REPRODUCTIVE BIOLOGY OF RIVER SWIMMING CRAB VARUNA LITTERATA

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ABSTRACT

This study aims to obtain information on the reproductive biology of these species, especially on sex ratio, fecundity, ovarian development, and breeding season. The study was conducted from August 2022 to July 2023 on every full moon with the highest tide in the estuary of Bengawan Solo River, East Java, Indonesia. The obtained samples were placed in the freezer and identified, and a dissection was performed to observe the gonads. There were 617 crabs with an average sex ratio of 1 male to 1.9 females. Mean fecundity of *V. litterata* was 45350±3182 egg/individual. In each sampling period, there were always females that were maturing gonads. The ovaries of females during the study were the early developed (stage III), late developed (stage IV), and mature (stage V), and no females in the immature (stage I) or underdeveloped (stage II). The highest mean of the Gonadosomatic Index was 0.14±0.3, and the result figured that *V. litterata* was in stage V. The highest GSI value was in June and December. This study illustrates that *V. litterata* has the potential to spawn every month and continuously for almost a year, with peak spawning between November and January.

Keywords: sex ratio, fecundity, ovarian development, breeding season, life below water

الحياتية التكاثرية للسرطان السابح النهري

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المستخلص

تهدف هذه الدراسة الحصول على معلومات عن الحياتية التكاثرية للسرطان السابح النهري، فيما يخص النسبة الجنسية، والخصوبة (عدد البيض)، و تطور المبيض و موسم التكاثر. أجريت الدراسة للمدة من آب 2022 إلى تموز 2023 في كل مرة يكتمل فيها القمر مع أعلى مد في مصب نهر بنغاوان سولو،وجاوي الشرقية، إندونسيا. تم تجميد العينات المستحصل عليها من ثم تم تشخيصها وسحب الرطوبة منها لمعاينة الغدد التناسلية. كان هنالك 617 عينة بمتوسط نسبة جنسية ذكر واحد إلى 1,9 انثى. بلغ متوسط خصوبة السرطان 45350 ±3182 بيض/فرد. كان هنالك في كل عينة إناث ذات غدد تناسلية ناضجة دائما. كانت مبايض السرطان في مرحلة مبكرة من التطور(المرحلة الثالثة)، و متأخرة التطور (المرحلة الرابعة)، وناضجة (المرحلة الخامسة)، ولم يكن هناك مبايض في السرطانات غير ناضجة (المرحلة الأولى) او غير المتطورة (المرحلة الثانية). كان أعلى متوسط لمؤشر الغدد التناسلية 0،14+/−0،3، و اظهرت النتائج أن السرطان كان في المرحلة الخامسة. بلغت أعلى قيمة لمؤشر غدد التناسلية في شهري حزيران و كانون الأول. أوضحت هذه الدراسة أن السرطان فيرونا لديه القدرة على التكاثر كل شهر و بشكل مستمر لمدة عام تقريبا، و إن ذروة التكاثر تكون بين تشرين الثاني وكانون الثاني.

الكلمات المفتاحية: النسبة الجنسية، الخصوبة، تطورالمبيض، موسم التكاثر، الحياة تحت الماء

