Journal of Aquaculture and Fish Health Vol. 13(3) - September 2024
P-ISSN: 2301-7309 / E-ISSN: 2528-0864
doi : 10.20473/jafh.v13i3.54757

New Records of the Egg Development Phase of *Varuna litterata* in the Lower Serayu River, Central Java

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Received : 2024-02-02
Accepted : 2024-07-07

Keywords :
Egg development, Fecundity, Migration, Varuna litterata

Abstract

One of the Brachyura crabs found in the lower reaches of the Serayu River is *Varuna litterata*, also known as the "herring bow crab". This crab usually inhabits shallow tidal areas and hides under rocks, logs, or dead leaves. It is a highly adapted crab found in a wide range of salinities. *V. litterata* is also a fishery product consumed in Thailand, the Philippines, and Indonesia. This research was conducted in the Lower Serayu River, Central Java, which consists of three observation stations, namely: 1) the first station is located above the dam with fresh water, 2) the second station is below the dam with fresh water properties and 3) the third station is downstream (estuary) of the Serayu River with brackish water. This research was conducted for four months, from October 2023 to January 2024. This study aimed to determine the level of egg development in *V. litterata*. The results showed that *V. litterata* experienced four stages in egg development and then hatched into zoea. At the initial development stage, *V. litterata* eggs will be purple; in the second stage, the eggs will turn reddish; in the third development stage, the eggs will turn orange to brownish; and in the fourth stage, the eggs will develop and become black. *V. litterata* that will spawn will migrate to brackish waters to hatch their eggs. *V. litterata* produces 20,708 - 85,886 eggs with an average egg diameter of 0.440 - 0.466 mm.

INTRODUCTION

The Serayu River is one of the longest rivers in Central Java, at 180 km and with 11 tributaries (Pranoto *et al.,* 2019). The headwaters of this river come from the Dieng highland area through the Tuk Bimo Lukar spring. The Serayu River basin is surrounded by mountains such as Mount Prahu, Sindoro, Sumbing, and Mount Slamet. The river empties into the Indian Ocean (Sukarjo *et al.,* 2023). The lower reaches of the Serayu River have high biodiversity, one of which is the brachyuran crab. Crabs from the Brachyura infraordo are one of the most diverse crustacean categories, with more than 7,000 species identified in 98 families (Tang *et al.,* 2018; Shi *et al.,* 2015). These crabs live in various habitats, including marine, freshwater, and terrestrial. These crabs play an essential role in the ecosystems they inhabit. One of the families of Brachyura is Varunidae, which currently consists of 160 species in 38 genera. The *Varuna* crab genus consists

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of two species, namely *V. litterata* and *V. yui* (Lee *et al.,* 2022; Lin *et al.,* 2018; Zhang *et al.,* 2022).

Varuna litterata, commonly known as the "herring bow crab", is a member of the Grapsidae family (Liu *et al.,* 2021; Fariedah *et al.,* 2021). These crabs have a high habitat distribution and are often found in mangroves, estuaries, and freshwater (Fariedah *et al.,* 2023; Waltham *et al.,* 2014). *V. litterata* usually lives in shallow tidal areas and often hides under rocks, logs, or dead leaves (Gayen, 2018). *V. litterata* often inhabits burrows along embankments, sides of ponds, rivers, and shallow banks. This crab is euryhaline, so it can live in various water salinities (Mahapatra *et al.,* 2017; Serdiati *et al.,* 2023). This adaptation allows *V. litterata* to survive in frequently changing environments, such as after floods, where the crab can be washed far out to sea and seen drifting with debris (Devi and Joseph, 2017). One of the distinguishing features of *V. litterata* is its paddle-like legs, which are used for swimming. Because of this feature, the crab is sometimes called the "Paddle Crab" (Schmidt *et al.,* 2020). *V. litterata* tends to prefer areas facing marine waters. This particular species is found across the Indo-Pacific area, spanning South Africa, India, Vietnam, Taiwan, Indonesia, Papua New Guinea, New Caledonia, and Australia (Jumawan *et al.,* 2022). In Indonesia, *V. litterata* is found in Halmahera, Maluku, North Sulawesi, and Yogyakarta (Susilo *et al.,* 2020; Cai and Ng, 2001).

