



Reproductive Biology of Land Crabs (*Gecarcoidea lalandii*) in Ly Son Island, Quang Ngai Province – Vietnam

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Authors' contributions

This work was carried out in collaboration among all authors. Authors HMS, LTTT, HSL, PMT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HMS and PMT managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The reproductive biology of the land crab *Gecarcoidea lalandii* was documented for the Ly Son Island, Quang Ngai Province, Vietnam. Approximately 30 crabs, collected every month in the 12-month period from August 2019 to July 2020, were analyzed gonadal development stages, sex ratio, spawning season, fecundity and size at first sexual maturity. Results showed that number of male and female crab in nature was not significantly different. Gonadal of the crab passed through five stages of maturation. The crab spawns continuously during the spawning season. In nature, the crab can mature whole year-round with a higher proportion from January to August and the peak maturation from June to July. The spawning season of this species is only on the raining period from June to July. Length at first sexual maturity of the crab was 53.91 mm of carapace width for female and 50.39 mm male crab. The absolute fecundity varied in 473,000-820,000 eggs ind⁻¹ and highly related to body weight. The current findings provide the scientific foundation for fishery resource management and artificial breeding of the land crab in Ly Son.

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1. INTRODUCTION

Land crabs (*Gecarcoidea lalandii* H. Milne-Edwards, 1837) distribute both tropical islands and continental maritime forests and may have large populations exhibiting high densities [1]. The roles for land crabs in the ecosystems of small islands, including the transfer of energy from land to sea and vice versa, preventing or reducing colonization by exotic plant species, hastening leaf litter breakdown reducing the number of carrion-breeding flies, aerating the soil, speeding erosion, aiding soil formation and providing drinking and breeding places for other animals [2]. The land crabs genus *Gecarcoidea*, belonging to the family Gecarcinidae, comprise only of 2 species and is confined to the Indo-Pacific region. The distribution of *G. natalis* is limited to Christmas Island and Cocos Island in the Indian Ocean [3]. In contrast, *G. lalandii* has a wider distribution in the Indo-West Pacific islands starting from the Andaman Island eastward [4]. Numerous studies have been conducted on *G. natalis* including physiological characteristics [5]; breeding migration and behavior [6,7]. In contrast, very few studies on *G. lalandii* have been conducted in terms of reproductive biology, only one study on this crab in Taiwan []. The results reveal that Ovigerous females have an average carapace width of 57 ± 7 (range in 42-78) mm. The breeding season is variable, depending on the beginning of the rainy season. Ovigerous females usually release their larvae during 7-10 days in the last quarter of the lunar cycle. Larval release shows a closer relationship with the timing of sunrise than with the high tide. This species exhibits an unusual larval release behavior while clinging to vertical rock faces in which the ovigerous females drop egg masses into the water without actually entering the water. This unusual larval release from above the water and the choice of surge channels as release sites are thought to be adaptations to life on land which may reduce the risks of mortality to ovigerous females [8].

Ly Son district locates from $15^{\circ}22' - 15^{\circ}23' N$ and $109^{\circ}05' - 109^{\circ}08' E$, at the North-Eastern of Quang Ngai Province (Fig. 1). The district comprises two islands named Lon and Be Island. Nowadays, Ly Son is one of the most tourist attractive areas in Quang Ngai Province. During 2017, more than 210 thousand tourists visited Ly Son, comprise of one-third tourists visited Quang Ngai Province.

Land crab (*G. lalandii*) is one of the famous seafood of Ly Son. The price of this crab is around 35 to 50 USD\$/kg during the tourism season. The increasing number of tourists visiting Ly Son results in a higher demand for this land crab. As the result, there is an increase in the impacts on the natural resource of this crab in Ly Son. A survey data conducted to evaluate the natural caught of crab in Ly Son has shown that the total number caught in 2015 was around 350 kg and decreased to less than 270 kg in 2019. Thus, there is a great need for a suitable management strategy of the natural resource of the crab as well as the strategy for also breeding of this crab supporting growth, reducing the impact on natural resources. However, information available on the reproductive biology of the crab in Ly Son is still unknown.

Hence, a detailed investigation on reproductive biology including gonadal development stages, sex ratio, spawning season, fecundity and size at first sexual maturity was conducted and the results are presented and discussed in this paper.

