Short Communication

The Embryonic Development of the Giant Freshwater Mountain Crab, *Isolapotamon bauense* (Ng, 1987)

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Abstract: The embryonic development of the giant freshwater mountain crab, *Isolapotamon bauense* (Ng ,1987) endemic to Sarawak, Malaysia which is an endemic species listed as Vulnerable under the IUCN redlist, is described for the first time in this paper. Mature female *I. bauense* were observed to spawn small clutches of large macrolecithal eggs typical of other Potamid primary freshwater crabs. Embryonic development occurred within the eggs corresponding to other primary freshwater crabs. The developing embryo goes through 3 major development stages within the egg before hatching into a fully formed crab hatchling at the end of the gestation period. Eggs had a gestation period of between 36-45 days from spawning until hatching. The findings of the present study provide useful insight into the life cycle and breeding biology of *I. bauense* which will be applicable for both conservation and aquaculture studies in the future.

Keywords: Giant freshwater crab, Borneo and embryonic development.

Abstrak: Perkembangan embrionik ketam gunung air tawar gergasi, *Isolapotamon bauense* (Ng ,1987) yang merupakan spesis yang endemik di Sarawak, Malaysia, yang tersenarai dalam senarai merah IUCN diterangkan buat julung kalinya di dalam kertas ini. *I. bauense* betina yang matang akan mengeluarkan sejumlah kecil telur "macrolecithal" seperti ketam-ketam Potamid air tawar yang lain. Perkembangan embrionik berlaku di dalam telur sepertimana ketam-ketam air tawar primer yang lain. Embrio yang sedang berkembang melalui 3 peringkat fasa perkembangan utama dalam telur sebelum menetas menjadi anak ketam kecil yang terbentuk sepenuhnya pada akhir tempoh pengeraman. Telur mempunyai tempoh pengeraman antara 36-45 hari dari pengeluaran sehingga penetasan.

Introduction

Primary or true freshwater crabs are an important ecological component of ecosystems of which they are found in (Dobson et al., 2007). The main feature distinguishing primary freshwater crabs from terrestrial and marine crabs is the direct development of offspring, whereby larval development happens within the eggs which hatch directly into young crabs (Sternberg and Cumberlidge, 2001; Ng, 2017). There are currently three families, 14 genera and 48 species of primary freshwater crabs recognized in Sarawak (Ng et al., 2008; Grinang et al., 2016). Under the IUCN Red List of Threatened Species there are four species of Sarawakian primary freshwater crabs listed as endangered and two as vulnerable (IUCN, 2021). The largest of the primary freshwater crab's endemic to Sarawak is *Isolapotamon bauense* (Grinang et al., 2016, Lirong et al., 2020). Taxonomically, the genus *Isolapotamon, Bott* (1968) is relatively well understood with seven species native to Sarawak. (Ng and Yang, 1986; Ng, 1987; Ng and Tan, 1998). *I. bauense* is currently classified as "Vulnerable" under the IUCN Red list (IUCN, 2021). *I. bauense* is an aquatic potamid crab narrowly endemic to

the Bau District in Kuching, Sarawak with populations recorded in restricted habitats of mountainous regions in the areas of Singai, Serumbu, Krokong and Serapi (Ng, 1987; Grinang et al., 2016; Grinang et al., 2017). There is a current lack of research into ecological and conservation aspects of primary freshwater crabs in Borneo (Mcfarlane et al., 2011; Zhang et al., 2020). All species of *Isolapotamon* are large in size (Grinang et al., 2016) but *I. bauense* is the largest Potamid crab in South East Asia, capable of reaching a maximum carapace width of 9.5cm (Lirong et al., 2021). It's large size, abbreviated life cycle and good meat quality makes it an interesting prospective species for commercial aquaculture. Current research on *I. bauense* has mainly focused on the taxonomic and morphological characteristics of the species. There have been few studies concerning the basic biology of this species and currently no prior reports on the embryonic development of *I. bauense.* Thus, this paper presents a first report of the embryonic development of *I. bauense*.