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INTRODUCTION

The riverine ecosystems in Indonesia play a crucial role in ensuring food security, as their rich biodiversity provides sustainable food sources, such as fish and aquatic invertebrates, which form a fundamental part of the local diet. Additionally, these ecosystems support rural communities by enhancing fisheries, improving water quality, and contributing to agricultural productivity and environmental sustainability (27, 36, 39). *Varuna litterata* is a species of crab from the Varunidae family, having the local name "kampat." *V. litterata* is widespread in Indo-Pacific waters to the east coast of Africa and Polynesia (8, 32). *V. litterata* usually inhabits mangrove forests, estuaries, freshwater, and shallow tidal areas. *V. litterata* is also commonly found under rocks, logs, and dead leaves and in burrows along pond dams, creeks, and shallow banks (24). *V. litterata* is also an organism that inhabits mangrove areas with sandy substrates rich in organic matter and prefers areas close to the sea (22). Moreover, they prefer areas close to the sea with good tidal currents or directly opposite the sea and can be found up to 32 km upstream (7, 13). *V. litterata* has been caught for consumption and utilized as a source of animal protein (22). Research conducted by (5) showed that *V. litterata* contains several important fatty acids such as palmitic acid (16:0), stearic acid (18:0), behenic acid (22:0), oleic acid (18:1ω9), linolenic acid (18:2ω6), linoleic acid (18:3ω3), EPA (20:5ω3), DHA (22:6ω3), arachidonic acid (20:4ω6). This species is also rich in chromium, which helps insulin metabolize sugar, so it is good for people with hyperglycemia to consume (5). The increasing demand causes the capture of *V. litterata* to be carried out continuously and wildly because there is no precise regulation on the rules of capture of *V. litterata*. The status of *V. litterata* is currently included as a species that has yet to be evaluated (not evaluated) in the I.U.C.N. red list (27) Still, continuous large-scale fishing operations, in the absence of precise regulation of fishing rules, in addition to the factor of pollution constitute the most important risks threatening the existence of *V. litterata* in it is habitat in the future. Studying biology related to reproductive data can be used as a reference in cultivation activities; of course, it will help overcome the threat of overexploitation because the availability of *V. litterata* can be met from cultivation, besides cultivating *V. litterata* as a fulfillment of community food security sourced from animal protein. Regarding the reproductive biologies studies, publications are still truncated and are still focused on the discovery of *V. litterata* in different regions or waters (35). There has been no research focusing on the reproductive biology of this species. The research results have yet to provide detailed information about the reproductive biology of *V. literate* (12), so they cannot be used as a reference for maintenance efforts in a controlled environment. This research was conducted to complete information on the reproductive biology of *V. litterata* especially on sex ratio, fecundity, ovarian development, and breeding season. Fecundity can change because many factors, such as age, feeding, size, habitat, and water conditions can influence them. Fecundity also differs even in the same species but in different habitats with different temperatures, and salinity (21). The value of fecundity can be an organism's reproductive potential (21, 33). Ovarian development is a process that occurs in oogonia in female individuals and includes repeated mitotic division, growth, and maturation by the yolk. The reproductive cycle or period will be known by knowing ovarian development, which is undoubtedly related to reproductive patterns (Garcia. When the breeding season forecast is known, it will help understand the right time for females to migrate and spawn safely (1), so it will benefit management programs and aquatic protection for aquatic animals, including *V. litterata*, from uncontrolled capture.

MATERIALS AND METHODS

Sample collection.

The sampling location was at one of the mouths of the Bengawan Solo River, which is enclosed by the Java Integrated Industrial and Ports Estate (J.I.I.P.E.) industrialization area and dense residential areas, and several brackish water pond areas on the right and left sides of the river. The estuary of the Bengawan Solo River becomes the only river used to distribute domestic and industrial

waste wastewater. Fishing communities also use the river to catch fish. Crab samples were captured using a set net in the Bengawan Solo River estuary on the north side of Java Island opposite Madura Island in East Java with coordinates 7° 5' 56.5188" S, 112° 37' 28.4664" E every full moon from August 2022 to July 2023. Furthermore, from September to February we term the rainy season and March to August is the dry season. Crab samples were identified based on the square shape of the carapace, symmetrical cheliped size and shape, and long and slender walking legs, while the last walking leg forms like a paddle. Male crabs have a conical abdomen, while female individuals have a rounded abdomen. (25, 38). The obtained crab samples were then brought to the Laboratory of the Faculty of Fisheries and Marine Science, Universitas Brawijaya, to continue with further observations on reproductive aspects. The number of females and males was first calculated before weighing the crabs using analytical scales and measuring the width of the carapace using a caliper with an accuracy of 0.05 mm. Gonadal observations were made after the crabs were unconscious by putting them in a coolbox filled with ice. Animal anatomy was performed by opening the upper carapace using scissors; the opened carapace was then photographed for macroscopic gonad analysis. Where:

GW = gonad weight,
BW = body weight,
GSI = Gonado Somatic Index,
CW = carapace width

Sex determination

Sex determination is based on the shape of the abdomen of each crab (Fig. 1). Female crabs have a slightly rounded shape. In contrast, male crabs have a conical and narrow abdominal shape (24).

