

Mating behavior of the freshwater crab *Kingsleya attenboroughi* Pinheiro and Santana, 2016 (Crustacea: Brachyura: Pseudothelphusidae)

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ABSTRACT

Freshwater crabs exhibit complex behavioral patterns, including those related to mating, which is generally characterized by the approach of the male towards the female. *Kingsleya attenboroughi* Pinheiro and Santana, 2016 is a pseudothelphusid freshwater crab endemic to Brazil. As with other species from this family, *K. attenboroughi* is nocturnal. The main objective of the present study is to describe the mating behavior of *K. attenboroughi* and to evaluate if the molt cycle stage influences mating. The crabs were allocated to pairs and filmed for 24 hr. The mating behavior of the species was characterized by the physical positioning of the male under the female, with an elaborate courtship occurring prior to copulation. The molt cycle appears to influence mating since mating occurred predominantly between pairs in which males were in the inter-molt stage and females were in the post-molt stage.

KEYWORDS

Behavior, Brazil, Neotropical crab, reproduction, Semiárido

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INTRODUCTION

Pseudothelphusid crabs and particularly the species in the genus *Kingsleya* Ortmann, 1897 are generally found in freshwater stream environments characterized by rapids, with rocky and sandy beds, as well as leaf litter and submerged branches and are the most diverse of the two Neotropical true freshwater crab families (Magalhães, 2016). Crabs in this family are part of a diverse group of invertebrates that play an important role in the structure of freshwater aquatic communities, contributing to both energy flow and nutrient cycling (Hill and O’Keeffe, 1992).

The increase in studies on freshwater crabs in the recent decades is noticeable (Yeo *et al.*, 2008; Cumberlidge *et al.*, 2009; Pedraza *et al.*, 2015; Carvalho *et al.*, 2018), and mostly they address taxonomic and zoogeographic aspects (Magalhães, 2016). However, there is an absence of studies on the reproductive biology, mating system and sexual behavior in pseudothelphusids, especially in the Americas.

Freshwater crabs exhibit complex behavioral patterns, including those associated with reproduction. Senkman *et al.* (2015), when studying the reproductive behavior of three trichodactylid freshwater crab species, reported an aggressive approach pattern in males towards females during mating.

Mating in freshwater crabs is characterized by the male approaching the female, where the pair then place themselves in a sternum-sternum position (Pace *et al.*, 1976; Gherardi and Micheli, 1989), which may occur in an aquatic or terrestrial environment (Liu and Li, 2000; Chua *et al.*, 2014), and with different copulation duration times (Micheli *et al.*, 1990). Moreover, differences in molt stage at the time of copulation exist, with females in the post-molt and males in the inter-molt stage being observed in *Travancoriana schirnerae* Bott 1969 (Sudha Devi and Smija, 2013), while *Candidiopotamon rathbunae* (de Man, 1914), and *Johora singaporensis* (Ng, 1986), have been observed to copulate while both are in the inter-molt stage (Liu and Li, 2000; Chua *et al.*, 2014).

Information on the behavior of neotropical freshwater crabs, especially pseudothelphusids, are rare. One exception is about the behavior of *Kingsleya attenboroughi* Pinheiro and Santana, 2016

under lab conditions addressed by Nascimento *et al.* (2020). Aspects of reproduction, more specifically detailed descriptions of their mating phases and the identification of the molt stage at the time of copulation, can facilitate the understanding of the evolution of their sexual systems and their reproductive success.

The purpose of this study is to describe *K. attenboroughi* mating behavior, as well as to evaluate whether the molt cycle stage influences the mating of this species.

MATERIALS AND METHODS

Collection and transport

Thirty crabs were manually captured between September and November 2018 and used in the study. The captures occurred at night at Farias stream, Arajara District, Barbalha, state of Ceará, Brazil (07°20’07.6”S 39°23’58.8”W). Following capture, the crabs were individually placed in plastic pots with water from the collection site and transported alive to the Laboratório de Piscicultura do Instituto Federal do Ceará, Campus Crato.

Acclimatization

The crabs were individually maintained in acclimatization aquaria (30 x 40 x 40 cm) for approximately 10 days before the start of the experiment and were fed with fish flesh every 24 hours, always at the same time, with waste removal and partial water change being performed every 2 days.

