones, Le Vay and Mohd (1993) speculated that information is needed on feeding behaviour, feed ingestion, digestion, energy needs of the shrimp and the nutritional content of the feed to determine feeding requirements of shrimp.
Shrimp feeding research have mostly been developed in ponds, evaluating the different types of rations or feeding management and the results have focalized variables that are indicative of feeding responses, such as weight gain, survival and feed conversion rate (Martinez-Cordova, Porchas-Cornejo, Villarreal-Colemaures, Calderon-Perez & Narango-Paramo 1998; Nunes & Parsons 1998; Cuzon, Lawrence, Gaxiola, Rosas & Guillaume 2004).

One of the approaches for optimizing feed use in shrimp ponds involves determining the circadian rhythm of the animals' digestive enzyme activity. Feed is then offered at times that are more favourable to its consumption and metabolic use (Molina, Cadena & Orellana 2000; Gamboa-Delgado, Molina-Poveda & Cahu 2003). Akyiama and Polanco (1997) recommended four-feedings per day, with each farmer adjusting ration according to pellet water stability. According to the authors, feed attractability proprieties are lost in about 2 h after being introduced into the water.

Studies on feeding frequency and its effects on shrimp growth are conflicting. Sedgwick (1979), Robertson, Lawrence and Castille (1993) and Tacon, Cody. Conquest, Divakaran, Forster and Decamp (2002) showed a positive effect on shrimp growth when feed frequency was increased. On the other hand, Velasco, Lawrence and Castille (1999), Smith, Burford, Tabrett, Irvin and Ward (2002) and Carvalho and Nunes (2006) found no direct relation between these two factors.

According to Huntingford (1991), maximum gains can be obtained in the commercial exploration of living organisms through specific methodology obtained in researches in the area of animal behaviour. Even though the behavioural activities of shrimp have been sporadically investigated in the laboratory (Rodriguez & Naylor 1972; Hindley 1975; Wickham & Minkler III 1975; Robertson et al. 1993; Primavera & Lebata 1995; Guerra & Ribera 1996; Pontes 2006), those related to feeding behaviour in general have only explored hierarchical aspects of those animals' responses to chemical stimulants (Costero & Meyers 1993; Pittet, Ellis & Lee 1996), and few studies have fallen within the area of applied ethology (Pontes & Arruda 2005a, b).

The study of the behavioural activities of *L. vannamei* in relation to feed offered in trays may help guide feed management in shrimp farming. Thus, the purpose of this paper was to study the behavioural activities of juvenile marine shrimp *L. vannamei* in relation to commercial pellet feed delivered in feeding trays at different frequencies over the course of the day.

### Materials and methods

*Litopenaeus vannamei* juveniles, aged 2 months old with a mean body weight of 8.04 ± 1.5 g (n = 135), were obtained from Papeba shrimp farm, located 52 km from the city of Natal, in the northeastern region of Brazil. The experiments were carried out under laboratory conditions, using glass aquariums (50 × 30 × 40 cm), containing 30 L of seawater in a closed-water recirculation system with constant aeration and continuous filtration through gravel and crushed oyster shell. Water temperature remained at 28 ± 1 °C and salinity at 34 g L⁻¹ throughout the experiment.

The aquariums were covered with a glass lid to avoid the loss of animals, which were exposed to a 12:12 h light–dark cycle (light from 06:00 to 18:00 hours), using white fluorescent lights (32 W) for the light phase and red incandescent lights (15 W) for the dark phase (Rodriguez & Naylor 1972; Hindley 1975), with a light intensity of 180 and 11x respectively.

Five shrimp (41 m⁻²) were placed in each aquarium. To permit behavioural recordings of each individual, four of the five shrimp were marked with different-coloured silicone rings on the ocular peduncle. They were fed pellet ration (35% crude protein), delivered in transparent acrylic trays (4 × 3 × 2 cm), at 10% of the tank biomass, and partitioned at frequencies of three (06:00, 12:00 and 18:00 hours), four (06:00, 10:00, 14:00 and 18:00 hours) or seven (06:00, 08:00, 10:00, 12:00, 14:00, 16:00 and 18:00 hours) times per day. In all treatments, the first feeding occurred immediately after the start of the light phase (06:00 hours) and the last after the start of the dark phase (18:00 hours). Ration was placed into the feeding trays through a 1.27-cm-diameter PVC tube to avoid losses and the remnants were removed from the water after 1 h of exposure.

Behavioural recordings began after a 9-day acclimation period. Ingestion of the material, involving the passage of feed from the oesophagus to the pyloric stomach, was recorded every 60 s using the instantaneous focal sampling. The following variables were recorded using continuous focal sampling: (1) feed ingestion, (2) latency to access the feeding tray, (3) latency to start eating. The focal animal sampling
records all instances of determined behavioural categories during an established amount of time (windows), in intervals (instantaneous sampling) or continuously (continuous sampling) along the observation window (Martin & Bateson 1999).