Sex ratio determination

The ratio of female and male crabs was determined using the formula (28),

sex ratio=

Fecundity

The gravimetric method is currently the most used to estimate fecundity, determined by total gonad weight and a sample of the gonad weight by subdividing three gonad subsamples and weighing with an accuracy of up to 0.001 g. Fecundity is obtained by multiplying the gonad weight by the number of eggs per gonad sample divided by the gonad weight (37).

Gonado somatic Index

To calculate the GSI value, the gonad weight is divided by the crab weight and expressed in grams using the following formula (9),

GSI= Gonado somatic Index
WG= wet weight of gonad (gram)
WC= wet weight of crab (gram)

Investigating the breeding season

The breeding season can be estimated by the gonad maturity level and GSI values each month. This study assessed the breeding season by counting female crabs with matured gonads each month and showing them in percentage.

Data analysis

The obtained data were then copied and correlated with a computer spreadsheet to ensure accuracy and grouped based on the objectives of the research findings.

RESULTS AND DISCUSSION

Sex ratio

There were 617 crab specimens obtained from August 2022 to July 2023. *V. litterata* will be easily found and caught when the moon is full, and the river is experiencing high tides.

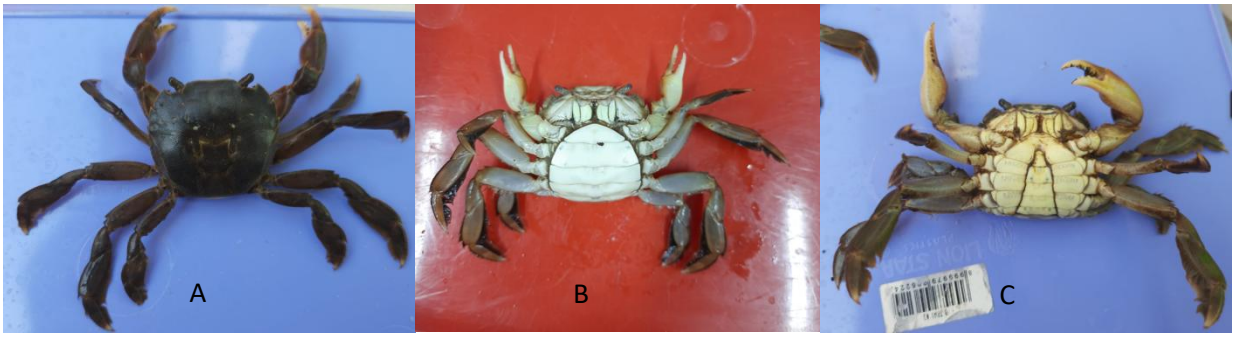

Figure 1. Different sexes of *V. litterata*. (A) Dorsal view of *V. litterata*, no difference in dorsal appearance for males and females, (B) Female crab with rounded abdomen, (C) Male crab has a conical and narrow abdominal shape.

V. litterata will swim in the water, while if the water is at low tide they will hide in burrows that they dig in the riverbed. Male and female individuals are distinguished by the shape of the abdomen (Fig. 1). Based on the sex ratio calculation, the highest number of female crabs was found in April and October. Conversely, we detect fewer female crabs in June and December. The sex ratio obtained is 1: 1.19 or 1 male compared to 1.19 females (Table 1). This ratio differs slightly from the findings (24) on the sex ratio of *V. litterata* obtained from the Sundarbans, India, which is 1: 0.66. This difference may be due to differences in water characteristics in the Sundarbans, India, with the location of this study. The emergence of an unbalanced sex ratio is due to differences in compatibility between male and female individuals with the aquatic environment, such as water temperature, salinity, and dissolved oxygen. The behavior of female individuals which migrate from rivers to the sea to hatch eggs influenced the sex ratio value, as stated by (7, 10, 21).