Experimental design

The tests were performed in observation aquaria (30 x 40 x 40 cm) connected to a single closed circulation system with a 250 L water reserve under continuous aeration, and an average water temperature of 23.89 °C (± 0.90), with a 12 hours light and 12 hours dark photoperiod controlled by a digital timer. The aquaria were designed to provide crabs with both an aquatic and a terrestrial environment. The images were recorded by medium resolution cameras (HL, model HM/22/52), positioned 0.35 cm above the aquaria. All crabs used in the experiment had all of their appendages intact and were free from injury. Each pair used in the experiments was chosen to have

a similar body size. Each pair was filmed for 24 hours. During the tests the crabs were not fed. In order to observe whether the molt cycle influences the sexual behavior, the pairs were divided into 4 groups, *i.e.*,

A: four pairs, B: five pairs, C: three pairs, D: three pairs according to their molt stage (Tab. 1). Each pair was observed in a separate aquarium.

Table 1. *Kingsleya attenboroughi* Pinheiro and Santana, 2016, pair arrangements in the four groups (A–D).

Groups			
A (4 pairs)	B (5 pairs)	C (3 pairs)	D (3 pairs)
Males and females in inter-molt	Males in inter-molt and females in post-molt	Males and females in post-molt	Males in post-molt and females in inter-molt
Copulation by group			
One pair copulated	All pairs copulated	No copulation	No copulation

RESULTS

Sexual behavior

Based on the sexual behavior observed for *K. attenboroughi*, the stage of the molt cycle (post-molt – inter-molt) influences mating, with the pairs preferentially mating when the female was in the post-molt stage and the male in the inter-molt stage.

The five pairs in group B exhibited mating (Tab. 1). Three of these five pairs copulated twice during the experiment. Only one of the four pairs in group A showed mating behavior. All the courtships and mating performed by *K. attenboroughi* recorded in this experiment occurred in the aquatic portion of the aquaria. The other three pairs in group A, as well as the three pairs in group C and the three pairs in group D, did not exhibit any sexual behavior (Tab. 1). Among the pairs that copulated, the males exhibited similar courtship and copulation behaviors, with a difference only in the time devoted to each phase. Just one of the four pairs in group A exhibited mating behavior. A single pre-copulation and copulation event was recorded, where the pre-copulation lasted 2.05 min and the copulation lasted 3.49 min.

In group B, the mean time of pre-copulation was 1.49 (\pm 1.02) min and the mean time of copulation was 5.09 (\pm 4.41) min. No mate guarding behavior was observed from either sex.

Pre-copulation: searching – “dancing” – physical contact

The males move around the aquarium in search of what appears to be the female’s field of vision (searching), then performs well-crafted and

continuous movements (“dancing”). Right after these movements, the males move to face the females and initiate physical contact, when the males touch the female’s pleon and/or dorsal region using their second pair of pereopods. In cases where copulation did not occur, the females exhibited aggressive behavior in response to the male’s physical approach, attacking the males using their chelipeds and moving quickly through the aquarium, away from the male. In cases where copulation occurred, the females keep still and accept the males.

The act of “dancing” in front of the females performed by the male crabs is very well defined and repeated several times before copulation. The crabs move up and down rapidly several times from the substrate using their pereopods, while slightly opening and closing the chelipeds as they move sideways (Video S1).

Copulation: pair alignment

After the physical contact initiated by the male, touching the abdomen and/or dorsal region of the female, the male is positioned under the female, so that the pleon faces upwards in contact with the female’s pleon, with the female being positioned on top of the male, with her abdomen facing downwards. The male then intertwines his pereopods and holds the female in place with his chelipeds, which are positioned on the dorsal region of the female near the ocular region. Subsequently, the pair’s abdomens open slightly, with the pair remaining in this position for a time period ranging from 0.0 to 5.09 (\pm 4.41) min (Video S2).

Post-copulation: pair separation

The pair separates after copulation and the male remains on his dorsal region in contact with the substrate, lethargic, for a few seconds. During the experiments no agonistic behavior, cannibalism and/or attempted forced copulations were observed.

DISCUSSION

The copulatory behavior observed for *K. attenboroughi* appears to follow the same pattern as other decapods where the pairs place themselves in a sternum-sternum position (Pace *et al.*, 1976; Gherardi and Micheli, 1989). Prior to copulation occurring, the male places himself in the female's visual field, performing a type of dance, followed by touching of the female's pleon and/or dorsal region, establishing a form of communication. In Brachyura, communication during the reproductive period can occur through visual, chemical, acoustic and tactile stimuli, which can also act synergistically (Sastry, 1983). For *K. attenboroughi*, it was observed that visual and tactile stimuli exert an influence on the mating/sexual activity of the species, since the male exhibits elaborate courting.