Materials and methods

Mature female crabs were collected from 3 principal sampling sites known to be the habitat of *Isolaptamon bauense* in the Bau region namely: Gunung Serumbu (01° 25' 55" N, 110°13' 27" E), Gunung Singai (01° 50' 39" N, 110°17' 25" E) and Gunung Podad (01° 35' 58" N, 110°12' 75" E) as shown in Figure 1.

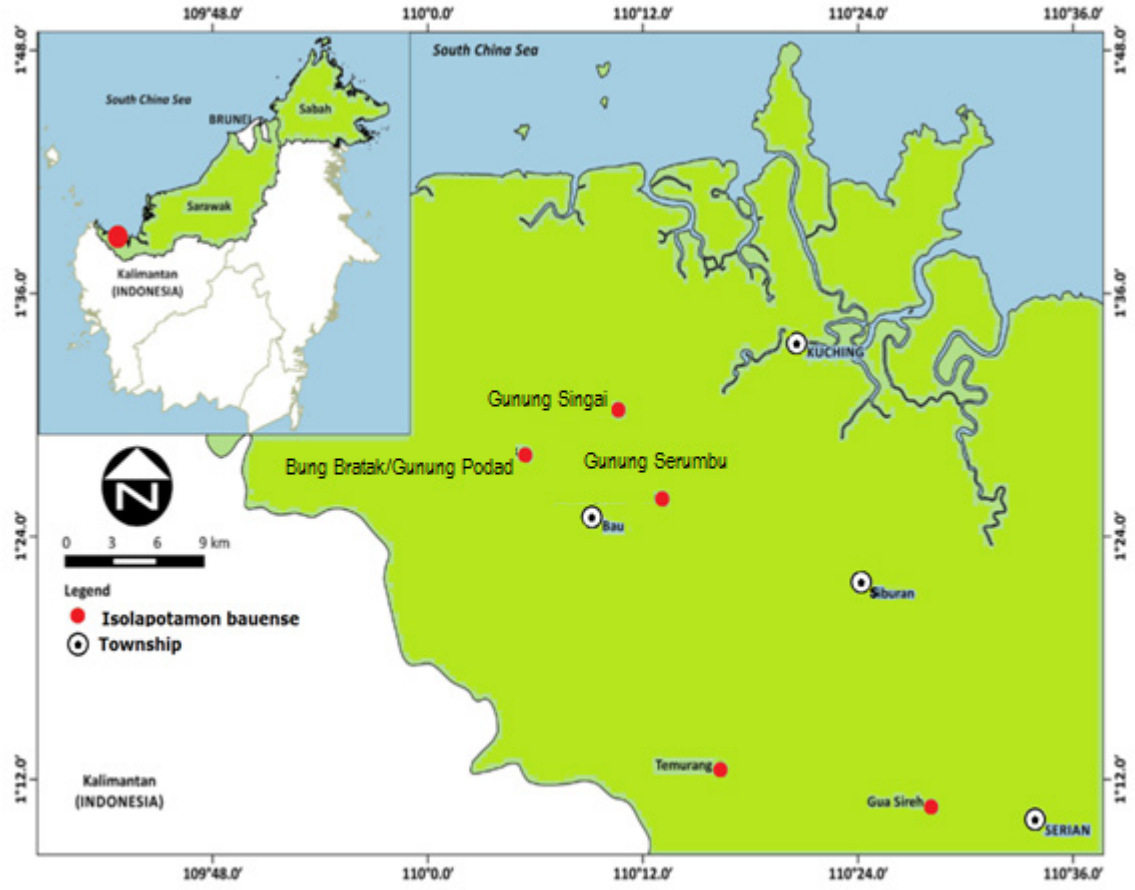

Figure 1: Map displaying the three main sampling sites of the study

Subsequently live experimental animals were transported to the Aquatic Nutrition Laboratory, Department of Animal Science and Fisheries, Faculty of Agriculture and Forestry, Universiti Putra Malaysia Bintulu Campus (UPMKB) where the breeding trials were conducted. After a one-week