Although many studies are conducted on the brachyuran crab fauna worldwide, there are relatively few compilations of studies conducted on *V. litterata*, especially in Indonesia. *V. litterata* is a vital fishery product for Southeast Asian people (Deyashi *et al.,* 2019). *V. litterata*, commonly referred to as "talangka" in local parlance, is harvested and utilized as a food source in rural regions, particularly in countries like the Philippines. The attached eggs on the females' abdomen are highly prized as a delicacy (Jumawan *et al.,* 2022). Thailand also catches *V. litterata*, which is very common in markets during the spawning season. Thailand consumes 18,000 tons of *V. litterata* annually; only about 12,000 tons can be produced domestically yearly. Therefore, at least 6,000 tons of *V. litterata* must be imported annually from Myanmar and Cambodia (Suppapan *et al.,* 2017). Fishing activities also occur along the lower reaches of the Serayu River. People catch and eat these crabs. As happened in the Philippines, continuous fishing activities reduce the population and necessitate sustainability. Sustainability efforts are also required to maintain the *V. litterata* population. This research aims to determine the level of egg development in *V. litterata*.

The level of gonadal maturity in *V. litterata* has been studied by Fariedah *et al.* (2021) in Gresik District, East Java. However, the research was incomplete and did not find *V. litterata* with mature gonads. Knowledge of crustacean ovary development has a vital role in understanding population dynamics, especially in the context of reproduction (Linhartová *et al.,* 2018; Souza *et al.,* 2017; Rahman and Ohtomi, 2018). Therefore, this knowledge is essential for designing and implementing management strategies to maintain the long-term sustainability of exploited populations (Shinozaki-Mendes *et al.,* 2012).

METHODOLOGY

Ethical Approval

No animals were harmed or mistreated during this study. Captured animals were collected, appropriately treated, and approved during the proposal seminar at the Faculty of Fisheries and Marine Science, Brawijaya University Malang.

Place and Time

This research was conducted in the lower reaches of the Serayu River, Central Java. Downstream of the Serayu River, a dam blocks the waterway. Observation was carried out at three stations: 1) The first station is above the dam with fresh water. 2) The second station is below the dam with fresh water, and 3) The third station is below the brackish water (Figure 1). The coordinates of the study site were 07°01′52″ - 07°31′54″ S and 108°50′16″ - 110°04′20″ E.

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The observation station was chosen to ensure an ideal location for the reproductive biology of *V. litterata*. This study was conducted for four months, October 2023 - January 2024.

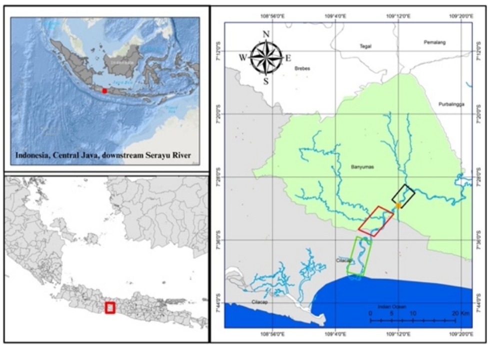

Figure 1. *Varuna litterata* sampling station. Station 1: black box, station 02: red box, station 03: green box.

Research Materials

The material used in this research was *V. litterata*, which was obtained from catches in the lower reaches of the Serayu River. *V. litterata*, both male and female caught, will be brought and observed. The materials used in this research were a vernier with an accuracy of 0.01, a microscope (Olympus, Japan), a set of surgical tools, and a digital scale with an accuracy of 0.01. The sampling technique used a gill net with a mesh of 2 mm, a length of 4 m, and a height of 1.5 m.

Research Design

This study used a group randomized design (GRD) at each station. Observations were made directly in the field and laboratory. Sample collection is carried out every two weeks at each observation station to determine the level of egg development in *V. litterata*. *V. litterata* catches were collected and analyzed to determine the relationship between carapace width and fecundity. Crab samples from each station were collected from the catch packed in ice and transported to the Jendral Soedirman University, Central Java laboratory for further analysis.

