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Short Communication

Evaluation of different maze systems for the determination of feed attractability for longarm river prawn *Macrobrachium tenellum*

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ABSTRACT. Previous studies have shown that crustacean feed attractability has an enormous importance, because the consumption, quality and feed conversion rate can be improved, by reducing the time of residence pre-ingestion and the leaching of nutrients. For this reason, different protocols and methods has been developed to measure feed attractability. This study evaluated the use of maze type Y, and rectangular systems to determine the attractant power of a commercial feed on longarm river prawn *Macrobrachium tenellum*. The Y type maze system without barriers, and with three different types of barriers (with or without access to the area where a commercial feed) was evaluated. Ten prawns were placed in the acclimation chamber of the system at 28°C for 60 min before the start of the experiment. To start the test, commercial feed (20% of prawn biomass) was placed into the system feeding area, then the acclimation chamber was open to allow for the free prawn movement, evaluating the feed attractability by measuring the time for the first "hit", total number of hits, and the number of prawns which entrances to the feeding area during 15 min. Similar tests were performed with a rectangular type maze system, comparing the atractability results obtained in both systems. The results presented here highlight the importance of the genus behavior and the selection of protocols and systems, as well as the materials used in its maze system construction, for attractability testing in *M. tenellum*.

Keywords: *Macrobrachium tenellum*, prawn, feed formulation, attractants, feeding behavior, Y type maze.

The elaboration of attractive animal feed for species in aquaculture, allows to minimize searching time and maximize the proportion of ingested feed and thereby reduce costs, to promote feed conversion rates, and to reduce feed waste (Nunes *et al*., 2006; Tantikitti, 2014). The evaluation of the feed attractability is basic to improved feed ingestion under low visibility conditions in cultivation ponds. Freshwater prawns of the genus *Macrobrachium* have the chemosensory systems for feed location and intake (Harpaz *et al*., 1987; Suresh *et al*., 2011). The study of feed chemoattractability in crustaceans has shown different results that can be due specific species behavior, but also could be attributed to the experimental methodological inconsistencies (Sánchez *et al*., 2005). There are different maze aquariums systems where organism behavior under one or several attractant stimuli has been tested (Montemayor-Leal *et al*., 2005; Ali *et al*., 2007; Suresh *et al*., 2011; Sacristán *et al*., 2014), including Y type and rectangular type maze systems.

All types of systems (static or with water flow, with or without barriers, with free feed or into a container) are used to observe the attractant preference of organisms towards a particular feed, ingredient or specific type of chemical compounds (commercial attractants, animal and plant extracts, amino acids, nucleotides, amines, pheromones, etc.). In general, the responses are quantified by animal behavior reactions and/or the number of organisms that arrive at the source of chemical stimuli in a certain time.

Most of the studies on this subject have been directed to penaeidae shrimp (marine); however, on *Macrobrachium* genus the evaluation of feed attractants is limited. Because *M. tenellum* is one of the most economically important native species in Mexico (Pérez-Velázquez *et al*., 2011), this work aims to evaluate different attractability systems to establish a proper methodology for studies of chemoattraction of feed and feed ingredients in prawns. To date, there are no studies that evaluate systems of feed attractability in prawn of the genus *Macrobrachium* and take into account the behavior of the genus in the obtained results.

Attractability tests were held at the facilities of the Laboratorio de Calidad de Agua y Acuicultura Experimental de la Universidad de Guadalajara, en Vallarta, Jalisco, Mexico. Juvenile organisms of longarm river prawn *M. tenellum* were used (1 to 2 g of weight) collected in the stream El Zarco, in Puerto Vallarta. The prawns (70) were acclimated for 15 days in glass aquariums of 40 L capacity, with waterfall filters (Elite®) and temperature of 28°C, with a feeding regimen of commercial shrimp feed (Camaronina®, Purina®, protein 35%, humidity 12%, fat 8%, crude fiber 5%, ash 10%, nitrogen free extract 30%) on a fixed feeding schedule of 10:00 to 13:00 h. Prawns in intermolt phase were selected to avoid any possible interference of this phenomenon on the process of perception, as it is noted by Montemayor-Leal *et al*. (2005). The prawns were fasted for 24 h prior to attractability bioassays in order to emphasize the response (Nunes *et al*., 2006; Jaime-Ceballos *et al*., 2007) in all experiments, water was kept at 28ºC, and with a 100% water exchange in every trial.