2. MATERIALS AND METHODS

2.1 Sample Collection

The land crabs were collected from the Fishermen's Catch in Ly Son Islands (Fig. 1). from August 2019 to July 2020. The carapace width of the 348 crabs ranged from 36.04 to 92.14 mm, average of 60.98 ± 7.99 mm and the weight ranged from 15.02 to 225.60 g, average of 74.04 ± 32.37 g. Around 22-36 crabs were collected each month due to a rate species. The number of crabs collected each month is presented in Table 1.

The crabs were transported to the laboratory at the Institute of Oceanography, Vietnam for analysis. At the laboratory, the crabs were killed using the ice-slurry immersion method then weighed and measure the carapace width. The reproductive gonad of the crab (testes or ovaries) were then dissected and weighed for further analysis.

2.2 Sample Analysis and Data Collection

Whereas the maturity stages of females were recognized based on the macroscopic appearance of the ovary in the body cavity and

microscopic structure of ova, in males only the macroscopic appearance of testes was considered. Gonadal development stages of crabs were determined by methods of [9] using an Olympus BX50 microscope at 10 X and 40 X magnification.

Histological analysis of testes and ovary was performed following the method described by [10]. Ovary or testes of each gonadal development stage of crab were dissected and fixed in 4% buffer formalin for 24 h. After dehydrating by passing the tissue through a series of alcohol solutions of 70, 85 and 98%, the samples were vacuum embedded in paraffin. The histological sections (4-5 μ m) were stained for general morphological purposes with hematoxylin and eosin (H&E). The samples were photographically analyzed and documented using the Olympus BX 50 microscope at 40 X magnification.

The spawning season of the species was determined based on the availability of mature and spent individuals in the commercial landings during different months and the monthly gonadosomatic indices (GSI). The GSI was calculated [11,12] using the formula:

$$\text{GSI} = 100 * (\text{GW}/\text{BW})$$

Where GW is the weight of gonad and BW is the weight of crab.

To estimate the length at first sexual maturity (L_m), females were grouped separately into 5 mm class intervals and crab in stage III and above were considered mature. Length at first sexual maturity (L_m) was defined as the length at which 50 percent of all female crab having ovaries/testes at an advanced stage of development according to [13]. The proportion of the female crab has ovaries at the advanced stage of development (P) of each size group was adjusted by the correction factor as the biggest size group was 100%. The linear relationship between the size group and $\text{Ln}(1-P/P)$ was determined and the L_m was calculated at $P = 0.5$.

The sex ratio was determined by the ratio of the number of males and females. A Chi-square test was used to test the difference between the number of males and females in the natural population of the crab. Fecundity, defined as the number of eggs per batch produced by a female, and the weight of the spawning egg (brood) mass attached to the pleopods during embryonic

development were estimated. Embryonic stages were categorized by the development of color [9]: (1) initial or 1st stage, which is yellow or orange-yellowish; (2) intermediate or 2nd stage, which is brown; and (3) final or 3rd stage, which is black-grey or black. The masses at each stage were measured following the procedure described by [14] before removing each egg batch. Egg-bearing pleopods were removed carefully; the wet weight of the whole egg mass (eggs + pleopods) was measured to the nearest 0.1 g using an electronic balance (Model: GL-602, Bogor, Indonesia). Before the removal of the eggs, the egg-bearing pleopods were immersed in 400 ml of 1M KOH for 12 h. The separation process was completed by scraping the pleopods clean and removing all setae. These organs were then weighed, and their combined weight was subtracted from the whole egg mass to give the mass of the eggs alone. Three replicate subsamples of ~0.2 g were randomly taken from each wet egg mass, and the number of eggs in each subsample was counted under a stereomicroscope mounted over a counting tray. The mean number of eggs per unit subsample weight was counted; this value was then scaled up to estimate the total number of eggs per individual egg batch.

The absolute fecundity was estimated using the formula:

$$\text{Absolute Fecundity (F)} = (\text{weight of ovary/weight of sample}) \times \text{number of oocytes in the sample.}$$

The relationship between fecundity - weight statically determined using the following formula:

$$\text{Log F} = a \text{ Log X} + b$$

Where F is demoted fecundity, X is the weight (gam), a and b are constant.