Fecundity

Fecundity is the number of eggs produced by the female and can be counted if the eggs are on the pleopod. In this study, berried females were only found in December, May, and June. The claculated fecundity of *V. litterata* was in the range of 38987 to 49982 eggs, with a female's carapace width between 21 - 36 mm. The average fecundity was 45350, with the average ratio of fecundity and carapace width being 1597 eggs/1 mm carapace width.

Table 1. Monthly variations in the sex ratio of *V. litterata* randomly collected from Bengawan Solo River estuary, East Java

Month	Total	Total Male	% of Male	Total Female	% of Female	Sex Ratio
Aug	52	33	63.46	19	36.54	1:0.58
Sept	66	40	60.61	26	39.39	1:0.65
Oct	62	20	32,26	42	67.74	1:2.1
Nov	40	12	30.00	28	70.00	1:2.3
Dec	27	19	70.37	8	29.63	1:0.42
Jan	41	26	63.41	15	36.59	1:0.58
Feb	68	37	54.41	31	45.59	1:0.84
March	66	24	36.36	42	63.64	1:1.75
April	74	28	37.84	46	62.16	1:1.64
May	47	13	27.66	34	72.34	1:2.6
June	32	26	81.25	6	18.75	1:0.2
July	42	26	61.90	16	38.10	1:0.62
Total	617	304		313		1: 1.9
Chi square value = 62.34, Degree of Freedom = 11, p value = 3.40×10^{-9}						

Fig. 2 shows a positive significant correlation between carapace width and fecundity value (R= 0.95; $p< 0.05$), meaning that the wider the carapace, the higher the fecundity value. With almost the exact width of a carapace, *Eriocheir sinensis* has a fecundity that reaches 100000-600000 eggs (4). Compared to other Brachyurans, the fecundity of *V. litterata* is

low. *Callinectes bellicosus* has 2730217 eggs, while *Scylla serrata* has 1574000 eggs, and *Scylla olivacea* has 2400000 eggs (16, 31, 37). Low fecundity illustrates that *V. litterata* has low reproductive potential, thus affecting stock availability and recruitment of the species. To date, no one has reported the fecundity of *V. litterata*. Fig. 3 shows the difference in fecundity in December (the end of the rainy season) and May and June (the end of the dry season). Seasonal differences are thought to affect water conditions and the availability of natural food for *V. litterata*. *V. literata*'s fecundity is generally higher in the dry season than in the rainy season. The difference in fecundity is likely due to the low water flow from upstream to the estuary in the dry season, which makes the availability of natural food favored by *V. literate* more abundant. In the rainy season, the river often receives a lot of water flow from upstream, so the availability of natural food, which is *V. litterata*'s prefereable food, is replaced by other types of natural food with different nutritional content. In general, Brachyuran (*Uca virens*, *Goniopsis cruentata*, and *Hemigrapsus sanguineus*) have diverse fecundities influenced by differences in species, habitat, diet, and season, including primiparous or multiparous (6, 15, 18). Differences in fecundity between species are closely related to carapace width, habitat, and primiparous or multiparous status. Some Brachyurans have higher fecundity when multiparous, as with *E. sinensis*, which has higher fecundity in the second or third spawning season (4). In this species, no information has been found on the fecundity of the first, second, or third breeding because the crab samples were not cultured but taken from the wild.