The physical placement of individuals during sexual activity facilitates copulation. Male *K. attenboroughi* are positioned underneath the female with their abdomen facing upwards during copulation. In *in situ* observations of *Johora singaporensis* Ng, 1986 (Potamidae) mating, Chua *et al.* (2014) reported that the male is positioned underneath the female. The opposite occurs in *Dilocarcinus pagei* Stimpson, 1861 (Trichodactylidae) where the male is positioned on top of the female at the time of copulation (Senkman *et al.*, 2015). Thus, given the results observed in the present study, *K. attenboroughi* seems to follow the pattern observed in Potamidae regarding male positioning in relation to the female at the time of copulation. Which shows divergent strategies among different freshwater crabs. Although Potamidae and Trichodactylidae present divergent patterns regarding the positioning of the sexes during mating, in both groups mating always occurs in the aquatic environment, as observed in *K. attenboroughi*.

In this study, *K. attenboroughi* did not exhibit mate guarding behavior. According to Wilber (1987), *T.*

schirnerae males only mate with females that they guard and do not guard the females they do not mate with. Guarding behavior by males is usually associated with the female's molt condition when she has decalcified her carapace at the time of copulation (Hartnoll, 1969; Salmon, 1983). This differs from the present results, where copulation occurred predominantly among pairs in which the females were in a post-molt stage and guarding behavior was not observed. This suggests that more detailed studies are needed to find out what leads males in this species to not exhibit this kind of behavior.

For *T. schirnerae*, mating occurs between post-molt females and inter-molt males (Sudha Devi and Smija, 2013). For *C. rathbunae*, 41 of 43 mating pairs were between rigid integument partners (Liu and Li, 2000). The present results point to a similarity with the first study since a predominance in copulation between pairs where males were in the inter-molt stage and females in the post-molt stage was observed. According to Hartnoll and Smith (1979), although mating between inter-molt males and post-molt females is common in Brachyura, it is not universal.

During *K. attenboroughi* courting, the males exhibit a "dance". A similar type of behavior has also been observed in the freshwater anomuran *Aegla platensis* (Schmitt, 1942), where males display by standing on the tip of their pereopods, and performing a type of dance, with open chelipeds, and ending with rapid and successive beating of their abdomen (Almerão *et al.*, 2010). The similar "dance" behavior in *K. attenboroughi* appears to be exclusively associated with mating as it was only exhibited by mating male crabs.

Kingsleya attenboroughi males gradually approach females without presenting aggression; however, the opposite can occur in other freshwater crabs. Senkman *et al.* (2015) observed an aggressive behavioral pattern during courtship and mating and reported the occurrence of forced copulation by males when studying the reproductive behavior of three Trichodactylidae species (*D. pagei* Stimpson, 1861, *Z. collastinensis* Pretzmann, 1968 and *T. borellianus* Nobili, 1896). *Candidiopotamon rathbunae* males have been seen to be aggressive, initially grabbing the female by the chelipeds, turning her with the help of their pereopods and forcibly mounting her (Liu and Li, 2000). There is no courtship behavior in

many crab species; the male simply grabs the female and adjusts her into the appropriate mating position (Warner, 1977). A more aggressive behavioral pattern exhibited by male crabs has been classified as “seek and intercept” (Christy, 1987); however, this does not seem to be the case with *K. attenboroughi* since the male exhibits a slow and subtle approach toward the female with no apparent record of aggression.

This study is a step towards a better understanding of the sexual behavior and mating system of *K. attenboroughi* and an attempt to infer possible sexual behavior patterns for freshwater crabs of the family Pseudothelphusidae. *Kingsleya attenboroughi* individuals were seen to present an elaborate courting behavior prior to copulation where the male exhibits a “dance”; mating is characterized by the physical positioning of the male under the female; the male apparently controls the mating event; no aggressiveness is observed during the courting and copulation phases; no mate guarding behavior before or after copulation is displayed; copulation occurs preferentially between inter-molt males and post-molt females, showing that molt cycle stage can influence mating; and finally, no forced copulation or cannibalism was observed between the individuals.

SUPPLEMENTARY MATERIAL

Video S1. Dancing - <https://youtu.be/BN1GFzzninM>

Video S2. Mating - <https://youtu.be/WVtQErHh84c>

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