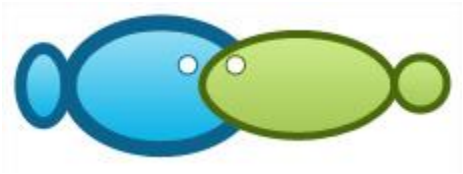

Short note: The fecundity and egg size of the freshwater crab (*Isolapotamon bauense* Ng, 1987) from Sarawak, Borneo

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Abstract. The Island of Borneo harbors a high species richness of freshwater brachyurans, many of which are endemic in the region and their existence is threatened by anthropogenic activities. Our knowledge on the fundamental aspects which are required for species conservation assessment is currently limited. For instance, information on fecundity of most Bornean freshwater crabs is almost non-existent despite this being an important predictor of vulnerability to extinction. This paper describes the fecundity and egg size of the giant freshwater crab (*Isolapotamon bauense* Ng, 1987) from Kuching Division in Sarawak, Borneo. The fecundity of the crab is between 26 and 81 eggs, with the egg diameter ranging between 3.7–4.2 mm. The macrolecithal eggs are attached to the pleopods of the female abdominal brood pouch; they are bright orange and are comprised of two layers of membrane, with a thicker outer layer and a thinner inner layer, encapsulating the large embryonic yolk sac.

Key Words: brachyuran crab, *Isolapotamon bauense*, reproductive biology, vulnerable species, endemic species.

Introduction. *Isolapotamon bauense* Ng, 1987 is the largest true freshwater crab in Malaysia and is endemic to the Kuching-Serian region in Sarawak. In addition to the extreme endemism, population density of the species is so low that it has acquired the "Vulnerable" species status in the IUCN Red List of Threatened Species (Esser & Cumberlidge 2008). Currently, not much government and non-government efforts has been done to conserve the species, particularly due to limited information to justify funding application.

Fecundity is often defined as the physiological reproductive potential of an individual (Bradshaw & McMahon 2008), that serves as a key determinant to ascertain the efficiency of population replacement, especially for decapod crustaceans (Cobo & Okamori 2008). The brachyuran crabs represent more than 7,000 valid species from 98 families, which have colonized a wide range of aquatic systems, some taxa have even successfully populated terrestrial habitats (Ahyong et al 2011; Ng et al 2008). Fecundity provides valuable insights into the evolutionary and reproductive strategies of a species (Carlos et al 2005). The recording of egg-bearing females is a useful index for determining the reproductive cycle of a species (Knudson 1960; Valter & Claudia 2008; Muzaffer & Ibrahim 2000). For decapod crustaceans, fecundity can be defined as the number of eggs produced by a female (Hartnoll 2015).

Freshwater crabs are the most species-rich of all decapod crustacean groups and show high levels of endemism (Esser & Cumberlidge 2008; Ng et al 2008). The taxonomy and systematics of freshwater crabs has gained significant interest due to

high taxa richness and endemicity (Klaus et al 2009). The freshwater crabs of Borneo comprise more than 95 species in three families, all of which are endemic to the island (Grinang 2016; Ng & Grinang 2018; Ng & Ng 2019). Among the three political boundaries in Borneo, the crab fauna of Sarawak has been relatively extensively studied for 49 species (Grinang 2016; Ng & Grinang 2018). *Isolapotamon bauense* is the largest true freshwater crab in Malaysia and can attain a carapace width of more than 9 cm (Grinang et al 2016). Anecdotal evidence from indigenous people have claimed that *Isolapotamon bauense* can grow to over 10 cm in carapace width. Currently only a few ecological characteristics of this species have been reported, which include geographical distribution, sex ratio, growth pattern and population size (Grinang et al 2018). This crab has been harvested by the Bidayuh community as a supplementary source of protein to their diet for millennia (Grinang et al 2017). Given the current lack of knowledge regarding many aspects of the reproductive biology of this species, any new information is important to reassess and evaluate the current sustainability of the population, conservation status, as well as the potential for aquaculture.

This short note aims at reducing the information gap about *Isolapotamon bauense* by determining its egg size and estimating its fecundity. Information obtained from this study will be used to develop propagation techniques destined for wild population restocking programs and for aquaculture.

Material and Method

Collection of live crabs. The population density of *Isolapotamon bauense* is significantly low. Only six individuals of mature non-berried females were successfully collected from an intermittent stream in the Bau district. Crab sampling was conducted at night along a 65 m sector of a stream, with minimal disturbance to the habitat. Bait (dead fishes) was used to attract the crabs from the burrows and then the crabs were caught by hand. The crabs were put temporarily in plastic buckets for transport to the Sarawak research facility, of the academic institution of Universiti Putra Malaysia Bintulu.

Setting up of terrarium and observation. The mature *Isolapotamon bauense* females were kept together at ambient room temperature in a HDPE tank (179 cm length, 119 cm width, 60 cm height) equipped with adequate aeration and submersible pump, with overhead filter to provide circulation as well as filtration. Substrate and shelter in the form of river gravel and river rocks protruding above the water line was provided to mimic conditions in their natural habitat. The tank was filled to a depth of 10 cm and the crabs were fed once a day at 6 pm with freshly chopped fish meat, at a rate of 5% body weight per feeding. 50% water exchange was carried out each morning with any excess food and feces being siphoned off and all the filter media rinsed.

After 7 days in captivity, one of the females spawned. A second female spawned after 16 days in captivity and a third female spawned after 48 days in captivity. Five eggs were gently removed with forceps from the pleopods of each female crab and measured using a scale bar and subsequently observed and photographed under 20X and 60X magnifications using a digital microscope. Removed eggs were fixed in 10% formaldehyde and stored in 70% alcohol. The total number of eggs attached to the pleopods of both carrying females was counted.