Work Procedure

Laboratory Analysis

In the laboratory, the sex of *V. litterata* is determined by examining the morphology of the abdomen. Males exhibit a long, narrow, inverted "T"-shaped structure, whereas females have a wider and rounder half-moon shape. The size and color of the outer egg mass from mated females are observed and categorized into four distinct stages based on the classification proposed by Fariedah *et al.* (2021). The dimensions of each specimen's carapace, specifically carapace width (CW) and carapace length (CL), were determined using a caliper with a precision of 0.01 mm. Additionally, the total body weight (TW) was measured using a digital scale with an accuracy of 0.001 g. The measurements for CW involved assessing the distance between the tips of the ninth anterolateral tooth, while CL was determined by measuring the distance between the frontal notch and posterior margin, following the definitions provided by Jumawan *et al*. (2022). Samples collected totaled 1,032 *V. litterata* consisting of 436 males and 596 females.

Sex Ratio

The sex ratio was determined by dividing the count of female specimens by the count of male specimens within a specific size range. This calculation aimed to determine if the observed ratio significantly deviated from the expected 1:1 ratio. The chi-square test (X^2) was employed to examine potential differences between male and female *V. litterata* (Wimalasiri and Dissanayake, 2016).

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Stages of Egg Development

The visual examination method was employed to distinguish the developmental phases in female crabs. Using Triay-Portella *et al.* (2014) macroscopic egg staging criteria, five distinct gonadal developmental stages were recognized in females. The external features of female crabs were meticulously examined concerning each of these gonadal developmental stages.

Fecundity

To evaluate fecundity, female *V. litterata* with eggs deposited on brightly colored abdomens, either yellow or orange, were chosen. The pleopod carrying the eggs was delicately extracted, and the total weight of the egg mass was precisely measured to 0.08 g. Employing the gravimetric method, three subsamples of around 2 mg each were extracted from various locations within the egg mass and then weighed with an accuracy of 0.1 mg. The number of eggs was tallied under a dissecting microscope, and the fecundity rate (F) was computed following the methodology outlined by Wimalasiri and Dissanayake (2016) using the specified formula:

$$F = \frac{\sum_{i=1}^{n} \frac{Oi}{Wi}}{n} xW$$

Where :
Oi : number of oocytes in a subsample
wi : weight of subsample
n : number of subsamples
W : total weight of ovary

The relationship between carapace width and fecundity was estimated through linear regression analysis.

Egg Diameter

The gonads of the crabs found were measured at four different stages for their external diameter, taking into account their physical appearance. Each stage involved taking 100 eggs as samples, which were then measured for diameter using an ocular microscope scale. Mean egg diameter (±SD) was calculated for each egg stage as done by Wimalasiri and Dissanayake (2016).

Data Analysis

All statistical analyses were tested using SPSS software version 26. Before analysis, the data variance was tested for homogeneity with the F test. The significance of differences was considered at the 0.05 probability level.

RESULTS AND DISCUSSIONS

The research results found a total of 1,032 *V. litterata* individuals, of which 214 (20.73%) were females, 436 (42.24%) were males, and 382 (37.05%) were unknown, while only 57 individuals carried eggs in their stomachs. Overall, the results showed that the mean (±SD) carapace width and length were more significant in females (26.382 ± 7.19 mm; 25.822 ± 7.62 mm) compared to males (22.594 ± 8.11 mm; 21.846 ± 7.62 mm) while the mean body weight of males (9.647 ± 8.84 g) was higher than females (7.513 ± 5.55 g). The mean (±SD) carapace width and length of *V. litterata* carrying egg on its abdomen were (30.878 ± 6.26 mm; and 31.689 ± 6.17 mm). The average weight of *V. litterata* was (12.031 ± 5.60 g) while the average egg of *V. litterata* found showed (1.178 ± 0.60 g). The results also showed that carapace width, carapace length, and body weight of male and female crabs were significantly different (Mann-Whitney test, $p < 0.05$) (length (Sig 0.00), width (Sig 0.010), and weight (Sig 0.001).