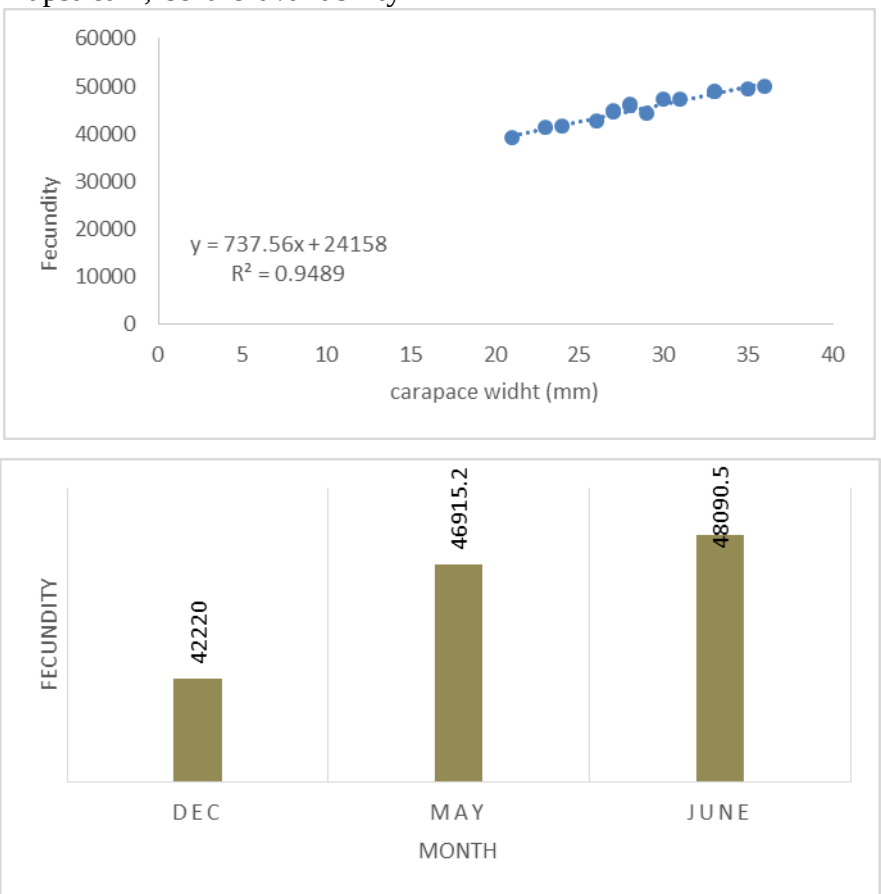

Figure 2. Relationship between carapace width (ICW) and fecundity of *V. litterata*

Figure 3. Ovarian development stages of *V. litterata* collected from Bengawan Solo River, East Java (A: Early-developed (stage III); B: Late-developed (stage IV); C & D: Mature (stage V))

Ovarian development

The ovary of *V. litterata* is in the cephalothorax cavity, wrapped in a membrane layer. The membrane that covers the ovary can change color with a higher level of gonadal maturity so that the eggs containing vitellogenin cannot be seen with the naked eye (Fig. 4). The stage of ovarian maturity is divided into five stages. Stage I (immature), the ovary at this level is still difficult to see, its shape is thin and transparent. In stage II (under development), the ovary is pale yellow and still thin. At stage III (early development), the ovary is yellow, and the membrane is green-yellowish. The ovary is solid yellow-blackish at stage IV (late development). In the stage V (mature), the ovary is empty again, with many eggs in the pleopod in the abdomen (26). This study shows color changes in *V. litterata* ovaries based on the level of gonadal maturity. At the third stage of gonadal maturity, the ovaries were bright yellow brownish, and greenish with a soft texture. The volume of the ovary still did not fill the cephalothorax cavity (Fig. 4A). At the fourth level of gonadal maturity, the ovaries are brownish green or blackish brown with a denser consistency (Fig. 4B). Many eggs are on the pleopod at the mature stage and ovary is empty again (Fig. 4C&4D). In previous studies, there was no clear information about ovary color changes in this species, the color change in the ovary is slightly different from the color change of the ovary in other types of crabs of different genera or families, such as the *Portunidae* family in the genus *Scylla* (28) and the genus *Portunus* (17). This is due to the character of the ovary of *V. litterata*, which is wrapped by a layer of membrane that is darker than the color of the ovary. Color changes in ovaries are closely related to the process of vitellogenesis during oocyte maturation in response to endogenous estrogen (20). During the sampling period, macroscopic observations showed no *V. litterata* ovaries in maturity stages I and II at various carapace sizes. (Fig. 5).

