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IN SITU OBSERVATIONS OF MATING BEHAVIOUR OF THE SINGAPORE FRESHWATER CRAB *JOHORA SINGAPORENSIS* (CRUSTACEA: BRACHYURA: POTAMIDAE)

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ABSTRACT. — Little is known about the reproductive behaviour and biology of the critically endangered Singapore freshwater crab, *Johora singaporensis*. Here, we describe, for the first time, the mating behaviour of this endemic species based on in situ observations. Sub-adult females were observed mating with larger males. A better understanding of its reproductive behaviour and biology will aid significantly in conservation efforts.

KEY WORDS. — *Johora singaporensis*, crustacean, decapod, conservation, reproduction, behaviour, critically endangered

INTRODUCTION

The Singapore freshwater crab, *Johora singaporensis* (Ng, 1986), is one of four species of primary freshwater crabs that are native to Singapore. It is an endemic potamid that is present only in a few hill streams (Ng et al., 2014). This semi-terrestrial crab forages both in water and on land (Ng & Yeo, 2013), and like many freshwater crabs, is more active at night. The species is listed as critically endangered globally by the International Union for Conservation of Nature (Esser et al., 2008), and is also recognised as one of the top 100 most-threatened species in the world (Baillie & Butcher, 2012). Although there have been published studies on its taxonomy (Ng, 1986, 1987) and biogeography (Yeo et al., 2007), the ecology of this species remains poorly understood (Ng & Yeo, 2013; Ng et al., 2014), which can present a significant impediment to conservation efforts. For instance, little is known about the reproductive behaviour and biology of this freshwater decapod. Here we contribute to addressing this gap by presenting our in situ observations of the mating behaviour of *Johora singaporensis*.

OBSERVATION

Two mating pairs were observed on the nights of 19 Feb.2014 (ca. 2120 hours) and 24 Feb.2014 (ca. 2040 hours) during full moon and third quarter lunar phases, respectively, in a hill stream at Bukit Batok, Singapore. The sandy-bottomed stream had steep, rocky banks, and was strewn with moss-covered stones. Moderate amounts of riparian vegetation and canopy cover were present. Both mating pairs were observed to be in shallow, flowing water (< 15 cm), in the open. In both cases, the crabs copulated in a sternum-to-sternum position, i.e., with their ventral sides facing each other, with the smaller female [estimated carapace width (CW) ca. 10 mm and 13 mm, respectively] on top of the larger male (CW ca. 12 mm and 14 mm, respectively). In both instances, the male lay on the substratum on the dorsal surface of its cephalothorax (Fig. 1). Both pairs remained motionless throughout the duration of the 15-minute observation, and the amount of sediment covering both individuals in the first pair suggests that the crabs had remained stationary for a considerable amount of time prior to being observed. The females of both mating pairs were sub-adults. Sub-adult females have a relatively narrow abdominal flap (Fig. 2A), which in mature females is greatly widened (Fig. 2B), forming the brood pouch in which eggs and juvenile crabs are carried. All mating individuals had hard integuments. Both mating pairs were collected while still coupled by DJJN as part of an ongoing ex situ breeding programme conducted by the National University of Singapore in collaboration with the National Parks Board, Singapore. The mating crabs separated shortly after collection.

DISCUSSION

Owing to their cryptic nature, little is known about mating behaviour of primary freshwater crabs. Mating typically occurs in a sternum-to-sternum position (Pace et al., 1976; Gherardi & Micheli, 1989), and has been observed both on land and in water (Liu & Li, 2000), Mating duration varies widely (Micheli et al., 1990). In *Candidopotamon rathbunae*, mating females were slightly larger than their partners (Liu & Li, 2000). However, in

Fig. 1. Mating pair of *Johora singaporensis*. The larger male (ca. 12 mm CW) is positioned beneath the smaller female (ca. 10 mm CW). (Photograph by: Kenny W. J. Chua).