Relative fecundity was calculated using the following formula:

$$S = F/W$$

Where S is relative fecundity W is crab weight (g).

3. RESULTS

Maturity stage ovary and testes of the Land crab in Ly Son Island are described in Table 2. Histology of the ovaries of the crab is present in Fig. 2.

Ratio of male and female of Land crab in Ly Son Islands was 1:1.2 (Fig. 3). Number of male and female was not significantly different $\chi^2 = 2.94 < 6.64$ (df = 1, $p < 0.01$).

The crab at mature (III) and ripe stages (IV) of ovaries presented all year round. However, percentages of crab at maturation stages of III, IV and V were higher during the period from February to August than that during the period from September to February (Fig. 4).

Percentages of the advance stage of ovaries (IV and V) in May and June were the highest (60.88% and 61.11%) and the lowest was in October and November (33.33%) (Fig. 4). GSI of the female crab in Ly Son Island was the highest from February (9.26 ± 1.07) to March (9.44 ± 2.24), and lowest was from September (2.61 ± 1.49) to October (2.77 ± 1.27) (Fig. 5a). For the male crab, the highest GSI was from February (8.83 ± 0.63) to March (8.57 ± 2.17), and the lowest was from October (1.12 ± 0.80) to November (2.11 ± 1.52) (Fig. 5b).

Length at first sexual maturity was 53.91 mm in carapace width for the female crab (Fig. 6a) and 50.39 mm for the male crab (Fig. 6b). The absolute fecundity of the crab in Ly Son varied in 473,000–820,000 ovaries/individual with an average of $685,152 \pm 102,707$ ovaries individual⁻¹. The relative fecundity varied in 4,141–7,614 with an average of $5,760 \pm 1,159$ ovaries gram⁻¹ of female crab. The relationship between fecundity and the carapace width of the crab was presented as in Fig. 7.

4. DISCUSSION

Understanding of the reproductive biology of a crustacean is very important for fishery resource management and aquaculture practices. This is the first attempt to investigate the reproductive biology parameters of the land crab (*Gecarcoidea lalandii*) in Viet Nam and the second study on the reproductive biology of this species in the world. The first study on the reproductive biology aspect of this species was conducted in Taiwan by [8]. This report focused on population data, breeding season, and larval release timing and behavior, with particular focus on the unusual larval release behavior in Taiwan [8]. By contrast, the present study reveals the results on gonadal development stages, sex ratio, spawning season, fecundity and size at first sexual maturity of this species in Vietnam. In this study, the gonad of the land crab *Gecarcoidea lalandii* was divided into 5 stages of maturity. This is normally observed in the tropical crab having the year-round reproduction cycles. At mature or ripe stages, besides the main component oocytes (oocyte at early vitellogenic and advanced vitellogenic stage), the primary growth oocytes also exist at the considerable number. This was the evidence that the crab spawns continuously during the spawning season. In Taiwan, at least some *G. lalandii* females can reproduce twice within a reproductive season [8]. By contrast, in the land crab *Epigrapsus politus* in Japan, females produced only one brood within a single breeding season [15].



Fig. 1. Study area. The map shows the location of Ly Son district, including two Islands: Lon and be Island, beyond in the Quang Ngai Province, Vietnam

Table 1. Number of crabs collected each month during 2019 – 2020

Month	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul
No	22	32	30	30	30	31	23	30	30	31	23	36