For the first chemoattractability bioassay, a Y type maze system was used (Fig. 1a). The aquarium Y type maze system has an acclimatization section (R), in which prawns are placed before starting tests. The R area has a retainer gate that can be removed to give to the pawns access to the neutral zone (N), after that there are two arms (sections A and B) with or without a dividing walls (DW) as a barrier, forming at the end of the arm the feed chamber (C), in where the feed materials to be tested are placed. In this bioassay, no DWs were used, in order to give free access to the prawns to sections A and B. Ten prawns were placed in the section R in the Y type maze system, to carry out its acclimatization (constant aeration and temperature of 28°C) for one hour prior to the beginning of the experiment. A camera (GoPro® Hero 3) was placed to register prawn behavior. The commercial feed was placed as attractive material (20% of the total biomass of organisms) in section C, using an aeration stone attached to a pump to promote the dispersion of the soluble molecules of the feed along the Y maze for 10 min. After removing the retainer gate of section R, the video recording was held for 15 min. Each test was performed in triplicate. The videos were subsequently analyzed with Quick Time Player (version 10.1). The videotaping was conducted to quantify the number of prawns accessing to section C for 15 min.

The second bioassay was performed to assess the effectiveness of three different DW in the Y type maze system (Fig. 1b): Each DW were rectangular (15×19 cm) with the following designs and materials: a) mesh with a conical shape plastic (PET) access port (6 cm in diameter for input and 2 cm of diameter for output), so the prawns would have access to the tested feed; b) acrylic with small holes (3 cm in diameter) to allow the water flow, and with truncated pyramid-shaped acrylic access port (5×4 cm of entrance and 3×2 cm for the exit) that allows, to the prawns access to feed; c) acrylic with small holes (3 cm in diameter), but without entrance, with no access to feed. The feed attractability was evaluated through the analysis of the videos, recording the total number of "hits" (contact of the prawn with their legs or *rostrum* with the DW during the 15 min of testing) (Fig. 1a). Also, it was established the time required for the first "hit". Additionally, the number of prawns accessing to section C for 15 min (only for the DW that have access entrance) was recorded.

Another test was performed with a conventional rectangular type maze system (Jaime-Ceballos *et al*., 2007) divided by two internal barriers (forming three compartments), which have an opening at the top (Fig. 2a). Ten prawns were placed in the central part of the maze system, having access to the other two sections through the openings. The feed was deposited randomly in one of the two feed areas of the aquarium (20% of the prawn total biomass). The videotaping was conducted to quantify the prawns that moved to each of the areas during 15 min.

The fourth test was conducted with rectangular type maze system used by Suresh *et al*. (2011) with a DW (opaque) between the A and N, as allow the prawns access to feed (Fig. 2b). A video camera (GoPro®) was placed at the front of the aquarium to register the prawn behavior. Ten prawns were placed in the R section for acclimatization at 28°C for 60 min, prior to the beginning of the trial. To start, the commercial feed (20% of the biomass of prawns) was placed at the end of the region A. After 5 min the gate of R section was removed, to allow access to the prawns to A section for 15 min. The videotaping was observed to record the total number of hits, the time required for the first hit, and the number of prawns which entered in section A, at the end of the 15 min trial. The evaluation of the attractability was repeated six times. The attractability results obtained in the rectangular type maze system were compared with attractability data obtained with Y type maze system with acrylic barrier and entrance; also the results of the use of barriers in the Y type maze system were analyzed statistically using one way variance (ANOVA) previous normality test (Shapiro-