acclimatization period, Methyl Farnesoate (Mf) (Echelon Biosciences, Salt Lake City, Utah, USA) was injected at a dose of 10 µl/g bodyweight into experimental animals through the arthrodial membrane of the coxa of the left cheliped to encourage spawning. The experimental animals (n=10) were kept together at ambient room temperature 24°C - 27°C in two High Density Polyethylene (HDPE) tanks (179 cm length, 119 cm width, and 60 cm height) with a water depth of 10cm. Tanks were equipped with adequate aeration (using a Hailea HAP 60 air blower system) and overhead filtration system (a Boyu UF-130 8watt submersible pump and overhead filter system) to provide circulation as well as filtration. Substrate and shelter were provided in the form of river gravel, cut lengths of PVC pipe and river rocks protruding above the water line. Crabs were fed once daily with freshly chopped fish meat and any excess food and crab exuvia was removed 2 hours after feeding through siphoning of 20% of rearing volume with new fresh water topped up after each cleaning cycle. Crabs were observed over a 60 day study period and any spawning females were moved to individual containers for egg monitoring and hatching. Hatching containers were lined with sterile moistened peat moss and humidity was kept high mimicking natural burrow conditions using two water saturated sponges in each unit. The hatching containers were exposed to the natural ambient lighting conditions of the laboratory fluctuating accordingly to the natural daylight cycle. Sponges were drained and re-saturated with freshwater every day. Embryonic development was observed by gently removing an egg from the pleopods of each ovigerous female using dissecting tweezers for viewing under a digital microscope (MUSTOOL Digital USB Microscope, 2-megapixel, 1000 X max magnification) at 7-day intervals until hatching occurred. Embryonic development was categorized into 4 general categories as adapted from Ramach et al. (2009): I- Initial (Yellow), II-(Intermediate), III –pre-hatching (dark brown with eye spot) and IV-(Crab 1).

Results

The embryonic development of *I. baunse* eggs from spawning until hatching is photographically chronicled (Figure 2). The first subsection (A) (Category I- Initial) shows the large bright yellow yolk of the newly spawned macrolecithal egg, the second subsection (B) (Category II- Intermediate) shows an egg 36 hours after spawning whereby the yolk has