Results and Discussion

Results. Off the six non-berried mature females caught, only three individuals successfully spawned during the 60 days study period. The first spawning female had a carapace width of 6.6 cm and a total of 81 eggs attached to the pleopods, whereas the second female had a carapace width of 6.1 cm and a total of 26 eggs attached to the pleopods and the third female had a carapace width of 5.7 cm and a total of 32 eggs attached to the pleopods (Figure 1). The third spawning female perished shortly after spawning and the eggs, though fully formed, were lacking the thick outer membrane, as

observed in the eggs of the other two females, and these eggs were not viable. The eggs were large, with a diameter of 3.7 to 4.1 mm (Figure 2). The newly formed eggs were composed almost entirely of the bright orange yolk. The viable bright orange eggs of the first two females consisted of a thick outer membrane with a thinner inner membrane forming a fluid sac encapsulating the entire yolk mass (Figure 3). Prior to spawning crabs were observed to spend time above water and clean their abdominal pouch using their legs. Table 1 compares the average egg diameter and fecundity between *Isolapotamon bauense* and some other crab species from both freshwater and brackish water.

Figure 1. Newly captured adult female of *Isolapotamon bauense*.

Figure 2. Berried *Isolapotamon bauense* female.

Figure 3. Newly formed (2 hours after spawning) macrolecithal egg *of Isolapotamon bauense* and the egg under 60X magnification.

Table 1
Comparison of average egg diameter and fecundity between *Isolapotamon bauense* and other freshwater and brackish* water crab species

References	Species	Carapace width (cm)	Egg diameter (mm)	Fecundity (eggs)
Present study	*Isolapotamon bauense*	5.7 – 6.6	3.7 – 4.2	26 – 81
Wu et al 2010	*Sinopotamon yangtsekiense*	-	3.5 – 4.1	70 – 100
Pace et al 1976	*Potamon fluviatile*	-	2.5	> 200
Herlinah & Septiningsih 2015	*Scylla olivacea**	12 ± 0.5	0.0035	> 2400000

Discussion. True freshwater crabs are brachyuran crustaceans which complete their whole life cycle in freshwater. They have evolved to adopt terrestrial, semi-terrestrial, or freshwater modes of living and reproducing by direct development without any free-living larval stage (Yeo et al 2008). *Isolapotamon bauense* belongs to the Potamidae family, which is one of the five families of crabs composed entirely of freshwater crab species (Klaus et al 2012). The life cycle of *Isolapotamon bauense* is through direct development, which excludes any external larvae stages, as found in marine and brackish water crab species (Mantellato and Fransozo 1999). The fecundity of an animal is an important parameter in estimating the reproductive potential and population size of a species accurately (Mantelatto and Fransozo 1999b). Our preliminary observation indicated *Isolapotamon bauense* has a low fecundity with large egg size. The inability of the third spawning female to successfully extrude eggs with the thick outer membrane could possibly be due to insufficient nutrients (such as naturally occurring micronutrients) obtained from the diet provided in this study over a longer period than the other two females. Increased egg size causes a decrease in fecundity of crustaceans (Steele and Steele 1991). Like other true freshwater crabs, *Isolapotamon bauense* produces a small number of large yolky (macrolecithal) eggs which attach to the female's pleopods in the abdominal brood pouch. In all true freshwater crabs the eggs attach to the mother's pleopods for the entire duration of development and hatchlings remain in the abdominal brood pouch for a period of time before being released as miniature versions of the adult crab (Wu et al 2010; Junzeng et al 2010). The life strategy of true freshwater crabs differs greatly from marine and brackish water crabs in that the number of eggs produced is small and all larval stages are encapsulated within the eggs. Parental care is a major strategy of true freshwater crabs which differentiates them from their marine brethren (Ng 1988).

Conclusions. The current results provide valuable preliminary insight into the aspect of fecundity and the reproductive biology of *Isolapotamon bauense*. This new information on the fecundity of *Isolapotamon bauense* may suggest that the conservation status of the species need to be re-assessed. Currently the only fully documented embryonic development studies in true freshwater crabs have been done on the Chinese true freshwater crab *Sinopotamon yangtsekiense* and the Mediterranean edible freshwater crab *Potamon fluviatile*. The incubation period for the Mediterranean edible freshwater crab *Potamon fluviatile* takes 46–47 days and 77 days for the Chinese true freshwater crab *Sinopotamon yangtsekiense*. The egg diameter and general characteristics of newly formed *Isolapotamon bauense* eggs were remarkably similar to *Sinopotamon yangtsekiense*. It is suggested that the incubation period for *Isolapotamon bauense* would also require a similarly extended incubation period. Consequently, ongoing research will be important to validate the current findings and to document the full embryonic development of this species to support its conservation and potential for aquaculture.

Acknowledgements. The authors would like to thank the Fundamental Research Grant Scheme (FRGS) for funding this research (FRGS/1/2018/WAB01/UPM/02/21) and YB Datuk Henry Jinep, as well as the people of Singai, Bau for their kind cooperation during the sampling works.

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Received: 01 January 2020. Accepted: 31 January 2020. Published online: 25 July 2020.
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How to cite this article:
Abit L. Y., Hassan M. Z., Latif K., Grinang J., Asif A. A., 2020 Short note: The fecundity and egg size of the freshwater crab (*Isolapotamon bauense* Ng, 1987) from Sarawak, Borneo. AACL Bioflux 13(4):1970-1975.