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ORIGINAL ARTICLE

Evaluation of the rheotaxis behavior of juveniles of the prawn *Macrobrachium tenellum* Smith, 1871 (Decapoda: Palaemonidae) under laboratory conditions

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ABSTRACT

Understanding the upstream migration of amphidromous prawns is important to address the impact of anthropomorphic activities in natural freshwater ecosystems. The ability of *Macrobrachium tenellum* (Smith, 1871) juveniles to overcome an artificial barrier was evaluated, simulating an obstacle in their upstream migration, under an experimental laboratory system. The prawns were collected from a stream located in Puerto Vallarta, Mexico (N = 1000). An experimental recirculation system was used, which consisted of a tank with a capacity of 600 L, a sheet of galvanized steel that acted as an anthropogenic barrier and two submersible electric pumps with flows of

0.12 L/s and 0.40 L/s. In this system, two inclinations of the barrier were tested, 45° and 90° in combination with the two flows. The results show that there are significant differences between the treatments with different inclinations, where a higher stimulation response was observed at the 90° angle. Within this inclination, the flow of 0.40 L/s produced the highest stimulation to climb the barrier in prawns between 30 and 60 mm in length. This study demonstrates the direct relationship between the slope and the effect of water flow of the anthropogenic barriers that hinder the migration process of *M. tenellum*.

Keywords
Anthropogenic barriers, amphidromy, Caridea, migration, upstream river

Introduction

Approximately 25 % of the described species of caridean decapods (Infraorder: Caridea Dana, 1852) are from freshwater habitats (De Grave *et al.*, 2008). A large number of these species migrate at some stage in their life cycle (*i.e.*, diadromy, but most notably amphidromy), mainly in the caridean families: Atyidae De Haan, 1849; Xiphocarididae Ortmann, 1895; and Palaemonidae Rafinesque, 1815 (De Grave *et al.*, 2008; Bauer, 2013). In this last family, the genus *Macrobrachium* Spence Bate, 1868, stands out due to its economic importance (New *et al.,* 2010; Rodríguez-Flores *et al.*, 2012).

Macrobrachium americanum (Spence Bate, 1868) and *Macrobrachium tenellum* (Smith, 1871) are species that support traditional fishing in coastal populations of rivers and coastal lagoons in many areas of Mexico, El Salvador and Guatemala (García-Guerrero *et al.*, 2013), where they are fished for self-consumption or local sale. *Macrobrachium tenellum* takes advantage of the rainy season to descend with the current flow of rivers and streams to coastal areas, where females spawn (Espinosa-Chaurand *et al.,* 2011; Vega-Villasante *et al.*, 2014). The early stages of development (from larvae to juveniles) inhabit brackish waters, and juveniles start an upriver migration towards freshwater areas (Bauer, 2011; Rodríguez-Uribe *et al.*, 2014).

The migration of amphidromous prawns has a very important ecological role, since it contributes to the energy flow in the form of biomass, generating an impact on trophic interactions (Novak *et al.,* 2017). Another important role is that on their way upriver they are prey for other organisms, as they are the main component in the diet of local fish and birds (March *et al.*, 2003). During this upriver migration, they often encounter impediments, mainly of anthropogenic nature (pollution, natural systems modifications, human intrusion and disturbance, invasive species, and mining), which can block and interrupt the migratory path of juveniles (Bauer, 2011), resulting in a decrease in their populations which, in the long term, represents a serious extinction risk for freshwater prawns (De Grave *et al.,* 2015). The effect of river control structures in the upstream migration of amphidromous prawns has been studied in general and Jarvis and Closs (2019), suggested that instream barriers inhibit the upstream migration of post-larvae and juveniles, but climbing abilities are common in those life-stages, allowing for creative solutions facilitating upstream migration. Olivier *et al.* (2013) tested water velocities and ramp angles that *Macrobrachium ohione* (Smith, 1874) juveniles are able to climb up; their results suggest that higher velocities (65 and 140 cm s^{-1}) and inclinations of 30 – 40° are favorable to increase climbing performance. Novak *et al.* (2017) studied the upstream migration of *Macrobrachium spinipes* (Schenkel, 1902) in Australia, and their two-year study revealed that juveniles migrate upstream during extended periods of declining discharge of the Daly River during the wet season. They also suggested that the migration of amphidromous prawns does not transport significant amounts of marine-derived energy and nutrients across the marine/freshwater ecotone. All these studies are important to understand rheotaxis behavior and to evaluate strategies to increase the climbing performance necessary to facilitate the upstream migration and prevent the extinction of amphidromous prawns.

Since information about rheotaxis and climbing performance in *M. tenellum* is lacking, this experimental laboratory study aims to provide information on the ability of *M. tenellum* juveniles to overcome natural and/or artificial barriers during their upstream migrations.