Digestive tract filling (DTF) (Pontes & Arruda 2005b) was recorded at the beginning and at the end of the observation session. Digestive tract filling was based on the amount of feed visualized by the naked eye in the proventriculus, which was classified according to the following filling levels (Pontes & Arruda 2005b): no feed = 1; low filling = 2; medium filling = 3 and completely full = 4 (Fig. 1a–d), used in order to indicate feed ingestion.

The latencies were determined according to Martin and Bateson (1999), considering the time elapsed between the presentation of the stimulus (ration) and the individuals accessing the tray and the onset of feed ingestion. Mean weight gain (g) was also recorded, based on the differences between final individual weight and initial mean weight.

A total of seven repetitions were performed for each treatment along 33 continuous days each, under similar conditions. Observations were made 4 day week 

\[-1\] in 15 min \n
\[h^{-1}\] windows, initiated immediately after ration offer, for a total of 28 individuals observed for each treatment. At frequencies of 3, 4 and 7 feedings day 

\[1\], the recordings occurred every 2, 4 and 6 h over a 12-h period totalling 105, 140 and 245 h of observation (total = 490 h) respectively. In all parameters, reliability tests performed by the observers produced 93% accuracy.

Given the non-adherence of the data to normal distribution and the heterogeneity of factor variances verified by Shapiro–Wilks and Levene tests, respectively, Kruskal–Wallis non-parametric variance analysis was used. When significant differences were found, the post hoc Mann–Whitney \[U\]-test was applied (Steel & Torrie 1988). A significance level of 5% was set to assess the results, which are graphically represented by their median and interquartile ranges (75–25%).

### Results

Statistically significant differences were observed in feed ingestion behaviour between treatments (\(H(2.33) = 296.05; P < 0.05\)), with the highest and lowest ingestion levels found at feeding frequencies three and seven times a day respectively (Fig. 2).

The time the animals spends to access the tray after feed offer was influenced by the feeding frequencies used (\(H(2.33) = 73.86; P < 0.05\)) (Fig. 3). While three offers per day induced lower latency for

![Figure 1](image1.png) Digestive tract filling based on the amount of feed visualized in the proventriculus, classified according to the following levels: (a) no feed; (b) low filling; (c) medium filling; and (d) completely full (Pontes & Arruda 2005b).

![Figure 2](image2.png) Feed ingestion behaviour in Litopenaeus vannamei according to feed offer at different frequencies. Different superscript numbers under the box plots denote significant difference (\(a < 0.05\), Mann–Whitney \[U\]-test).

![Figure 3](image3.png) Latency (s) for Litopenaeus vannamei accessing the tray after feed offer at different frequencies. Different superscript letters under the box plots denote significant difference (\(a < 0.05\), Mann–Whitney \[U\]-test).
accessing the tray, seven offers led to greater latency. Similar results were observed for consumption 

\( H(2.33) = 245.38; P < 0.05 \), where animals fed three times per day started ingestion more quickly than those receiving the remaining treatment frequencies (Fig. 4).

Digestive tract filling varied according to the treatments applied 

\( H(2.3258) = 245.2959; P < 0.05 \). Shrimp fed three times per day had higher levels than those recorded for the remaining frequencies, with seven times per day resulting in the lowest levels (Fig. 5).

The shrimp had varying weight gains according to the treatments applied 

\( H(2.45) = 17.51; P < 0.05 \). The animals given three and four feedings per day had significantly similar and higher levels than those fed seven times a day (Fig. 6).

**Discussion**

Our results showed that feed delivered in feeding trays three times per day resulted in greater ingestion and higher DTF as compared with frequencies of four and seven times. The animals accessing the tray and starting ingestion were inversely proportional to the number of daily feedings. Animals fed three times a day had similar results to those fed four times, whereas a frequency of seven times led to a lower weight gain.

Corroborating these results, Josekutty and Jose (1996) observed that juvenile *Penaeus monodon* had similar weight gain when fed three or four times per day, with values higher than those fed once and twice. However, Velasco et al. (1999) did not observe differences in the growth or survival of *L. vannamei* fed three, five, eight, 11 or 15 times over a 20-day period. Smith et al. (2002) studied the effect of four feeding frequencies (three, four, five and six feedings a day) on the growth and survival of the shrimp *P. monodon* fed with a commercial pellet feed placed on feeding trays. These researchers concluded that feeding frequency had no effect on growth rate, feed conversion ratio, shrimp survival and water-quality parameters, suggesting that there is no benefit from feeding *P. monodon* more frequently than three times per day when using a feed that is nutritionally adequate and has high water stability.