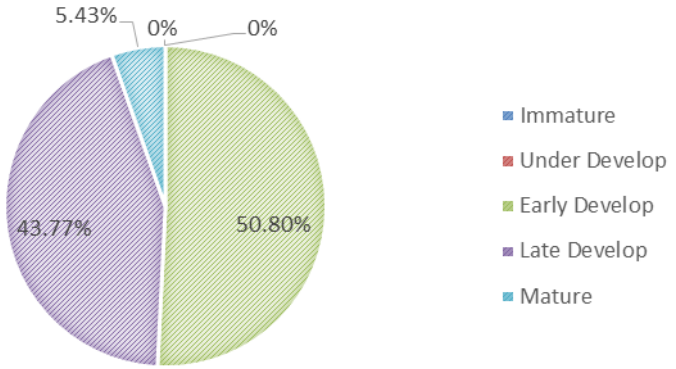

Figure 4. Ovary development stages of *V. litterata* after observation through the dissection.

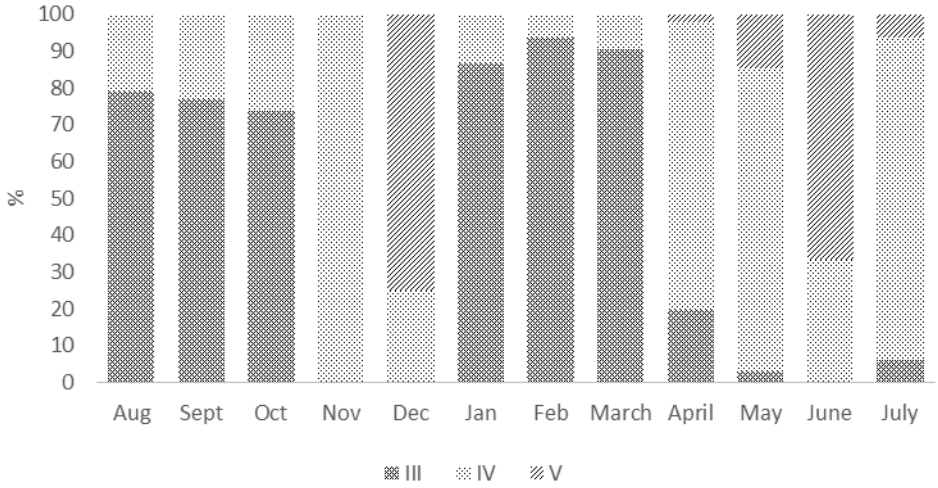

Figure 5. Monthly changes of ovaries at the three development stages of female *V. litterata*

This is thought to be because the sampling period was carried out at the same time every month, i.e., during the highest tide, so the condition of the ovaries outside the sampling period is unknown. The absence of stage I and II females is also related to the life cycle of *V. litterata*, which returns to the river to grow and develop; then, *V. litterata* will head to the sea to hatch its eggs. The ovaries of *V. litterata* during the sampling period were in three levels of gonadal maturity namely there was 50.80% at the third level of gonadal maturity or early development, 43.77% at the fourth level (stage IV) or late development, and the remaining 5.43% at the fifth level (stage V) or mature. The ovaries at the first and second levels, or immature and underdeveloped, are the same at 0%. Ovaries at the third stage (early development) were mostly obtained at the beginning of the dry season (July, August, September, October) and the start of the rainy season (January, February, March). At the end of the dry season (November) and towards the end of the rainy season (April, May), many ovaries at stage four (late development) were obtained. In December and June, transition months from the dry to the rainy season or vice versa, many ovaries with stage V (mature) are obtained (Fig. 6). Individuals with stage V were found more at the end of the dry season due to differences in river water temperature. Higher temperatures in the dry season and high sun intensity make the density of natural food in the river high, which seems reasonable for the ovary maturation process in *V. litterata*.

Gonadosomatic index

The gonadal somatic index is usually used to determine gonadal maturity by comparing gonadal weight to total body weight (26, 28). In this study, the average GSI was informed that it would increase with increasing gonadal maturity and reach the highest average of 0.14 at the fifth gonadal maturity level (stage V) (Table 2).