Materials and Methods

Specimen collection

Juveniles of *M. tenellum* were collected at Arroyo El Zarco (20°41'02"N 105°13'50"W), located in Puerto Vallarta, Jalisco state, western-central Mexico, in the canals that lead to the El Salado estuary.

The prawns were transferred, using a 1000 L tank with constant aeration, to the facilities of the Laboratorio de Calidad de Agua y Acuicultura Experimental (LACUIC) of the Centro Universitario de la Costa of the University of Guadalajara (CUC-UDG), where they were divided into two tanks with a capacity of 400 L each. Before starting the assays, prawns were fed for a period of seven days with commercial feed (Purina®; 35 % protein and 5 % fat). Partial water changes (30 %) and removal of feces and uneaten food were carried out on a daily basis.

Preliminary migration stimulation bioassays

To evaluate the rheotaxis behavior in juvenile prawns, water flows of 0.10 L/s–0.50 L/s were initially tested, and different types of ramp materials were also analyzed. Ramp materials, such as wood, concrete and galvanized metal sheets, that have been previously used in overpasses in water courses to facilitate the migration of aquatic fauna, mainly fishes and crustaceans (MARM, 2009) were considered. Two ramp lengths, 1.5 m and 2 m (Martínez de Azagra-Paredes and García-Molinos, 2003; MARM, 2009) and three inclinations (30°, 45° and 90°) were also tested. The tests were carried out with a total of approximately 1000 individuals measuring 10–70 mm (total length was measured from rostrum to telson with a Vernier caliper), with rest periods of 24–48 h between each trial. Preliminary experiments were run during seven non-consecutive days, in periods with different natural sunlight (morning, afternoon and sunset). A digital photometer (AMPROBE® LM631A) was used for monitoring the luminosity and a multiparameter (YSI® 550[a]) for measuring the water temperature. Light intervals from 0 (7:00 h, 20:30 h) to 1850 lux (14:00 h) were measured. An average of 10 tests with 15–50 min duration per day were carried out during a week, with a total of 70 tests. A log was kept where the most outstanding observations (high activity, migratory behavior, etc.) were noted.

Experimental design

With the results obtained from the preliminary tests, it was decided to use the following experimental system (Fig. 1). A circular tank with a capacity of 400 L, adapted from a Rotoplas® tank with a capacity of 600 L. A V-shaped cut was made on one edge of the container to place a galvanized sheet channel, attached to a structure that allowed the modification of the angle of inclination. A galvanized metal sheet was used as a simulation of an anthropogenic barrier (a V-shaped structure with a 10 cm wide internal central channel), which served to conduct the water flow. This channel was covered by a black cloth mesh (to provide texture to the metallic surface). The total channel length was 1.50 m. The inclinations on the ramp for the experiments were 45° and 90°. For the water flow, two submersible electric pumps of different capacity with different water flows were used: low flow (LF) 0.12 L/s and high flow (HF) with 0.40 L/s.

The four treatments evaluated were determined by combinations between inclination and current flow: 1) low flow with a 45° inclination, 2) low flow with a 90° inclination, 3) high flow with a 45° inclination, and 4) high flow with a 90° inclination. Three replicates of each combination were made resulting in a total of 12 trials. One hundred different juveniles were used each time. Each trial lasted 40 minutes, and during this time the prawns were under constant direct observation by a researcher.

The following parameters were evaluated: (1) total number of individuals that exceeded the total length of the channel (SI), (2) individuals that interrupted the journey and return to the pond (DI), (3) individuals that remained at the end of the test in the channel without reaching the upper end (PI), and (4) number of individuals that remained in the pond at the end of the test time without any obvious climbing effort (FI).