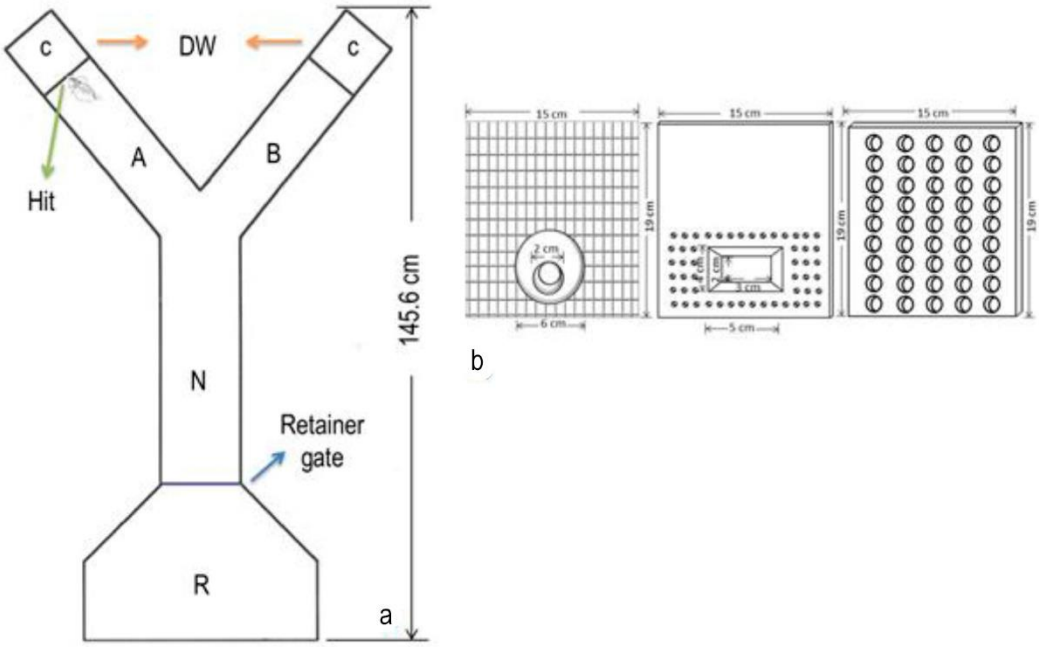

Figure 1. a) Y type maze system with the representation of the hit, b) barriers used in the experiment (from left to right): mesh with entrance, acrylic with entrance, and acrylic without entrance.

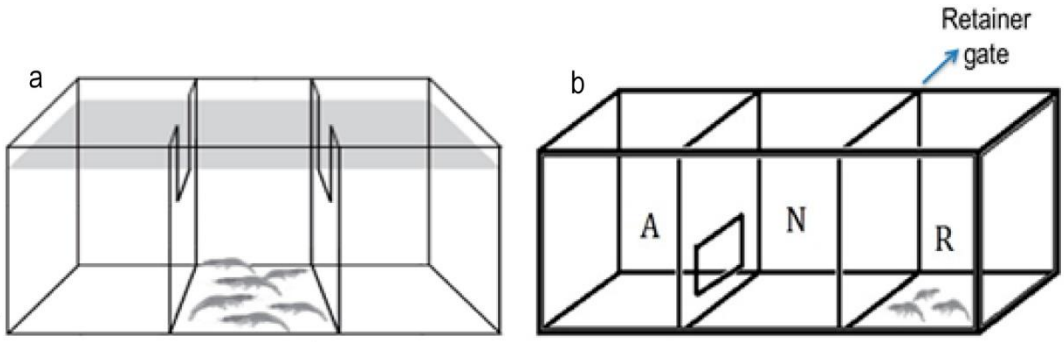

Figure 2. Rectangular type maze systems used in the third and fourth bioassay. a) According to Jaime-Ceballos *et al*. (2006), to start the test, the feed is randomly placed in right or left section, b) according to Suresh *et al*. (2011), to start test, feed is placed in section A, then the gate between R and N sections is removed.

Wilk; $\alpha = 0.05$) homoscedasticity (Bartlett; $\alpha = 0.05$). The differences were considered statistically significant when $P < 0.05$. The data were analyzed with the statistical program Sigma plot 11.0.

In the evaluation of the Y type maze system, with any of the barriers, no significant differences of attractability were found ($P > 0.05$) by evaluating the total number of hits, time required for the first hit and the number of prawns which entered through the barriers to feed section (C) (Table 1). In the comparison of the Y type maze system (with acrylic barrier and entrance) and the rectangular type maze system with a single barrier, significant differences were found in the number of prawns which entered into the feeding section, of each of the systems (Table 1).

The response of crustaceans to the food effectors have been studied due to its relevance in the understanding of feeding behavior and their applications in aquaculture (Sánchez *et al*., 2005; Smith *et al*., 2005; Nunes *et al*., 2006; Grey *et al*., 2009; Tolomei *et al*., 2013; Sacristan *et al*., 2014). It is necessary to emphasize that the literature about feed stimulants for prawns of the genus *Macrobrachium* is limited, compared to works that have been made about marine shrimp or other crustaceans. For attractability experiments carried out with *M. rosenbergii* a simple

Table 1. Comparation of attractability values (average ±SD) between tested maze systems.

Systems or aquarium		Number of observations	Time for the first hit (seconds)	Total hits	Number of entrances
Maze Y	without barriers	3	-	-	23.20 ± 6.26
	mesh	6	126.67 ± 115.20	17.67 ± 4.32	0.60 ± 0.89
Maze Y	acrylic with entrance	6	53.50 ± 35.54	22.67 ± 13.32	2.40 ± 1.67[b]
	acrylic without entrance	6	74.67 ± 33.25	24.67 ± 6.56	-
Rectangular system (Suresh *et al*., 2011)		6	108.00 ± 89.91	25.20 ± 12.56	9.80 ± 5.17[a]

The different superscripts indicate significant statistical differences ($P < 0.05$).

rectangular type aquariums, have been used; where the feed (stimulus) is placed at one end, while the organism is placed in the opposite extreme. Authors found significant differences among the treatments (evaluating attractants such as free amino acids, nucleotides and biogenic amines and pheromones) (Harpaz *et al*., 1987; Mendoza *et al*., 1997). Lee & Meyers (1996) highlighted some of the limitations of the used methodologies, commenting that there is a need to perform several tests under different conditions and type of aquariums to avoid misinterpretation and to improve the methodology to select the best ingredients to develop a stimulating useful feed.