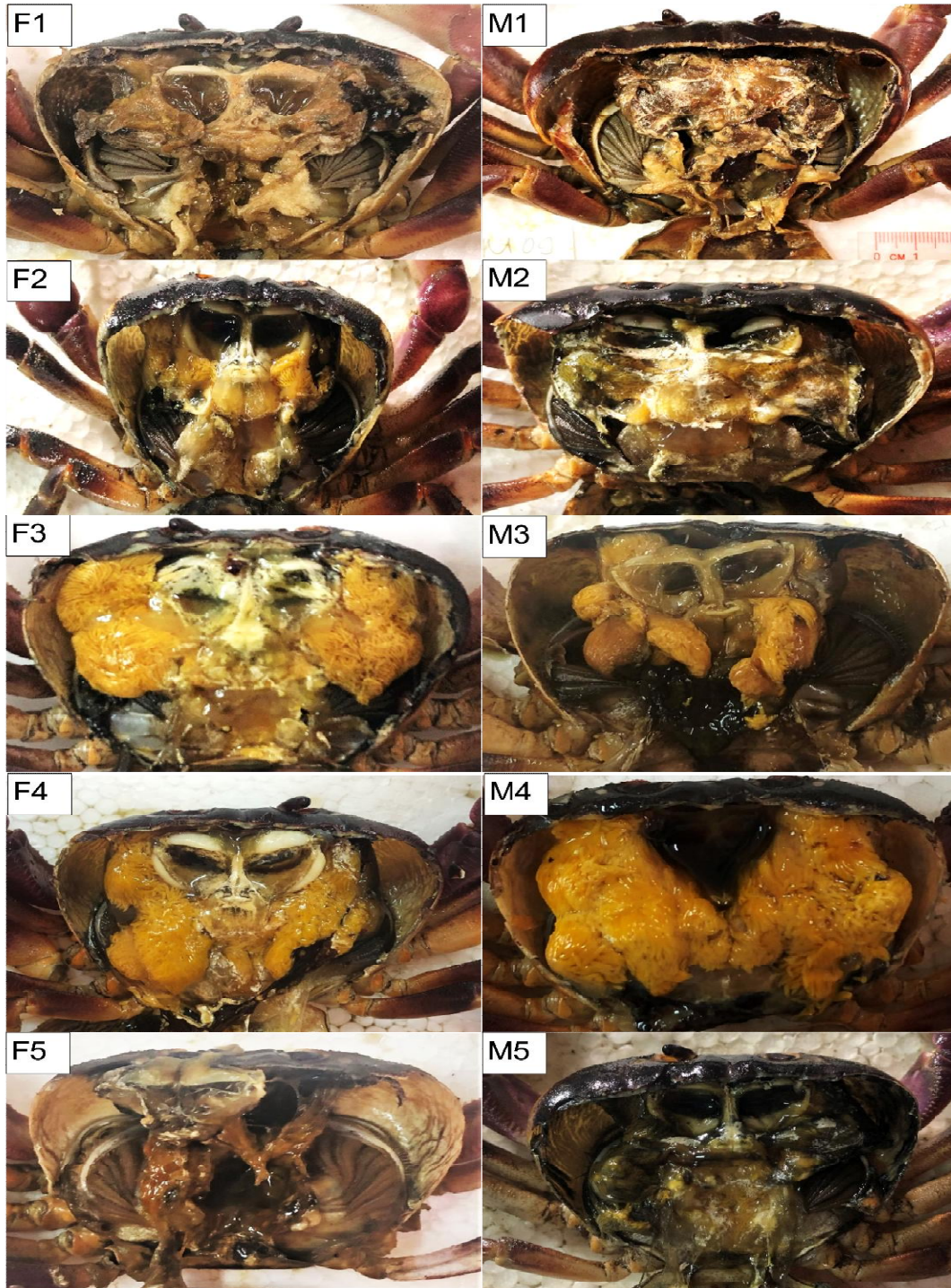


Fig. 2. Maturity stage of the ovaries and testes of the Land crab

Table 2. Maturity stage of the ovary and testes of the Land crab in Ly Son Island

Maturity stage	Particular of ovary and testes
Stage I, Immature	Ovaries and testes are thin, and glassy in appearance or white. It could not be distinguished ovaries and testes by snake-eyes (Fig. 2 - F1 & M1). The stage is observed in the crab size under the first maturation size
Stage II, Maturing	Gonad developing. Ovaries and testes are increasing in size, the Ovaries and testes are cream yellow in color (Fig. 2- F2 & M2).
Stage III, Mature	Ovaries and testes increase in size comparing to stage II. Ovaries and testes are yellow or orange, extending about 1/2 - 1/3 of the hepatopancreas area (Fig. 2- F3 & M3).
Stage IV, Ripe/Oozing	Ovaries and testes increase to the maximum in size comparing to stage III, extending all the hepatopancreas areas, and ready for the spawning. Ovaries and testes are orange or orange – yellow in color (Fig. 2- F4 & M4).
Stage V, spent	Ovaries and testes are rather flaccid (Fig. 2- F5 & M5) and become rather darker brown in color comparing to stage III and IV.