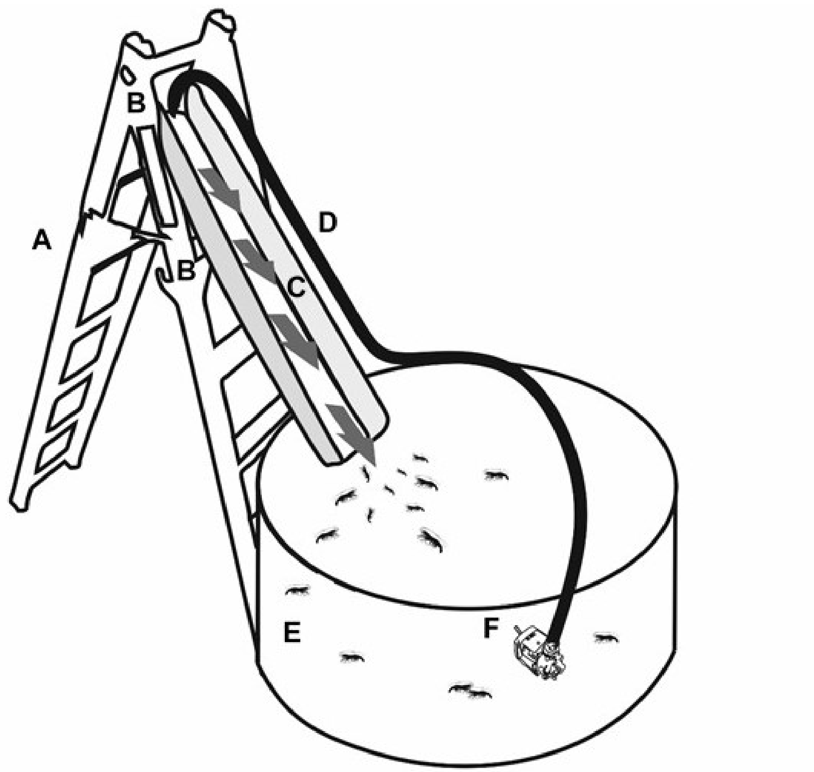

Figure 1. Experimental system used for migratory behavior bioassays of *Macrobrachium tenellum* juveniles. (**A**) support structure; (**B**) supports to adjust inclination; (**C**) galvanized metal channel with internal gutter of 10 cm; (**D**) water flow conducting hose; (**E**) circular tank, capacity 400 L with V cut; (**F**) submersible pump.

Statistical analysis

The data obtained was subjected to a two-way Analysis of Variance (ANOVA) analysis, using the statistical software SigmaPlot version 11.0 (Systat Software, Inc.). Subsequently, a Tukey´s test was applied to determine possible significant differences between treatments ($p < 0.05$).

Results

Preliminary tests

The results from the 70 preliminary trials showed that the galvanized steel sheet by itself was not attractive to the prawns, registering no climbing attempts. For this reason, we decided to cover the canal with a dark colored cloth mesh to facilitate climbing (Fig. 1).

The angle of inclination of the ramp was adjusted between 30° and 45°. In both cases, the duration required to reach the upper end of the barrier (1.50 m) varied between 30 to 40 minutes at high luminosity (1400–1850 lux). In the case of low lighting (0–35 lux) the journey was completed in 15–20 minutes. When the slope was increased to 90°, the prawns had more difficulty to climb up losing their grip and falling repeatedly back into the tank (about 1 in 3 times).

With water flow higher than 0.5 L/s, the prawns were easily detached by the water flow; therefore, we decided to adjust the final experimental design to a lower flow velocity (0.12 L/s). In addition, it was observed that the prawns needed 1 to 5 minutes to start climbing (once the water pump was turned on and they perceived the waterfall flow in the pond).

Juveniles between 30 and 60 mm in total length were most stimulated by the water flow and started climbing the barrier. Smaller juveniles were not stimulated, or if they were, they only gathered under the flow, but did not demonstrate barrier climbing behavior.

Climbing activities were recorded between 7:00 a.m. and 11:00 a.m. and from 5:00 p.m. to 7:00 p.m. Juveniles showed greater attraction to climbing and interaction with the channel. From midday to 4:00 p.m. the prawns did not show climbing activities.

Final essays

The two-way ANOVA ($F = 8.2$, $p < 0.05$) revealed that there were significant differences in the number of juveniles that climbed the total length of the barrier under different inclinations (45° and 90°), but when these were associated with the water flow; however, no differences ($F = 0.02$, $p > 0.5$) were detected in the water flow effect by itself. The Tukey test revealed that the water flow influenced the climbing behavior ($q = 4.0$, $p < 0.05$), as long as it was associated with the 90° slope, since at this inclination and with HF, more individuals exceeded the barrier. At a 90° slope with LF a smaller number of individuals managed to overcome the obstacle (Tab. 1).

Table 1. Results of the tests carried out for the different treatments on migratory behavior bioassays of *Macrobrachium tenellum* juveniles.

Inclination	45°		90°	
Flow	LF	HF	LF	HF
SI	5.0 ± 2.0 ^{Aa}	5.7 ± 2.1 ^{Aa}	2.3 ± 1.5 ^{Ba}	8.0 ± 2.0 ^{Ca}
DI	2.7 ± 0.6 ^{Aa}	10.3 ± 1.5 ^{Bb}	13.0 ^{Cc}	14.7 ± 2.5 ^{Cc}
PI	10.0 ± 4.4 ^{Aa}	7.0 ± 3.6 ^{Aa}	31.3 ± 4.0 ^{Bb}	46.0 ± 8.5 ^{Cc}
FI	90.0 ± 4.4 ^{Aa}	93.0 ± 3.6 ^{Aa}	68.7 ± 4.0 ^{Bb}	54.0 ± 8.5 ^{Cc}

SI: individuals who exceeded the total length of the canal; DI: individuals who interrupted the journey and returned to the pond; PI: individuals who remained in the channel at the end of the trial time; FI: individuals in the pond at the end of the test time. The superscript capital letters show significant differences in inclination according to the flow. The superscript lower case letters show differences ($p<0.05$) in flow depending on the inclination.