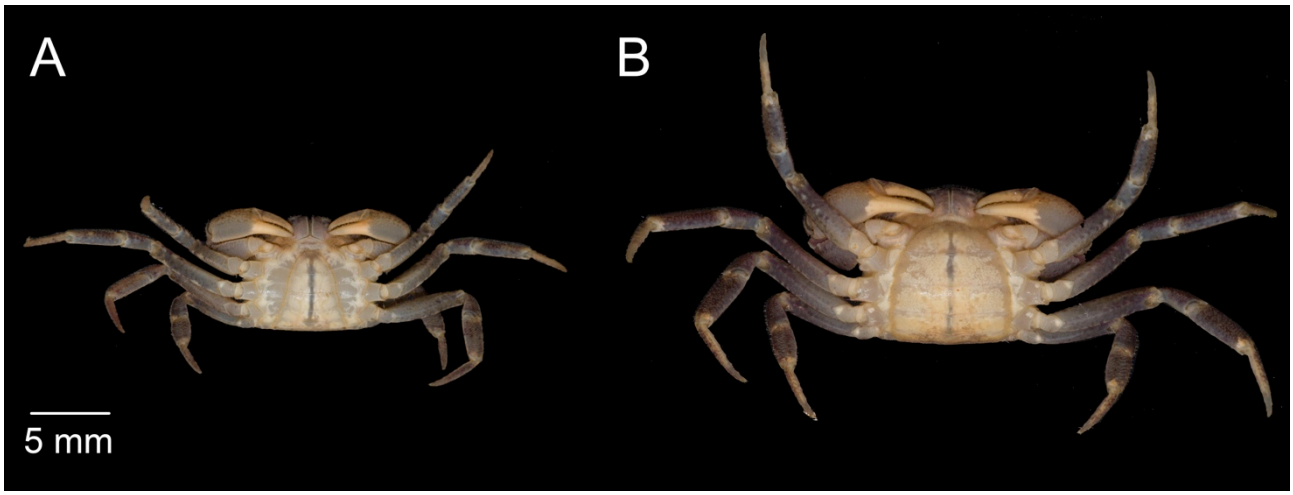

Fig. 2. Ventral view of *Johora singaporensis.* A, sub-adult female; B. adult female. The sub-adult female as a narrower abdominal flap than the adult female. (Photographs by: Kenny W. J. Chua).

Potamon fluviatile mating is generally between a larger male and a smaller female (Micheli et al., 1990). While more observations are needed, in *Johora singaporensis* the male is generally the larger partner.

Our observations indicate that in *Johora singaporensis*, mating occurs with the male positioned below the female. Similar ex situ observations have been made (unpublished data), and the same has been reported for *Somanniathelphusa sexpunctata* (see Ng & Ng, 1987). However, the reverse is true for some other freshwater crab species, where the female is positioned below the male (Pace et al., 1976; Micheli et al., 1990). More work is required to investigate the reasons for either mating position.

All observed mating individuals of *Johora singaporensis* possessed hard integuments, indicating that the crabs had not moulted recently, and were therefore in the inter-moult phase. Mating during the inter-moult phase also occurs in *Potamon potamios palestinensis* (see Gherardi & Micheli, 1989). However, females of *Potamon fluviatile* (see Micheli et al., 1990), *Candidopotamon rathbunae* (see Liu & Li, 2000) and *Travancoriana schirnerae* (see Sudha Devi & Smija, 2013) have been observed to mate during the soft post-moult phase. While post-moult mating occurs in brachyurans with immobile, calcified vulval opercula (Salmon, 1983; Diesel, 1990), mating is possible throughout the moult cycle for females of species with mobile vulval opercula or permanently soft vulval openings (Liu & Li, 2000). Further investigation is needed to determine the structure of female genital openings in *Johora singaporensis*.

Male freshwater crabs are known to mate with sub-adult females (Micheli et al., 1990), as was observed in the present study. While it is possible that these females are physiologically mature, they are unable to carry a clutch in their relatively narrow abdominal flaps (Corgos & Frerie, 2006), and are thus not considered to be functionally mature. Spermatophores deposited from these "premature" mating events might be stored in the spermathecae until the female reaches functional sexual maturity and is able to brood a clutch successfully. Other crustaceans have been reported to store sperm in their spermathecae (Sastry, 1983), with spermatozoa remaining viable for long periods—as long as 21 months—in *Spiralothelphusa hydrodroma* (see Adiyodi, 1988, as *Potamon hydrodromus*). Mating with immature females could be reproductively advantageous as encounters between crabs of opposite sexes in the wild have been noted to be infrequent (Gherardi & Micheli, 1989). Histological analysis of seminal receptacles will be required to confirm if sperm storage occurs in *Johora singaporensis*.

The in situ mating observations presented in this paper can aid the ongoing ex situ breeding programme for *Johora singaporensis*. More work needs to be done in order to gain further insights on the mating behaviour of the crab. A comprehensive understanding of its reproductive behaviour and biology will contribute to understanding its ecology, and help existing efforts to conserve this highly threatened species.

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