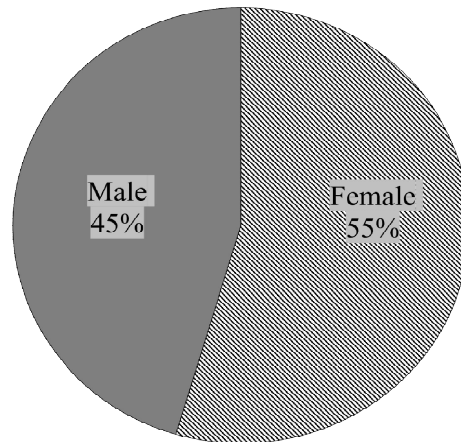


Fig. 3. Male: Female ratio of the land crab in Ly Son Islands

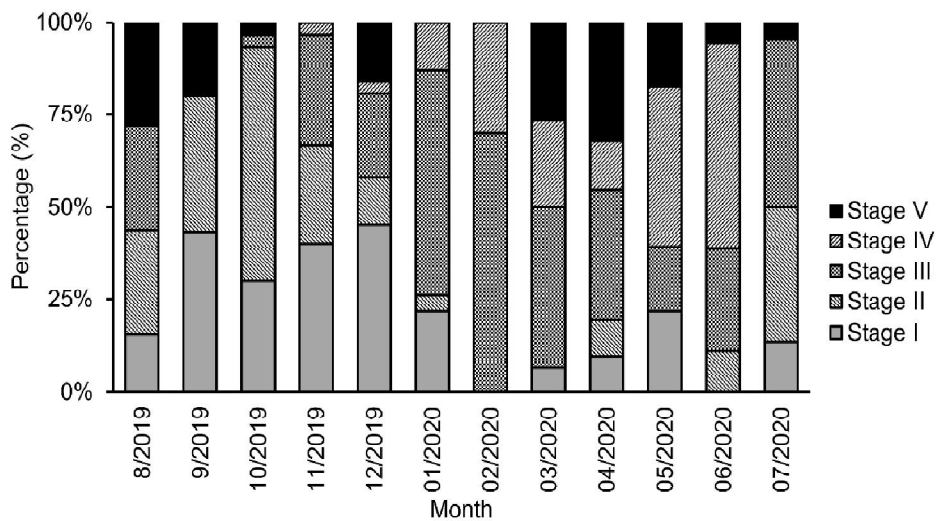


Fig. 4. Monthly percentage of different gonadal maturation stage in Land crab in Ly Son Island