Table 2. Frequency distribution of GSI for each development stage of female *V. litterata.*

GSI %	Frequency at each development stage %				
	Stage I	Stage II	Stage III	Stage IV	Stage V
<0.01	0.00	0.00	0.00	0.00	0.00
0.01-0.05	0.00	0.00	5.7	0.00	0.00
0.05-0.1	0.00	0.00	0.00	11.4	0.00
>0.1	0.00	0.00	0.00	0.00	2.4
No. of samples	0.00	0.00	157	139	17
Mean GSI	0.00	0.00	0.04	0.08	0.14
SD (±)	0.00	0.00	0.52	0.98	0.3

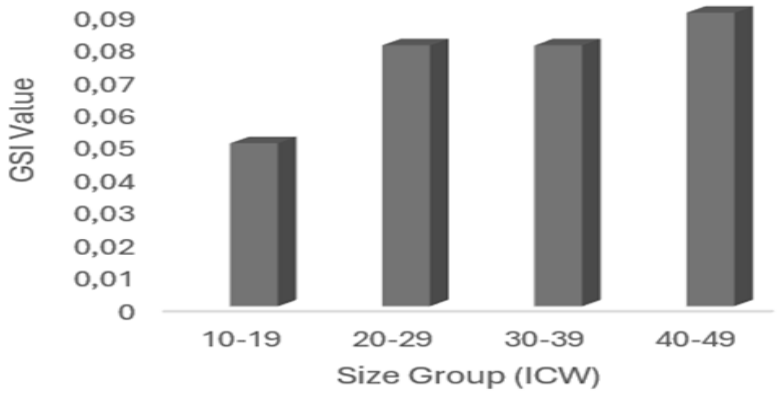

Figure 6. Gonado somatic index of female *V. litterata* by size from August 2022 to July 2023 from Bengawan Solo River estuary, East Java.

The average GSI will also increase with increasing carapace size; Fig. 7 explains that GSI is highest in the 40-49 mm size group. The relationship between GSI values with gonad maturity and carapace width is due to the process of gonad maturity vitellogenesis occurs, which affects the size of the egg diameter; when the size of the egg diameter gets bigger, it will require more space in the cephalothorax cavity. This discovery is identical to other studies (26, 31, 34) on other crab species, in which body size is associated with gonad maturity and weight. The process of vitellogenesis in the ovary begins to occur at level three, so the weight of the gonads will increase rapidly at the next level, reaching the highest value at level five and decreasing again after spawning (1, 19).

Breeding season:

The percentage of gonadally mature crabs and GSI values in each sampling month are shown in Figures 6 and 8. Ovaries undergoing vitellogenesis stages III-IV were found in almost every sampling month except in November and June, where no ovaries were found in stage III, while ovaries with stage IV were found in all sampling months. Stage V or mature ovaries were only found in November, December, April, May, June, and July. However, many were found in November and June (Fig. 6). This indicates that gonad development and spawning occur continuously in one year. The highest GSI value was in June, then slightly lower in December (Fig. 8). The breeding season of *V. litterata* is known to have two peaks, which are between November and January and will be repeated in May to July (Fig. 8). Not only *V. litterata* have two peaks of breeding season, but several other types of crabs also have two peaks of breeding season, namely in *Scylla olivacea*, *Carcinus maenas* and *Portunus sanguinolentus* (1, 11, 23, 29).