Sex Ratio

The research results conducted during the study showed differences in ratios each month (Table 1). Based on the month of capture, the sex ratio in *V. litterata* showed a significant difference (Sig<0.05), in December and January. The *V. litterata* found shows that the female population is greater than the male population.

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Table 1. *Varuna litterata* sex ratio.

Months	Sex Ratio (Male : Female)	Sig
October	0.86 : 1.16 (n = 370)	0.683
November	0.97 : 1.03 (n=299)	0.926
December	0.46 : 2.19 (n=198)	0.049*
January	0.50 : 2.02 (n=187)	0.026*

Note: The number of sexes between males and females is in parentheses; *Significantly different at 95%; Sig<0.05

Anatomy of *V. litterata* Female Reproductive System Macroscopically

The reproductive system of *V. litterata* exhibits bilateral symmetry and is situated on both the cephalothorax and the front portion of the abdomen. In this crab species, the reproductive system comprises a pair of H-shaped ovaries and a pair of vaginas (Figure 2a,b). Positioned dorsally within the cephalothorax, the ovaries are connected solely by a short transverse commissure. The anterior sections of the ovaries, known as the left and right anterior lobes, are located on the lateral side (Figure 2c). Beyond the transverse commissure, the posterior part of the ovary extends ventrally towards the heart, forming two parallel lobes situated above and on the lateral side of the median gut. These lobes stretch toward the cephalothoracic end or the commencement of the abdomen, typically reaching the third abdominal somite.

The back part of the ovary exhibits a ventrolateral extension resembling a pouch beneath the midgut and heart, known as seminal receptacles. These pouches are linked to the dorsal segment of the ovary, while the lower part connects to the vagina and possesses slender, rounded walls. *V. litterata*'s vagina is brief and slim, positioned on the third set of pereiopods, featuring two openings referred to as gonopores (or vulvas) (Figure 2).

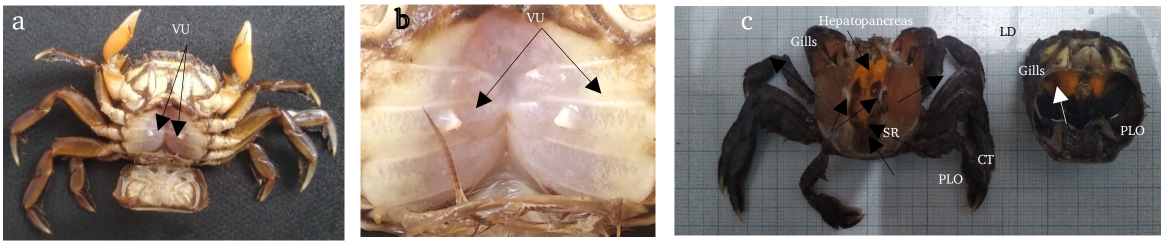

Figure 2. (a) The gonopore has movable parts to close and open the opening. It also shows a small space between the operculum and the outer edge around the vulva (VU). (b) vulva (VU). (c) Female reproductive system in the thoracic cavity; Left Anterior (LE) and Right (LD) Ovarian Lobes; Commissure Transversa (CT); Seminal receptacles (SR); Posterior Lobe of the Ovary (PLO); Anterior Lobe of the Ovary (ALO).

The anatomy of the reproductive system of crabs, in general, is almost the same as the research conducted by Guimarães *et al.* (2021) on tidal spray crabs (*Plagusia depressa*) and *Danielethus crenulatus* conducted by Farias *et al.* (2017). Female crabs have left anterior and right ovarian lobes, transverse commissure, and posterior lobes of the ovary. The gonopore can be moved. This section also shows a small gap between the operculum and the surrounding outer edge of the vulva. Crab eggs will attach to the pleopod.

Stages of Egg Development

During spawning, eggs are released through the gonophores and attached to the long, smooth setae on the pleopod endopods. Based on color differences, the development of eggs outside the body can be divided into four stages (Figure 3). Stage I eggs have a color ranging from purple, while stage II from reddish. Egg color development in stage III is from orange to brownish. Stage IV egg development has a black color. Macroscopically, the developmental stages (Figure 4) also have different appearances.