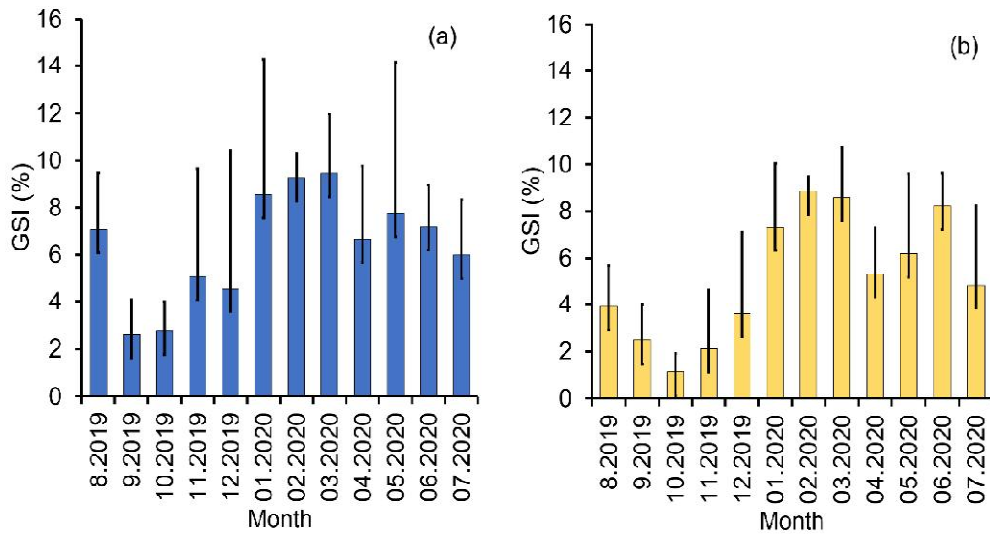


Fig. 5. GSI of female (a) and male (b) Land crab in Ly Son island

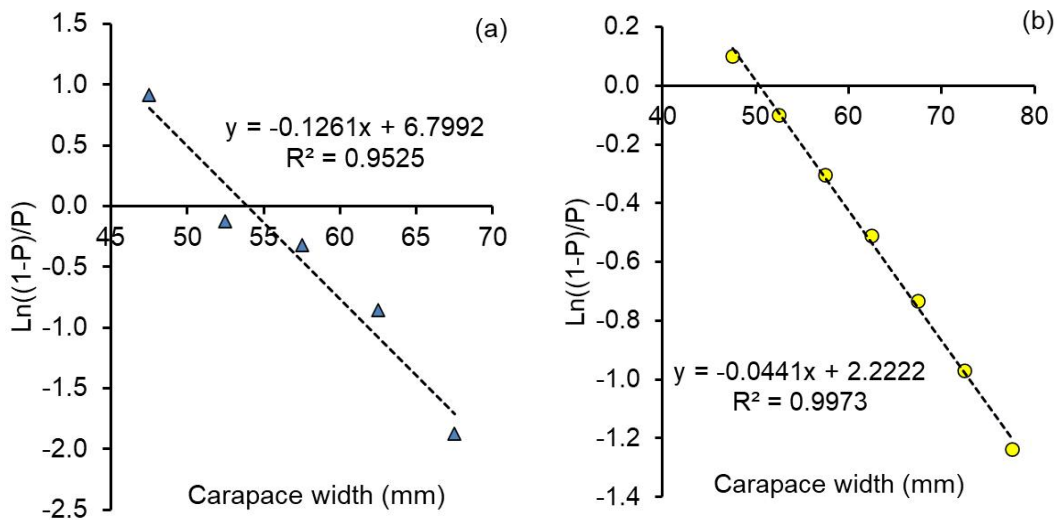


Fig. 6. Length at first sexual maturity of the female (a) and male (b) Land crab in Ly Son Island

In addition, the data on the changes in the percentage of maturity stages (stage III, IV and V) and GSI in the current study suggest that the crab can mature whole year-round with a higher proportion from January to August and the peak maturation from June to July. Even the females have gonad at stage III-IV occurs whole year-round, the ovigerous females only occur from June to July. This is the evidence that the spawning season of this species is only from June to July. This time is the rainy season in Ly Son. The results are consistent with the previous study of that *G. lalandii* in Taiwan have the reproductive cycles that begin at the onset of the

rainy season, and the breeding season lasts longer and ends almost at the same time as the rainy season tapers off [8]. The breeding ecology of *E. politus* was characterized as being short and occurring in a cooler season than that of other gecarcinids inhabiting the subtropical islands of the northwest Pacific [15]. The results imply that even the gonadal of the females develop to the mature stage (III-IV), the spawning occurs only when the crab met the suitable ecological parameters such as raining, humidity. The previous studies on the land crab also reveal that the breeding season also shows different from species to species. Species of

Cardisoma usually have a longer breeding season compared to other gecarcinids. *Cardisoma guanhumi* in south Florida breed from June or early July to December [16]. In Okinawa, the land crab *Cardisoma hirtipes* to release larvae from June to November [17]. On Aldabra, the spawning season *C. carnifex* (Herbst) on Aldabra I. lasts for 6 lunar cycles [18]. On the other hand, the breeding season of *Epigrapsus notatus* (Heller) only lasts for 2 months [19]. The breeding activity of *G. natalis* also lasts for 2 or 3 lunar cycles with only 1 marked by major reproductive activity. *Gecarcoidea lalandii* in Taiwan has a longer breeding season which lasts 4-5 months [8]. Ovigerous (egg-bearing) females *Epigrapsus politus* in Japan were detected from early September to late November, with the highest abundance occurring between late September and early November [15].