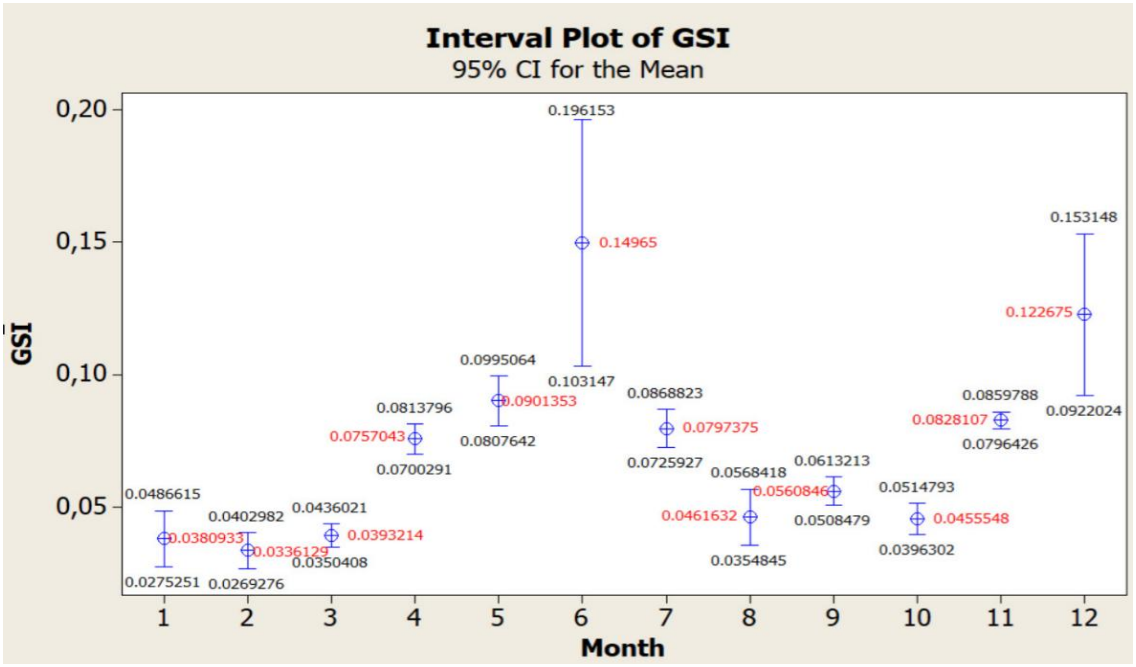

Figure 7. **GSI variation of *V. litterata* from August 2022 to July 2023**

There have been no previous reports on the breeding season of *V. litterata*. Breeding season may differ depending on geographical areas and different species. One water parameter that affects gonad development is salinity. In this study, salinity in November-December was low (18 ppt) because it was the peak of the rainy season. While in March-July, salinity was in the range of 22 and 25 ppt. These months are included in the dry season. Salinity above 20 to 25 is suitable for developing crab gonads in brackish water and marine areas. They will then be spawning in higher salinity, so they must go to the sea to lay their eggs (1). However, it is certainly not only salinity that affects brackish water crabs such as *V. litterata*. In addition to salinity, surface water temperature and availability of natural food are important factors for crab gonad development. Based on surface water temperature measurements during sampling, the water temperature in the months before the breeding period, August-October, was 31.5° C. This is almost the same as the water temperature between 31 and 33° C from March until July. High temperature is one of the main factors for the development of crab gonads with synthetic relaxation and secretion of reproductive hormones (30). Breeding season is also influenced by latitude and longitude and is also affected by the moon phase, which is closely related to seasonality and the availability of natural food in the waters (2, 3).

Conclusion

This study is the first to investigate the sex ratio, fecundity, ovarian development, and breeding season of *V. litterata* in the Bengawan Solo River estuary, Indonesia. Including waters in Indonesia**.** The results of this study indicate that although *V. litterata* has a low reproductive potential, based on ovarian development, *V. litterata* has a reproductive pattern that continuously lasts throughout the year by having a peak reproduction in certain months, such as at the end of the rainy season and the end of the dry season. Based on ovarian development in *V. litterata*, it can be said that *V. litterata* is a total spawner individual**.** The status of reproductive biology, including sex ratio, fecundity, ovarian development, and breeding season of *V. litterata*, is closely related to several factors. Species, size, age, and genetics are endogenous factors. Water conditions, habitat, and food availability in the water are exogenous factors that affect the reproductive status of *V. litterata*. This study is imperfect because it is still limited to reproductive biology data and has yet to see the relationship between the mentioned environmental factors. Further research will be better focused on the influence of the environment, such as water parameters and the presence of natural food,

on the potential development and reproductive patterns of *V. litterata*. The results of this study also recommend that the local government review the function and regulation of public waters.

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