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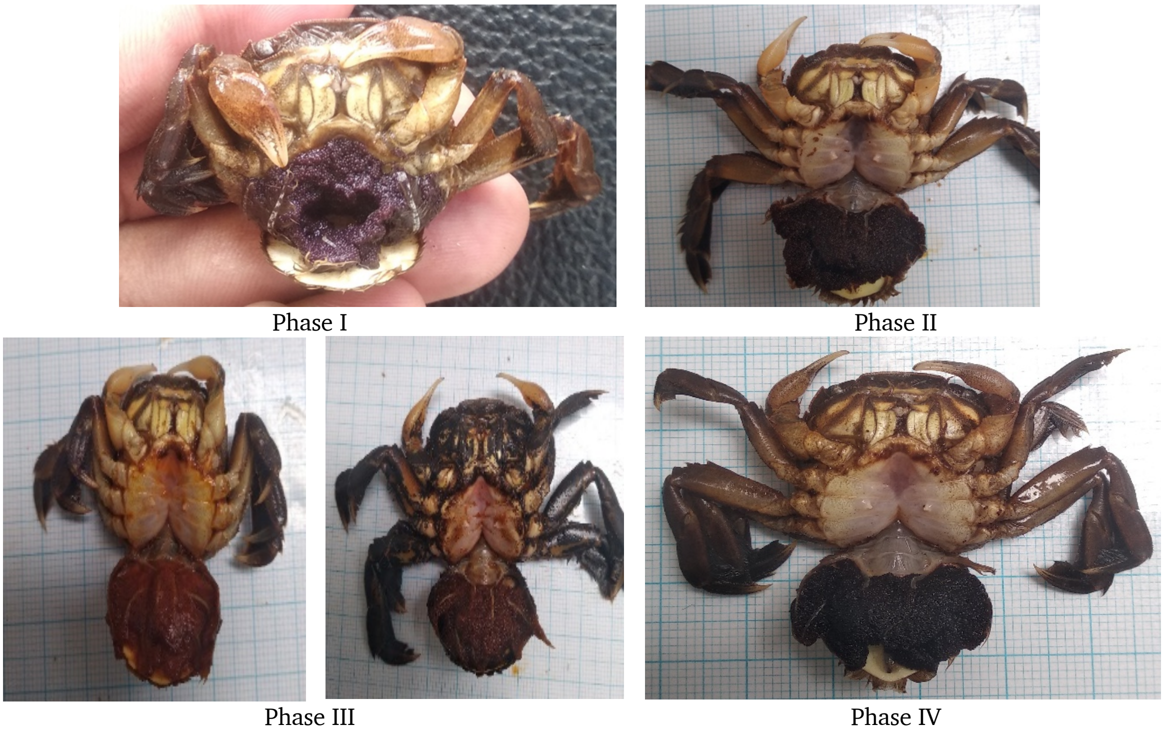

Figure 3. *Varuna litterata* egg development stage.

The developmental stages of *V. litterata* eggs (Figure 3) are similar to those of *Chaceon fenneri* crabs, initially light purple or burgundy, then changing to dark purple and purple-brown. In *C. quinquedens* crabs, the color is initially red-orange, then changes to brown, purple, and finally black (Wigley *et al.,* 1975; Haefner Jr., 1978; Erdman and Blake, 1988; Martínez-Rivera and Stevens, 2020). In *C. affinis* crabs, the egg development stages are in sequence: orange-red or light purple, burgundy, dark purple, brownish, brown to gray-black (Tuset *et al.,* 2011).