Similarly, Carvalho and Nunes (2006), in a study on the shrimp *L. vannamei* fed commercial pellet feed delivered exclusively in feeding trays two (at 07:00 and 17:00 hours), three (at 07:00, 11:00 and 15:00

![Figure 4](image_url) Latency (s) for *Litopenaeus vannamei* consuming the feed after it was placed in the tray at different frequencies. Different superscript letters under the box plots denote significant difference (\( \alpha < 0.05 \), Mann–Whitney U-test).

![Figure 5](image_url) Digestive tract filling index for *Litopenaeus vannamei* at different frequencies to feed offer. Different superscript letters under the box plots denote significant difference (\( \alpha < 0.05 \), Mann–Whitney U-test).

![Figure 6](image_url) Weight gain (g) of *Litopenaeus vannamei* according to feed offer at different frequencies. Different superscript letters under the box plots denote significant difference (\( \alpha = 0.05 \), Mann–Whitney U-test).
hours), four (at 07:00, 10:00, 13:00 and 15:00 hours),
five (at 07:00, 09:00, 12:00, 15:00 and 17:00 hours)
and six times (at 07:00, 09:00, 11:00, 13:00, 15:00 and
17:00 hours) per day; observed that no shrimp perfor-
mance benefit could be detected by adopting higher
diurnal feeding frequencies. A different result
was found by Tacon et al. (2002), who investigated
*Litopenaeus vannamei* for 56 days at frequencies of four times
in the light phase, four times in the dark phase or
eight times over the entire day (light and dark), and
found higher growth in shrimp fed eight times.

Martinez-Cordova et al. (1998), using three differ-
et feeding practices: (1) feeding tables, (2) feeding
trays and (3) complement to natural food, in the cul-
ture of shrimp *Litopenaeus californiensis*, concluded
that the best individual weight gain was obtained
with treatment (3), followed by treatment (2). An-
other experiment conducted to determine the ef-
effect of the mechanical feed dispersal device of feeding
trays showed that cultured shrimp fed by feeding
trays had greater mean weight and yield than those
fed by mechanical dispersal. The amount of feed
added, shrimp survival and feed conversion were
not significantly different between the two feeding
strategies. This study also showed reduced nitrogen
and phosphorous loss in comparison with me-
chanical feed dispersion (Casillas-Hernández, Magallón-
Barajas, Portillo-Clark & Páez-Osuna 2006).

Concerning preferential feed offer times, Molina
et al. (2000) observed *L. vannamei* fed ration twice a
day for 15 days at 8:00 and 16:00 hours; 10:00 and
18:00 hours; 12:00 and 20:00 hours and 14:00 and
22:00 hours, and found that shrimp fed at 12:00 or
14:00 hours consumed more feed and had heavier fi-
nal biomass. These authors verified that peaks of
amylase, lipase and protease enzymes occurred at
14:00 hours, regardless of feeding time. Nunes and
Parsons (1998) verified that for *Farfantepenaeus subti-
lis*, feeding at 14:30 and 9:30 hours induced greater
consumption as compared with 6:00 hours. Previous
results on behavioural and feeding activities as a
function of feed offer suggest that feed must be of-
fered in the light phase and at a greater proportion
7 h after sunrise (Pontes & Arruda 2005b; Pontes,
Arruda, Menezes & Lima 2006). Substrate explora-
tion behaviour, indicative of the search for feed, was
more intense around this time (Pontes 2006).

According to Krebs and Davies (1993), the overall
success of an individual at passing on its genes may
depend on finding enough food, choosing a good
place for reproduction, attracting many mates, and
so on. In solving any of these problems an animal
makes decisions, which can be analysed in terms of
an optimal trade-off between appropriate costs and
benefits. For a foraging animal, for example, curren-
ties might be energy and time. Cuthill and Houston
(1997) state that an organism must acquire and store
reserves not only for occasions when food is not
available, but to fuel periods when it cannot feed due
to performance of other behaviours. A full analysis of
daily feeding patterns is thus likely to have to take
into account not only temporal variation in the costs
and benefits of feeding but also temporal variation in
the costs and benefits of other behaviours.

In conclusion, the search for more effective feeding
management systems must take into account, among
the factors involved, optimizing the animals’ expres-
sion of their behaviour. In this sense, we registered
a shorter time to access the tray and feed consump-
tion, the higher DTF index and a greater weight gain,
associated with a three-times-per-day feed offer. These
results suggest that more spaced feeding over the
course of the day may stimulate the animals to
search for and ingest feed. In comparison with greater
frequencies, this practice assumes lower labour
costs and a more efficient use of the feed offered,
which could be converted into economic advantages
for shrimp producers.

Acknowledgments

We thank the National Research and Development
Council (CNPq), the Papeba shrimp farm and the
Department of Physiology of the Universidade
Federal do Rio Grande do Norte.

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