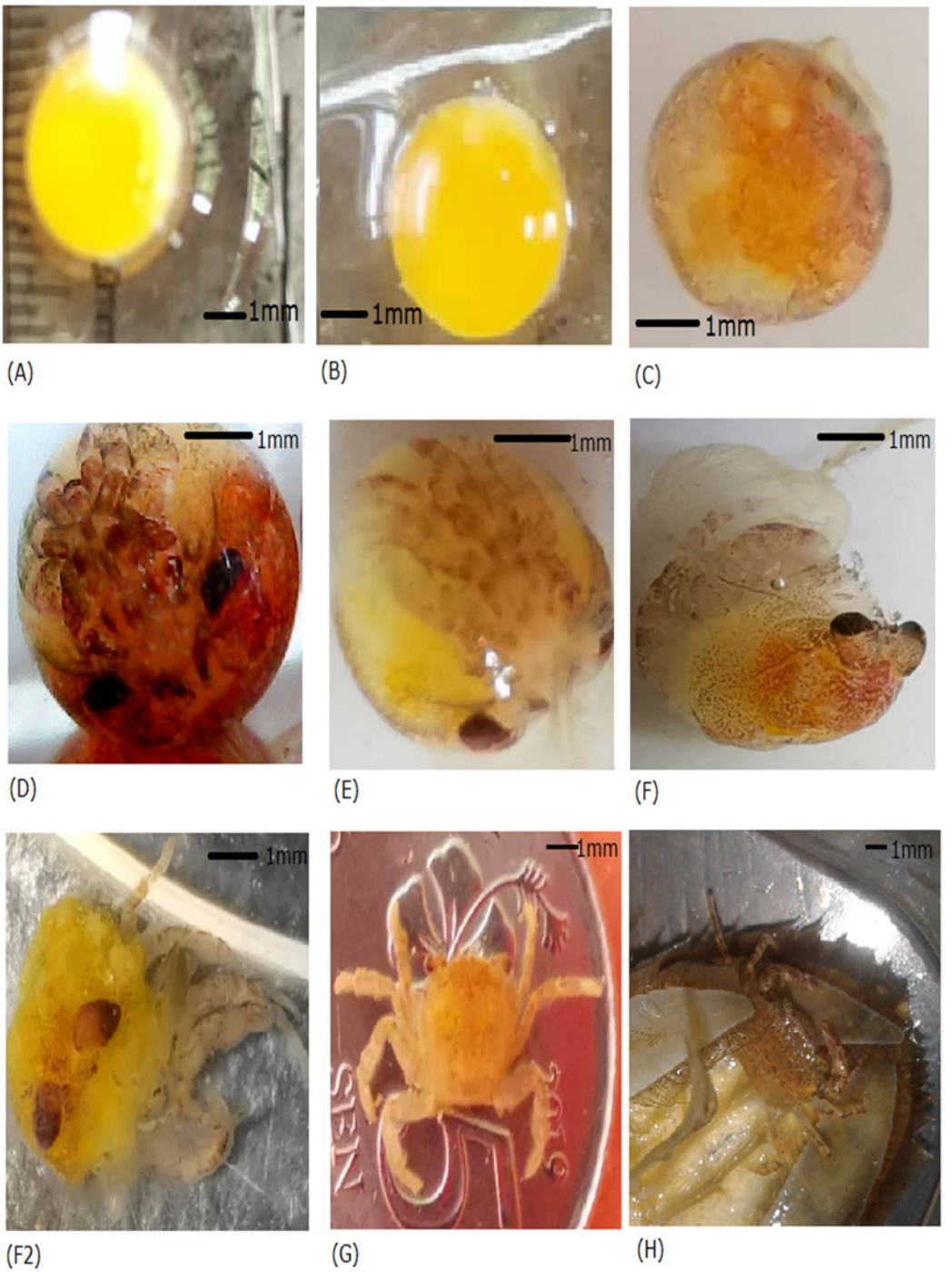

Figure 2: The embryonic development of *I. bauense* (I- Initial, II- Intermediate, III-Pre-Hatching, IV- Crab 1)

become a deeper orange hue and the blastula is visible on one side of the egg indicating embryonic development. The second stage of development continues on into the first and second week, as presented in the third sub-section (Category II- Intermediate) whereby the developing embryo can

now be distinguished. By the third week eyespots and appendages are now visible within the egg as shown in fouth sub-section (D) (Category III-Pre-Hatching) which corresponds to the zoeal stage of development and by the 4th week of embryonic development the imprisoned megalopa stage can be ascertained by the appearance of chelipeds. By the 5th week of embryonic development (Category III-Pre-Hatching) the egg juvenile crab can be discerned by the appearance of stalked eyes, chelipeds and folded telson within the egg membrane. By the 36th day of gestation some of the crablings begin to break out of the egg membrane as shown in the sixth sub-section (F). The hatching process takes a little over 30 minutes with the emerging hatchling having a soft gelatinous exoskeleton (the seventh sub-section (F2) with curly pereiopods which hardens and straightens within an hour after hatching. The first crab stage (Category IV- Crab 1) as shown in eighth sub-section (G) has an average carapace width (CW) of 3.5 mm. Under captive conditions it was observed that the hatchlings remained in the abdominal apron of their mother for 14 days, during which they underwent several molting cycles (2-4 molts) before being released with a size of between 4-5 mm as shown in Figure 2 (H). Once the young crabs had been released from the abdomen of the mother, maternal care ceased and one female was observed cannibalizing her newly released offspring.

Discussion

The earliest scientific record of the embryonic development in a Potamid crab species was by Pace et al. (1976) for *Potamon fluviatile*. The present study records the first observation of the embryonic development of *I. bauense* bred under laboratory conditions. The embryonic development of this species corresponds closely to the embryonic development of other Potamid primary freshwater crab species such as *Sinopotamon yangtsekiense* as observed by Wu et al. (2010), with an extended gestation period (36-45 days) and zoeal development within the egg. Rachata et al. (2020) recorded a relatively short 12-day gestation period for a smaller species of true freshwater crab *Esanthelphusa nani,* although the embryonic development stages corresponded closely to *I. bauense*. Further studies are necessary to collect more information on the breeding biology of this species for use in both conservation and aquaculture purposes.

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