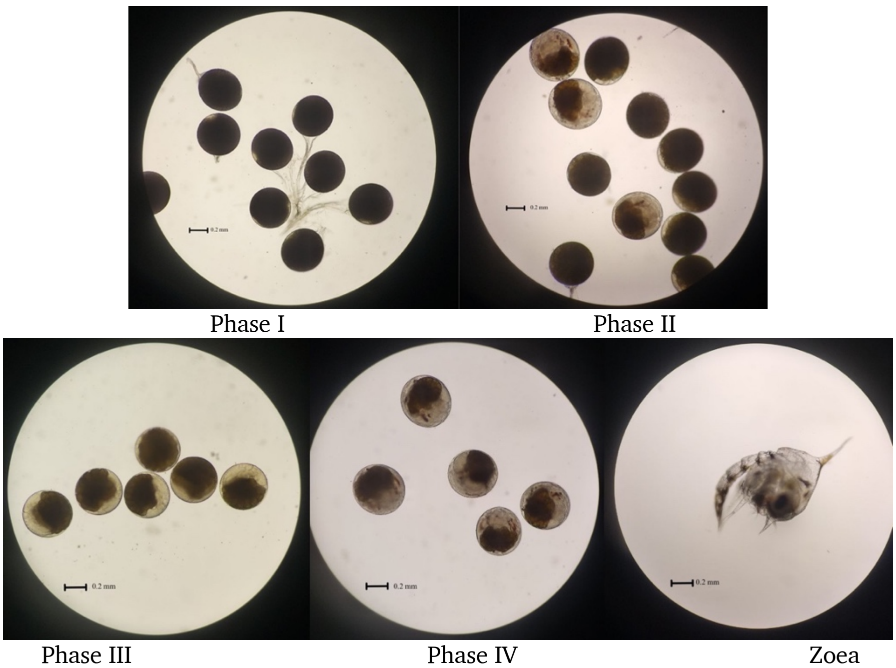

Figure 4. *Varuna litterata* egg development stages macroscopically at 1000 x magnification.

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Phase I is visible by round or oval eggs densely distributed around the pleopod setae. *V. litterata* eggs have a dark color, and the transparent egg membrane is tightly attached to the egg surface without having a particular color. In Phase II, it can be seen that a transparent egg membrane covers the surface of the embryo during division, and a precise division groove can be seen. The cell development and surface of the embryo show a uniform and dense state without massive structures. In addition, the embryo is arranged and wrapped by a thin blastoderm.

Phase III shows the spread of yolk granules covering the entire blastocoel under the blastoderm, resulting in the yolk sac formation. While the embryo is in this stage, one end of the yolk invaginates from the outside, causing a small crescent-shaped transparent area. This forms the archenteron and protostoma structures. In Phase IV, cells continue to grow and differentiate, leading to the morphological division of cells and tissues. The transparent region on the nauplius enlarges, the yolk color diminishes, and the yolk area becomes less conspicuous. Additionally, ongoing cell division and differentiation contribute to the gradual formation of the optic lobe rudiment, antenna rudiment, and mandibular rudiment. During this phase, the transparent area further expands, the yolk area decreases, and lines of eye spots emerge on both sides of the transparent region. The compound eye pigment area extends, and a telson becomes apparent. Subsequently, the larva exits the membrane.

Fecundity

Information relating to fecundity is essential in crab management to assess the reproductive potential of spawning stock biomass and the commercial potential of crab stocks. The research results showed that *V. litterata* has a fecundity of 20,708 - 85,886 eggs. The number of *V. litterata* fecundity found in this study is less compared to the type of *Portunus sanguinolentus* crab as research conducted by Wimalasiri and Dissanayake (2016) states the *P. sanguinolentus* crab can produce fecundity ranging between 112,017 and 1,380,223 eggs. Tuset *et al.* (2011) also researched deep sea red crab (*C. affinis*) and found that the crab produced fecundity ranging from 36,000 - 226,000 eggs (90 - 118 mm CW).

Research conducted by Viswanathan *et al.* (2019), also showed that mangrove crabs (*Scylla olivacea*) produced fecundity ranging from 1.16 - 3.53 million in females with sizes 76 - 135 mm CW. This study's findings also showed an increase in fecundity levels as carapace width and body weight grew (Figure 5). Morphometric parameters, such as carapace width, abdominal width, and body depth, can be considered good indicators for evaluating the reproductive potential of crabs.