Fecundity of the crab is determined by the number of ova of the mature group in the ovary. In the present study, fecundity of *G. lalandii*, was determined from the examination of 30 specimens. Fecundity of the land crab in the present study showed a high correlation coefficient with the total weight of the crab. The regression of fecundity and total weight can be expressed as $\text{LogF} = 0.451 \cdot \text{LogW} + 4.8928$ with $R^2 = 0.8678$. This finding suggests that total ovaries in a spawning season depend on the

crab size. The fecundity of the crab in Ly Son is higher than the fecundity of this crab reported for Taiwan. The number of developed larvae carried by females varies between 70,000 and 210,000 depending on the size of the female, with fecundity directly increasing with size up to 65 mm CW, then falling slightly [8].

Length at first sexual maturity of the crab was determined by the accurate method described by [13]. In the current study, female matures at a larger size (53.91 mm CW) than male crab (50.39 mm CW). For the male crab *Johngarthia lagostoma*, 60-70 mm CW both sexes are mature. The 61-70 mm CW class has a high proportion of ovigerous females, as do all larger classes and the smallest ovigerous female was 63 mm CW [20].

The results of the present study are primary basic data for natural resource management and conservation of land crab (*Gecarcoidea lalandii*) in Ly Son island. It is suggested that the catching of the crab is not permitted for the ovigerous females and the female crab smaller than 55 mm CW and male crab smaller than 51 mm CW. Besides, the catching time is inhibited from May to July. Moreover, the long-term strategy sustainable using the resource of this crab in Ly Son needs to be established.

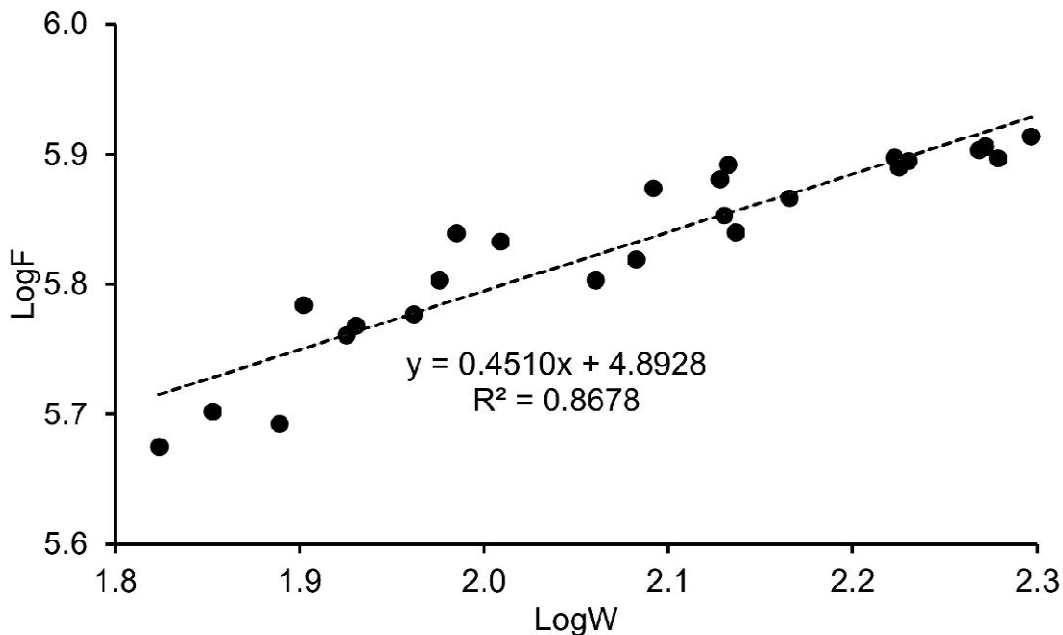


Fig. 7. Relationship between fecundity and weight of the Land crab in Ly Son island

4. CONCLUSION

The land crabs are the famous food of Ly Son District. Increasing the number of tourists has put high pressure on the crab resources both in abundance and biodiversity. Our findings of reproductive biology of the land crabs is the first scientific evidence both in Ly Son Islands and in Vietnam generally. In Ly Son, the carapace width at first sexual maturity was identified in female crab of 53.91 mm and in male crab of 50.39 mm, whereas the male : female ratio was 1:1.2. The crab can spawn continuously during the spawning season and can mature whole year-round but the peak maturation is from June to July. Therefore, to protect and conserve the land crabs in nature, it is involved in the spawning season in the master strategy of biodiversity protection in Ly Son Islands.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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