There was a significant difference ($F = 12.0$, $p < 0.05$) in terms of the individuals that interrupted their journey and returned to the pond. The results of the Tukey test ($q = 8.8$, $p < 0.05$) showed that the effect of flow levels depends on the inclination. The 90° slope had a similar effect on the two flows tested, but in the case for the 45° slope with LF, less juveniles interrupted the upward migration and returned to the pond (Tab. 1).

In the case of the individuals that remained in the channel at the end of the test time, the two-way ANOVA ($F = 7.7$, $p < 0.05$) showed significant differences between slope and water flow, and the results of the subsequent Tukey test ($q = 4.6$, $p < 0.05$) revealed that at the 90° slope the water flow directly affected the climbing behavior of the juveniles. The HF resulted in a higher number of prawns showing climbing behavior. In the case of the 45° slope, there was no difference between the two water flows tested.

Individuals that remained in the pond at the end of the trial time were similar in size ($F = 7.7$, $p < 0.05$). More juveniles without climbing behavior were found in the 45° ($q = 12.2$, $p < 0.05$) inclination treatments. Within the different slopes there was no statistical differences ($F = 3.3$, $p > 0.05$) comparing the water flows tested.

Discussion

In most of the cases treatments that combined the flows (high and low) with the 90° slope presented higher climbing activities compared to the 45° slope: the HF (0.40 L/s) produced the highest climbing activity. This could be due to the fact that migrations of these prawns are associated with the increase in the current velocity during the rainy season (June–October). Bauer (2011) mentioned that after larval development, the juveniles must migrate upstream to a freshwater habitat where they grow to adult stages. To carry out this migration, juveniles must find the mouth of a river or coastal stream to enter and travel considerable distances to their destination (Espinosa-Chaurand *et al.*, 2011). The experimental system designed for the evaluation of the migratory behavior of the prawn *M. tenellum* allowed us to obtain new data on the stimulation exerted by different water flows and slopes on juveniles.

Rodríguez-Uribe *et al.* (2014) reported a positive correlation between the current velocity (increase in cm/s of the flow) and the activity of individuals migrating through the gate of an artificial pond. This finding corroborates the results obtained in the present work. Juvenile prawns between 30 and 60 mm in total length were more rapidly stimulated by the water flow and started climbing the barrier. Smaller sized prawns were stimulated very little; and if they were, they only met under the flow, but they did not show climbing behavior. The slope did not seem to be an impediment for the prawns movement, since we recorded the same number of successful attempts to migrate over the total length of ramps at the two different slopes. However, taking into account the flow with the inclination of 90°, more prawns interrupted their path or were dragged by the water current with a HF compared to the 45° slope. Benstead *et al.* (1999) mentioned when juveniles encounter obstacles they can climb them even with vertical slopes (90°) or almost vertical slopes such as small waterfalls (Kikkert *et al.*, 2009) or floodgates (Rodríguez-Uribe *et al.*, 2014). However, there must be water flow above the barrier for movement to occur, since the unidirectional flow of water downstream is the factor that stimulates countercurrent swimming (Bauer, 2011). According to our results for *M. tenellum* stimulation this flow must be greater than 0.12 L/s and close to 0.40 L/s.

Our results show that between 7:00 a.m. to 11:00 a.m. and 5:00 p.m. to 7:00 p.m., juveniles had a greater incentive to climb and interact with the channel then during the time period from 12:00 p.m. to 4:00 p.m. Apparently, the phenomenon is associated with light intensity in the experimental set up; because in a natural environment these prawns show higher activity during nighttime to avoid predation by birds and fish. In addition, during those hours, the humidity is higher, the temperature is lower, and they suffer less desiccation (Benstead *et al.*, 1999; Bauer and Delahoussaye, 2008; Kikkert *et al.*, 2009).

The results of this study demonstrate the direct relationship between slope and flow effects of anthropogenic barriers that hinder the migration process of *M. tenellum*. In Mexico there are few field studies (Rodríguez-Uribe *et al.*, 2014) focusing on this topic and none under laboratory conditions with controlled variables. The results of the present study contribute essential information on how to build slopes and passages for fauna in canals, dams and reservoirs, since in most cases the needs of migratory species such as fish and crustaceans are not taken into account. It is necessary to expand the information on the effects of anthropogenic barriers on migratory behavior and physiological expenditure as a result of overexertion by amphidromic species that are hindered by these barriers (Bauer, 2013).

This study was carried out between February and March. Therefore, it is recommended to carry out additional tests and observations in the months after the rainy season in tropical regions (from June to October) and in a controlled environment, since there is an increase in temperature, and relative humidity. These variables can significantly affect the results of similar studies, according to the literature.

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