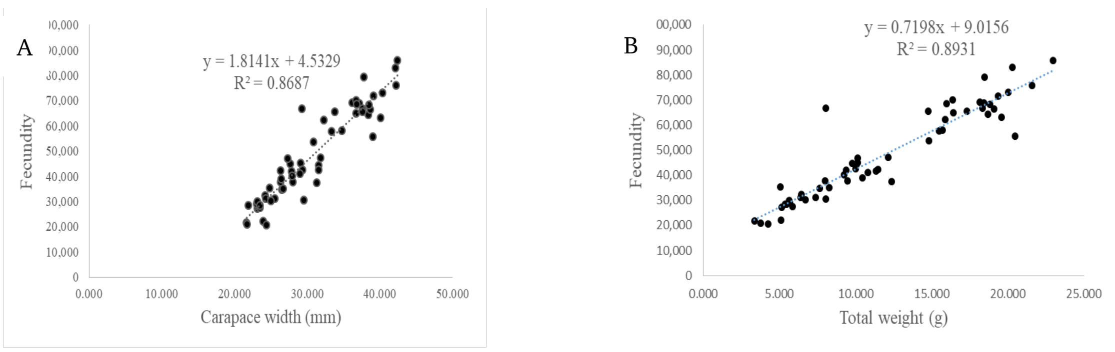

Figure 5. Relationship between fecundity and carapace width (A) and total weight (B).

Variations in fecundity strongly influenced by body size are common in crabs, as noted in several studies (Sukumaran *et al.,* 1986; Rasheed and Mustaquim, 2010; Safaie *et al.,* 2013; Soundarapandian *et al.*, 2013) as also occurs in other brachyuran (Haddon, 1994; Mantelatto and Fransozo, 1997; Litulo, 2004, 2005; Arshad *et al.,*

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2006; Zairion *et al.,* 2015; Swetha *et al.*, 2015; Sharifian *et al.,* 2016; Tureli and Yesilyurt, 2017). Nonetheless, it should be kept in mind that there may be essential variations in fertility levels throughout the year (Wimalasiri and Dissanayake, 2016).

Egg Diameter

The development of *V. litterata* eggs studied at this site showed similarities with the general embryonic pattern observed in several brachyuran crabs, including an increase in egg size (Table 2) from the newly laid stage to the hatching process (Walker *et al.,* 2006; Ates *et al.,* 2012). The results showed that the diameter of *V. litterata* eggs had an average size of 0.440 - 0.466 mm. This increase in egg size is caused by embryonic development, which causes changes in wet weight, diameter, and egg volume. A study conducted by Abit *et al.* (2020) also showed an increase in the diameter of *Isolapotamon bauense* eggs, namely 3.7 - 4.2 mm. Meanwhile, Swetha *et al.* (2015) found that the egg diameter of *Oziothelphusa senex senex* ranged from 1.95 - 2.21 mm. Ikhwanuddin *et al.* (2015) also researched *S. olivacea* and found an increase in egg diameter during egg development.

Embryos that developed to the blastula stage had an average egg diameter of 329.91 ± 6.62 μm. Embryos developing into the gastrula stage have an average egg diameter of 337.10 ± 8.37 μm. The next stage has an average egg diameter of 338.16 ± 6.57 μm. When the eye spots become crescent-shaped, and tissue formation occurs, the average egg diameter is 358.45 ± 14.80 μm. While before hatching, the average egg diameter is 377.26 ± 11.50 μm. In addition, research on coconut crabs shows that the average egg diameter in stage III ranges from 0.01 to 0.056 mm, while stage IV ranges from 0.01 to 0.061 mm (Serosero *et al.,* 2020).

Table 1. *Varuna litterata* egg diameter.

Stage of Development	Number of egg samples (n)	Mean Diameter (mm) (±SD)
I	n = 100	0.440 ± 0.060^{a}
II	n = 100	0.451 ± 0.047^{ab}
III	n = 100	0.454 ± 0.037^{ab}
IV	n = 100	0.466 ± 0.063^{b}

Table 2 shows that *V. litterata* eggs are tiny. Some of the crab's adaptation patterns are the small number of eggs and high fecundity. The diameter of *V. litterata* is slightly larger than that of the crab (*P. pelagicus*), which has an egg diameter of 0.324 mm (Gaffaroğlu *et al.*, 2013). The egg diameter in *V. litterata* is still smaller than that of the King crab (*Lithodes santolla*), which has an average diameter of 1.537 – 1.984 µm (Militelli *et al.,* 2019). This crab also has a smaller egg diameter when compared to *I. bauense*, which has an egg diameter of 3.7 - 4.2 mm (Abit *et al.,* 2020).