The first bioassay to validate the use of the Y type maze system was carried out according the methodology used by Nunes *et al*. (2006) who evaluated attractability of commercial ingredients in *Penaeus vannamei*, placing the ingredient freely at the end of each of the arms without using any kind of DW, allowing the entry of the prawn into the feeding area. However, based on what was observed in the present study, the agonistic behavior of the longarm river prawn *M. tenellum* must be considered, since they are territorial animals and the confluence of several specimens near the feed, causes aggressive behavior and the escape of some of them, without accessing C or otherwise taking a piece of food and withdrawing immediately (which causes that the source of stimulus is also displaced). For this reason, the feed attractability evaluation in *M. tenellum*, was tested using a maze systems with a DW.

In the second bioassay where different types of DW in the Y type maze were evaluated, it was noted that the longarm river prawn, tend to move around the aquarium, regardless of where the food was located. Coinciding with Sacristan *et al*. (2014), who finding no significant differences by incorporating, in different percentages, squid meal in feed for *Cherax quadricarinatus*, and proposed the hypothesis that freshwater decapods mainly find feed due to the time spent roaming in the aquarium.

In the third bioassay, it was used the rectangular type maze aquarium described by Jaime-Ceballos *et al.* (2007), who evaluated *Spirulina platensis* meal as an attractant for *P. schmitti* finding that 68% of shrimps moved to the *Spirulina* feed chamber, and kept eating the feed, while the rest moved to the control feed chamber. In our study was observed shows that the residence time of the prawns in the acclimation area was very brief, moving immediately to any of the two chambers (even when the feed had not been placed in the system). Therefore the use of this rectangular type maze aquarium was not considered suitable for the feed attractability evaluation for *M. tenellum*.

The fourth bioassay was conducted with a rectangular maze system with a single feeding chamber and having a DW with open access used by Suresh *et al*. (2011) to evaluate the attractability of different types of protein ingredients in *P. stylirostris*. In the comparison of the Suresh *et al.* (2011) system with the Y type maze system, just considering the number of entries into the feed chamber, is likely that used DW material interferes with the chemical signs perceived by the prawns, or the area of acclimation of the Y type maze system is very large. Because it is observed that when the prawns come to the DW their receptors play a fundamental role, since as soon as they detect the presence of it (both by tactile stimuli or light reflections generated by the acrylic barriers) most of the time go backward or move away (usual behavior demonstrated in the face of threat), this can be observed in the difference of the amount of prawns that enter the area C when the maze is used without DW. In addition, prawns preferably tend to stay in the R zone, which apparently is considered as a safe area than the Y arms areas, even if there is a higher concentration of chemical compounds of feed in arm areas.

It is possible that the results obtained in the present study could be related with the behavior of the longarm river prawn that is quite different from the marine shrimp. It is suggested that the process of adaptation of prawns from fresh water has resulted in many different

adaptations due to competition, predation, habitat complexity, biotic interactions and events of disturbance (Covich *et al*., 2015) provoking, in our point of view, not only speciation, but suggesting the evolution of an elaborated agonistic feeding behavior. According to Crowl & Covich (1994), freshwater decapod crustaceans have a high capacity to receive and interpret feed chemical and physical (tactile) stimuli and react with aggressiveness against others including its congeners in a way that confers an adaptive advantage to getting feed. In this sense the acceptance of the prawns to attractive molecules are not only influenced by the intensity of such chemical signals, but also by the environment in which animals are maintained (changes in the intensity and quality of light, the hydrodynamics or direct stimulation), and relationships between them.

In the evaluation by the hit as attractability indicator, due that no significant differences were found in Y type maze system with any of the three tested of DW, or in its comparison with the rectangular system, any could be used to determine the attractability of feed for *M. tenellum*. For evaluate attractability by the number of pawns that enter into the feeding chamber, it is recommended to use an opaque material in the DW (provoking less disturbing of normal behavior). Another option is not use DW, however it would be necessary to use some feed container, which allow dispersion of attractant chemical compounds. The results presented here highlight the importance of the genus behavior and the selection of protocols and systems, as well as the materials used in its maze system construction, for attractability testing in *M. tenellum*, to the search for adequate responses related to prawns feeding behavior.

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