Research conducted in the lower Serayu River at two observation stations (Station 1 with fresh habitat and Station 02 under the Serayu motion weir with fresh habitat) did not find *V. litterata* carrying eggs on its abdomen. *V. litterata* carrying eggs was found at the third observation station (Serayu River estuary with brackish habitat). It is not sure at what stage of the gonad development phase is needed so that *V. litterata* migrates to brackish waters. The research results show that *V. litterata* can hatch its eggs well at a salinity of 15-25 ppt. This condition supports the condition of the Serayu River estuary waters, which have a salinity of 17-25 ppt during the rainy season. When the rainy season occurs, the salinity conditions in the Serayu River Estuary will decrease and even become fresh. This will cause *V. litterata* to migrate in search of a suitable habitat to hatch its eggs.

The migration pattern in *V. litterata* spawning is similar to that of freshwater giant shrimp (*Macrobrachium rosenbergii*). Tan and Wang (2022) state that freshwater giant shrimp will migrate to brackish waters according to their natural tendencies. This results in the development of embryos in

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freshwater giant shrimp, which will take place in brackish water. This is the same as what happens to *V. litterata*. The migration pattern of *V. litterata* is similar to that of coconut crabs, which lay eggs by migrating to the sea to release their eggs. Female crabs lay eggs immediately after mating and attach them to the underside of their abdomen. They carry the fertilized eggs for several months before releasing the larvae into the sea at hatching (Serosero *et al.,* 2020).

V. litterata will spawn in brackish water, leading the author to hypothesize that mineral content is needed in the normal development of *V. litterata* embryos. Estuaries have a higher mineral composition compared to rivers. Wang *et al.* (2019) research on embryo and larval development in Chinese mitten crab showed that low concentrations of sodium chloride ions can delay cell division and even inhibit embryo development, while too high sodium chloride ions will damage cell division. Hangsapreurke *et al.* (2015) also argue that low concentrations of calcium chloride ions can result in thinning of the egg-wrapping layer.

The concentration of minerals such as calcium (Ca), sodium (Na), and potassium (K) are the main factors that affect salinity levels. This research conducted by Tan and Wang (2022), that the optimal levels of sodium chloride, potassium chloride, and calcium chloride in water are 169.2 mM/L, 3.6 mM/L, and 3.7 mM/L, respectively. Mineral content is necessary for shell hardening and is vital in maintaining ion channels' internal balance and function. Internal balance and ion channel activity play a vital role in embryo development.

CONCLUSION

The results showed that the development of *V. litterata* eggs is divided into four stages. At the initial development stage, *V. litterata* eggs will be purple; in the second stage, the eggs will turn reddish; in the third development stage, the eggs will turn orange to brownish; and in the fifth stage, the eggs will develop and become black. The results also show that the carapace width and weight of *V. litterata* are positively correlated with the number of *V. litterata* fecundity. *V. litterata* that matures gonads will migrate to brackish water. The results also showed that *V. litterata* produced as many as 20,708 - 85,886 eggs with an average egg diameter of 0.440 - 0.466 mm. Further research is needed to determine the optimal salinity and mineral requirements for good larval development.

CONFLICT OF INTEREST

There were no conflicts of interest between the authors while writing and publishing this manuscript.

AUTHOR CONTRIBUTION

Sorbakti Sinaga's contribution to this research was as a researcher who carried out data collection and data analysis, Maheno Sri Widodo and Yunita Maimunah as a valuable mentor in this research and excellent preparation of the manuscript.

ACKNOWLEDGMENT

We want to thank the Aquaculture Master's Study Program at Brawijaya University and the Aquaculture Study Program at Jendral Soedirman University for helping provide laboratories for this research. We would also like to thank all parties who contributed directly or indirectly to data collection in the field.

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